EFFECT OF POLYETHYLENE TEREPHTHALATE (PET) MICROPLASTIC ON THE BIOLOGICAL ACTIVITIES AND HEAT SHOCK PROTEINS OF *DROSOPHILA MELANOGASTER*

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Zoology

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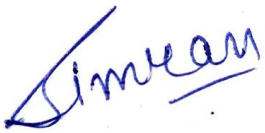
2025

DECLARATION

I hereby declared that the presented work in the thesis entitled "Effect of polyethylene terephthalate (PET) microplastic on the biological activities and heat shock proteins of *Drosophila melanogaster*" in fulfillment of the degree of **Doctor of Philosophy (Ph. D.)** is the outcome of research work carried out by me under the joint supervision of Dr. Yachana Mishra working as an Associate Professor at the School of Bioengineering and Biosciences of Lovely Professional University, Punjab, India and Dr. Mahendra P. Singh, Associate Professor, Dept of Zoology, DDU Gorakhpur University, Gorakhpur, India. In keeping with the general practice of reporting scientific observations, acknowledgments have been made whenever the work described here has been based on the findings of other investigators. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



(Signature of Scholar)

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CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled "Effect of polyethylene terephthalate (PET) microplastic on the biological activities and heat shock proteins of *Drosophila melanogaster*", submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the School of Bioengineering and Biosciences, is a research work carried out by Simran, 12108721, is a bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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ABSTRACT

Microplastic toxicity has become a major harnessing environmental concern, increasing evidence indicating its detrimental effects on terrestrial as well as aquatic ecosystems. These tiny plastic fibers and particles, measuring under 5 milli meters, are ubiquitous in the environment and can be consumed by a wide range of organisms, leading to various physiological stresses and toxic impacts. In addition to the physical hazards of ingestion, microplastics also serve as carriers for harmful chemicals, which can be absorbed by organisms, leading to oxidative stress, inflammation, and genetic damage. Although awareness of these issues is increasing, the detailed mechanisms through which microplastics cause toxicity, particularly in terrestrial species such as *Drosophila melanogaster*, remain insufficiently understood. This research aims to address this knowledge gap by offering new perspectives on the health risks associated with microplastics and enhancing our understanding of their broader ecological consequences. With the given microplastic contamination, it has become a global concern, prompting numerous research efforts to understand its effects. These prevalence rates underscore the extensive reach of microplastic pollution across various ecosystems, highlighting the urgency for further research into its impacts, including studies using model organisms. Studies have consistently shown that plastics have harmful consequences on aquatic life. While various types of microplastics have been studied, our research focused specifically on polyethylene terephthalate (PET) microplastics. This focus is due to the remarkable durability and resistance of PET to decomposition, leading to its persistent accumulation in the environment.

Our research aimed primarily to evaluate the toxicity of various concentrations (10, 20, 40 g/L) and durations of exposure (2, 24, 48h for larvae and 15 days for flies) to PET microplastics. Previous studies did not provide conclusive evidence on the concentration at which PET microplastics exert the most significant effects, making this assessment crucial. Additionally, there remains a gap in the understanding of the effects of PET microplastics on terrestrial model organisms, a gap our study sought to address. Key factors such as size, exposure duration, and the quantity of microplastic particles, both at the organismal and sub organismal levels, are considered critical in determining their impact.

In our study, the terrestrial model organism *Drosophila melanogaster* (Oregon R^+), owing to the significant genetic similarities it shares with higher animals, including 73% homology with human genes, was selected. We characterized PET microplastics ranging from 2 to 100 µm in size via FTIR, SEM and EDX and confirmed their accumulation within *Drosophila* via Nile red dye and confocal microscopy, which allowed us to further explore their toxicological effects. At higher dosages, we observed a decline in physiological and neuromuscular functions, as evidenced by impairments in locomotor activities such as climbing, jumping, and crawling. Additionally, our research revealed delayed development and a reduced survival rate in female flies exposed to the highest concentrations of microplastics. These experimental findings provide critical insights into the potential neurotoxic effects of MPs and their detrimental effects on the growth and development of *Drosophila*. Given the widespread presence of microplastics, further research is necessary to explore their consequences for living organisms comprehensively.

Our research also investigated the cellular and genetic toxicity of PET microplastics to *Drosophila melanogaster*. We observed significant cytotoxicity in the larvae's midgut alongside the induction of oxidative stress, as indicated by changes in total protein, protein carbonyl, MDA content, *Cu-Zn* SOD, and CAT activity. Specifically, we noted a decrease in total protein content and an increase in protein carbonyl, *Cu-Zn* SOD, CAT, and MDA levels after 24 and 48 hours of exposure. Notably, for the first time, our study revealed cellular damage to reproductive organs, including the ovaries of female flies and the accessory glands and testes of male flies. This damage was accompanied by a decline in reproductive health, leading to reduced fertility among the flies.

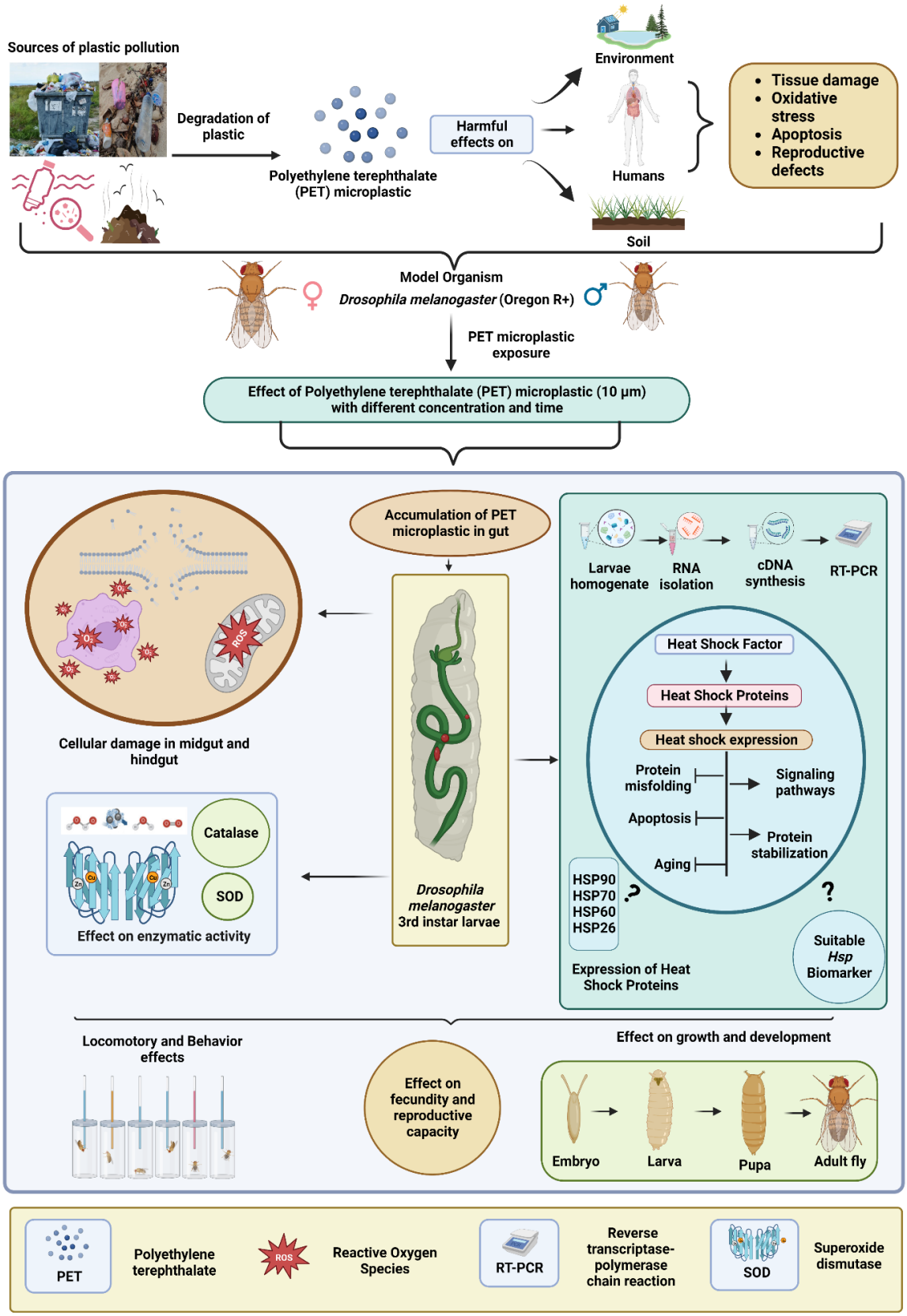
We conducted an in-depth analysis of stress-related genes, including *hsp 83, hsp70, hsp 60*, and *hsp 26*. Our results demonstrated an increase in the expression of *hsp 83* and *hsp 70*, while *hsp 60* expression was downregulated, and *hsp* 26 remained unchanged. Importantly, we identified *hsp 83* as a specific biomarker for detecting early stress caused by PET microplastics. This finding offers new perspectives on the primary defense mechanisms against the toxicity of PET microplastics.

The study contributes valuable knowledge to the increasing environmental threat posed by microplastics, emphasizing significant genetic alterations. The reactions involving heat shock proteins (HSPs) are crucial for preventing DNA damage and maintaining

cellular integrity. HSPs could serve as biomarkers to detect and monitor human exposure to microplastics and their genotoxic effects. Understanding the role of HSPs in response to microplastic-induced genotoxicity may lead to the development of therapies that increase cellular resilience by targeting HSPs for modification. The threat posed by microplastic toxicity to organisms is increasing substantially, and the implications of daily microplastic use cannot be ignored. The severity of microplastic pollution has driven an increase in toxicological research, including our own efforts to assess its impact at the cellular and genetic levels. At higher concentrations, microplastics have been observed to induce cellular and reproductive toxicity, corresponding to elevated oxidative stress, as determined by various oxidative stress markers. Moreover, research has highlighted the role of HSPs in the primary defense mechanism against microplastic toxicity. However, there is a notable gap in understanding the molecular mechanisms and long-term consequences of microplastic toxicity in *Drosophila*. These findings underscore the need for further investigations into the genetic alterations induced by microplastics, particularly regarding potential transgenerational effects that could pose significant risks to future generations.

Key words: - *Drosophila melanogaster*, Polyethylene terephthalate, Heat shock proteins, Toxicity

GRAPHICAL ABSTRACT



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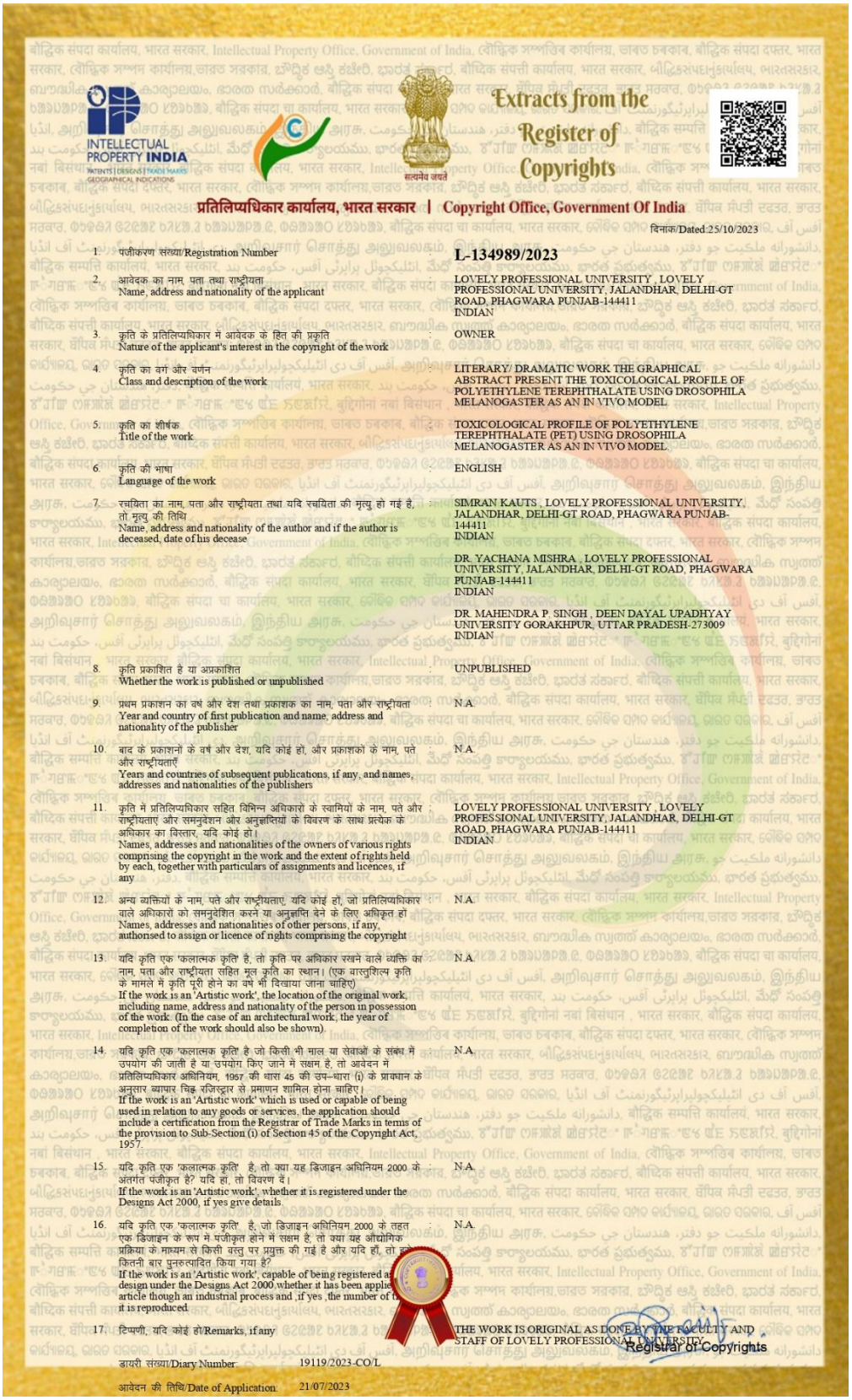

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SIMRAN

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CHAPTER 1

INTRODUCTION

In recent years, microplastic pollution has become a major environmental issue, significantly threatening ecosystems, wildlife, and human health (Ghosh et al., 2023). Microplastics is linked to multiple sources, including the degradation of larger plastic objects and the release of products that contain microplastics (Hale et al., 2020). Negligence in waste management and disposal practices play a major role in this issue, as plastic objects break down into smaller particles with time due to sunlight and environmental wear. Furthermore, everyday activities like washing synthetic fabrics, using personal care products containing microbeads, and the wear and tear of car tires on roads contribute to the significant release of micro and nano plastics into the environment (Dimassi et al., 2022). There are two types of plastic particles: microplastics and nano plastics. These particles have garnered a growing amount of interest because of their ubiquitous prevalence in the environment as well as the potential consequences they might have on human health and the ecosystem (Agamuthu, 2018). It is possible that these minuscule plastic particles are the product of the disintegration of larger plastic objects or that they are intentionally made at a smaller scale. The size range of microplastics is generally between 5 micrometers to 5 millimeters. Microplastics are small particles derived from plastic, and their formation can result from various factors, including the friction of synthetic fabrics, tire wear, the breakdown of plastic waste, and the disintegration of plastic products like bottles, bags, and packaging materials (Enders et al., 2015). There is proof of the presence of microplastics in a variety of ecosystems, such as the air, rivers, lakes, soils, and even seas. Through their processes of ingestion and accumulation, they have the potential to endanger aquatic life as well as terrestrial and marine ecosystems (Ma et al., 2020). People are becoming more concerned about the likelihood of microplastics being transferred through the food chain. Humans may also be exposed to microplastics through the ingestion of seafood and other food products (Pironti et al., 2021). The size of nanoplastic is on the nanoscale, often ranging from one to one hundred nanometers. Nanoplastic are even smaller than regular plastic particles. An additional breakdown of microplastics might result in the formation of nanoplastic (Enfrin et al., 2020). Nanoplastic can also be produced as a consequence of manufacturing operations that are carried out on purpose, such as the fabrication of nanosized plastic particles suitable for certain purposes. Owing to their small size, nanoplastic can penetrate the structures of

cells and may interact with biological creatures that are distinct from those of other substances (Shen et al., 2019). Ongoing research is being conducted in several specific areas, including the behavior and destiny of nano plastics in the environment, as well as their potential toxicity (Wang et al., 2021). Because of small size, their capacity to be carried over extensive distances and their propensity to introduce themselves into biological systems are worrisome. Owing to their potential to disrupt food webs and ecosystem processes, both microplastics and nano plastics present problems for ecosystems and biodiversity (Machado et al., 2018). Microplastics are more prevalent than nano plastics and present significant environmental risks due to their persistence in the environment, resistance to degradation, and the difficulty of removing them (Nowack et al., 2007). In current years, the issue of MPs has gained prominence as a key environmental concern, bringing attention to their sources, dispersion, and widespread impact on human and environment (Xiang et al., 2022). As we confront the challenges of plastic pollution, the presence of microplastics across various environmental compartments requires urgent attention and in-depth investigation (Ogunola et al., 2018). This introduction aims to offer a thorough exploration of the definitions, origins, environmental impacts, and potential consequences of microplastics on human health. To develop effective mitigation strategies, it is crucial to understand the sources of microplastics (Gong and Xie, 2020). Microplastics originate from the breakdown of larger plastic items such as bottles and packaging materials, which are key sources of these particles (Barnes et al., 2009). Additionally, they are produced through the wear of synthetic fabrics, tire degradation, and the breakdown of plastic waste. The wide range of sources contributing to microplastic pollution highlights the need for a comprehensive strategy to tackle the issue at its source (Devendrapandi et al., 2024). Once released into the environment, microplastics can disperse across ecosystems, contaminating water bodies like oceans, rivers, and lakes, as well as soil and even air. These particles pose significant risks to both terrestrial and aquatic life (Bradney et al., 2019). The ecological consequences are amplified as microplastics are absorbed and accumulate in organisms, potentially triggering cascading effects throughout the food web (Huang et al., 2021) as shown in Figure 1.1.

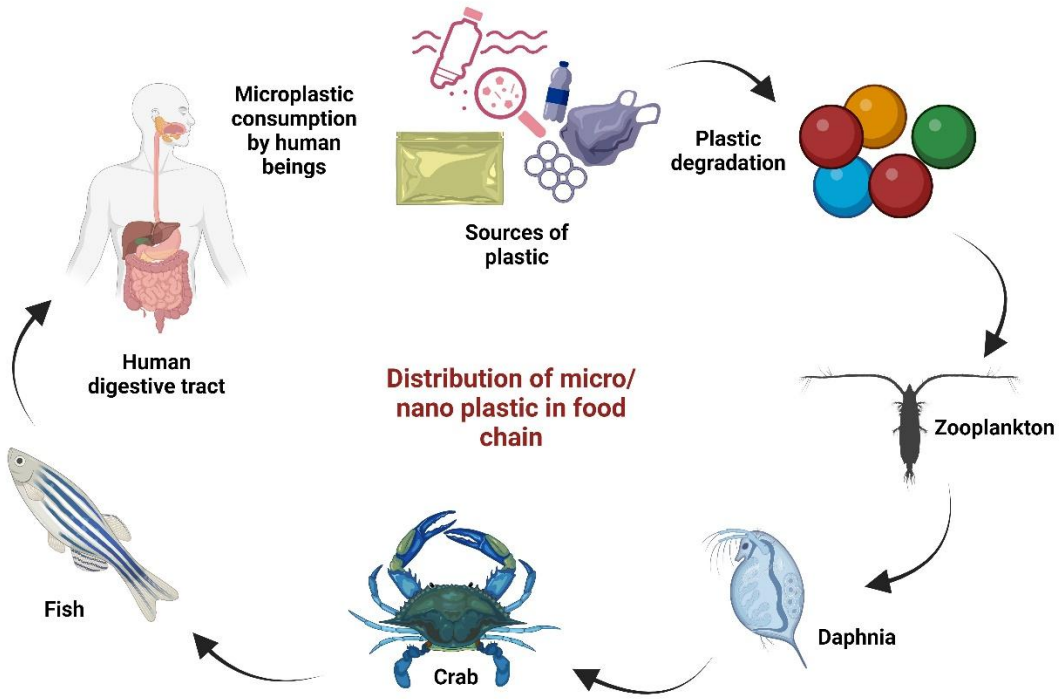

Figure 1.1: Schematic representation of microplastic consumption by humans through the food chain

A better understanding of the magnitude of the environmental problem that we are now facing through research into the complex mechanisms by which microplastics contribute to the disruption of ecosystems is essential (Prinz and Korez, 2020). The spectra of microplastics have become a worldwide environmental problem in this age of ubiquitous plastics. Microplastics make their way into a wide variety of habitats, ranging from the depths of seas to the summits of mountains, it is highly important to have a comprehensive grasp of the prevalence rates of microplastics to assess the scope of the problem accurately (Vincent and Hoellein, 2021). The pervasiveness of plastic pollution has spread beyond geographical bounds, resulting in the creation of a complex web of environmental damage (Da Costa et al., 2020). Microplastics, which are characterized by their small size and insidious nature, have become an all-encompassing worldwide issue in the middle of this catastrophe (Filella, 2015). These minute particles, which are often undetectable to the human eye, have entered ecosystems with an ease that has never been seen before (Landrigan et al., 2020). As a result, researchers worldwide have begun to explore the prevalence rates and dispersion patterns that constitute the

foundation of this silent threat. In recent years, microplastics have expanded their reach beyond the confines of specific pollution hotspots and have now reached a genuinely global scale (Hale et al., 2020). Gyres have been amassing enormous amounts of microplastic particles, which has led to oceans, which are considered to be the birthplace of life on Earth, becoming reservoirs of these particles (Issac and Kandasubramanian, 2021). Nevertheless, pollution is not confined to habitats that are found in the ocean (Kim et al., 2023). According to the prevalent research (Wu et al., 2019), microplastics could be detected in water bodies, soils, and even the atmosphere. This suggests that there is a complex web of dispersion that transcends the boundaries that are traditionally established. Assessing the presence of microplastics on a worldwide scale is fundamentally difficult because of the wide variety of ecosystems, the procedures that are used, and the many sources that contribute to contamination (Horton et al., 2020). Direct comparisons across studies are more difficult because of differences in the sampling methodologies, analytical methods, and size categorizations used in each study. There are two types of microplastics, primary and secondary (Auta et al., 2017). Primary MPs, such as microbeads in personal care products, are directly released into the environment. In contrast, secondary MPs result from the breakdown of larger plastic items. (Winkler et al., 2022). Primary microplastics are intentionally made for particular objectives. According to the International Union for the Conservation of Nature, there are seven principal sources of microplastics (Komyakova et al., 2020). These sources include synthetic fabrics, vehicle tyres, road markings, personal care items and cosmetics, plastic pellets, marine coatings, and city dust (Kole et al., 2017). Additional sources of microplastics include activities that take place in the ocean, such as shipping, fishing, and tourism, as well as microplastics that are carried by the air. Rivers are often regarded as the most significant conduits by which microplastics are transferred from inland regions to seas (Fan et al., 2022). Microplastics may also originate from plastic debris that is found in urban drainage systems and sewage effluents, as well as from the faulty management of inland regions that are blown into the sea via rivers and from plastic garbage that is discharged directly into the environment as a result of beach-related tourism (Dris et al., 2018)

1.2 Prevalence rate of microplastics

Studies indicate that microplastics are virtually ubiquitous, with detection in water the air we breathe (Logannathan et al., 2023). Oceanic environments, in particular, bear a heavy burden, with estimates suggesting that millions of tons of microplastics are present in seas (Choudhury et al., 2022). Coastal regions and urban areas tend to have relatively high concentrations due to human activities and waste disposal. A recent study revealed the geographical distribution of microplastic debris, revealing significant contributions from Southeast Asia, North America, and Africa (15.9%), (17.2%), and (8.7%) respectively (Duis and Coors, 2016). Importantly, this data is limited to primary microplastics from specific sources and do not provide a comprehensive overview of microplastic release into aquatic ecosystem (Akdogan and Guwen, 2019). Numerous studies have identified secondary microplastics, emphasizing their prevalence resulting from the continuous fragmentation of plastic items (Patli et al., 2021). The findings of the present study align with this observation, revealing the dominance of secondary microplastics in aquatic ecosystem (Zhao et al., 2019). While primary MPs are notably relevant in certain industries, such as cosmetics care products, their production is limited. Nevertheless, microplastics have been widely documented across diverse environments and geographical regions (Akdoven and Guven, 2019). Different studies have been performed in different countries on microplastics, as shown in Figure 1.2 (a), and on different organisms, as shown in Figure 2(b).

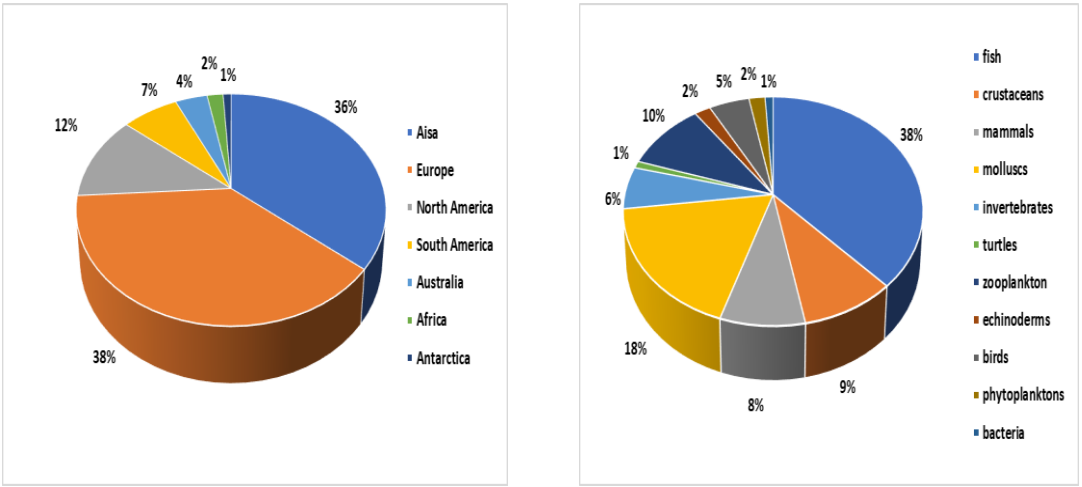

Figure 1.2: - Graphical representation of studies showing the prevalence rates of microplastics in different countries and different organisms (Ajith et al., 2020).

1.3 PET production and prevalence rate

PET microplastics are among the most widespread pollutants across diverse environments, largely due to their extensive use in consumer goods. These microplastics are pervasive in various ecosystems, ranging from urban waterways to remote locations such as the Arctic and the deep sea microplastic concentrations show notable variation by region, with higher levels typically detected in urban and coastal areas (Citterich et al., 2023). Recent researchers applied a novel analytical technique to assess PET-based microplastics in 286 indoor dust samples across twelve countries, finding PET MPs concentration in all samples of 38 to 120,000 µg/g, with a median concentration of 5,900 µg/g. The concentration MPs in these dust samples ranged from 29 to 110,000 µg/g, reflecting considerable variability among different countries studied (Dewika et al., 2023).

South Korea recorded the maximum concentration of PET microplastics of 25,000 µg/g, followed by Japan at 23,000, Saudi Arabia at 13,000, Greece at 9,700, and Romania at 9,100 µg/g respectively. In contrast, lower median levels of microplastics in dust were found in the United States (8,900 µg/g), Kuwait (8,600 µg/g), Vietnam (3,900 µg/g), China (3,700 µg/g), Pakistan (1,900 µg/g), India (1,600 µg/g), and Colombia (1,000 µg/g). One sample from China had the highest concentration at 120,000 µg/g, accounting for 12% of the total dust sample mass (Dewika et al., 2023). In 2016, global PET production was estimated at 53 metric tons. Exposure to PET has been associated with adverse effects, irritation, discomfort, tearing, and blurred vision. PET breakdown in the environment and in human systems can release TPA, which is thought to have lower toxicity; however, the widespread production and resulting exposure to TPA remain a concern. Indoor dust ingestion is recognized as a significant pathway for human exposure environmental contaminants (Sojobi et al., 2016). In India, 1,692 kilotons (kt) of PET waste were generated in 2021-22, with 38% (640 kt) of food and beverage packaging and the remaining 62% (1,053 kt) from non-food and mixed packaging applications. During the same period, India produced an estimated 2,301 kt of virgin PET resin, primarily for purposes, maintaining growth in production, imports, and exports since 2007, with a temporary decline due to COVID-19 lockdowns (Nandi et al., 2023). Since the pandemic, PET demand has recovered, driven by increased packaging needs in the food and beverage to a rise in PET imports and exports. In 2021-

22, approximately 46% (686 kt) of India's bottle-grade PET chips were exported, with the remaining 807 kt untypically, supplemented by 185 kt of imported bottle-grade chips. About 21% (173 kt) of PET flexibles produced in India were exported, while the remaining 635 kt was consumed domestically, along of imported flexible-grade PET chips (Shanker et al., 2023).

In our current research, we used the specific microplastic polyethylene terephthalate to determine its toxicological profile and effects on terrestrial model organisms. Owing to their extensive use, these goods significantly contaminate the environment as they degrade into microplastics (Joseph et al., 2024). It is very resistant to deterioration from the environment. PET microplastics may linger in the environment for extended periods of time after discharge, which helps them accumulate in different ecosystems. Concern over the possible health hazards linked to PET microplastics is increasing (Santomasi et al., 2024). Numerous species, including humans, may consume these small particles, potentially having harmful effects. The goal of research is to examine the potential health concerns posed by these microplastics and how they interact with biological systems (Bharadwaj et al, 2024). The toxicological effects of the microplastics themselves may be exacerbated by these pollutants, which can be ingested by creatures (Piccardo et al., 2020). Research is being conducted to determine how organisms disseminate and acquire PET microplastics. Determining their possible negative effects and long-term exposure requires an understanding of their biodistribution. Concerns about public health and regulations have increased as a result of increasing knowledge of microplastic contamination (An et al., 2024). Public health recommendations and policy actions aimed at lowering exposure and limiting hazards are informed by research on PET microplastics. PET microplastics are of great scientific interest because of their distinct physical and chemical characteristics. Researchers are examining the processes by which they degrade, how they interact with living things, and whether they may be harmful to molecules and cells (Lu et al., 2024).

The essential component of all metabolic processes that result in ROS production is oxygen. These creatures have evolved a variety of strategies to eliminate ROS and prevent oxidative damage to their cells (Palma et al., 2024). Oxidative stress in cells occurs when the balance between ROS production and the neutralization of ROS molecules increases. Increased ROS cause lipid, protein, and DNA damage. It may also

result in a number of illnesses, including atherosclerosis and HD (Hong et al., 2024). Cellular stress is caused by oxidative stress. Mitochondria are the principal residences and targets of ROS. As the powerhouses of the cell, mitochondria produce ATP for cellular processes. In mitochondria, the electron transport chain (ETC) generates ATP (Manoharan et al., 2024). Owing to its imperfections, the ETC experiences electron leakage. Cellular ROS are the result of additional reactions between these released electrons and molecular oxygen. These ROS specifically target ETC complexes I and III, which increase the production of ROS, deplete ATP, and ultimately cause the cell to die (Rasouli et al., 2024). Genetic alterations, environmental exposures, or both can function as triggers for the generation of mitochondrial ROS in cells. The term "cellular toxicity" describes the harmful effects that environmental stressors or toxic chemicals have on the structures and activities of cells, which can eventually result in cell death or damage (Obeagu et al., 2024). Comprehending the processes underlying cellular toxicity is crucial for formulating effective treatment plans, reducing environmental risks, and guaranteeing medication safety. These are the main ways in which cellular toxicity might manifest itself. Direct interactions between genotoxic chemicals and DNA can result in strand breakage, chromosomal abnormalities, crosslinking, and mutations (Bilal et al., 2024). While some chemicals produce ROS that indirectly damage DNA, others create DNA adducts. Certain toxicants can change the cellular environment, mutate amino acids, or interfere with chaperone activities to impede protein folding. Proteins that are misfolded can combine to generate hazardous oligomers and fibrils (Ladeira et al., 2024).

1.4 Induction of stress and the necessity of HSPs in organisms

Several physiological and environmental conditions can cause cellular stress, as shown in Table 1.1.

Table 1.1: Causes of heat shock proteins and their effects on organisms.

Causes	**Effects**	**References**
Thermal Stress	High temperatures have the ability to denature proteins, causing them to aggregate and become less	(Kayastha et al., 2024)

	functional. Heat shock response pathways are triggered in response by cells.	
Oxidative Stress	Proteins, lipids, and DNA are damaged when there is an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defenses of the cell	(Houldsworth, 2024)
Heavy Metals	By binding to biological proteins, metals such as arsenic, mercury, and cadmium can cause structural and functional changes	(Alazoumi et al., 2024)
Toxins and Chemicals	By interfering with metabolic processes and causing damage to cellular components, exposure to toxic chemicals can cause cellular stress.	(Zahaba, 2024)
UV Radiation	UV radiation has the potential to directly damage DNA and produce reactive oxygen species (ROS), which can stress cells**.**	(Wei et al., 2024)

HSPs are a group of proteins that are triggered by stress (Nawaz et al., 2024). HSPs are classified based on their molecular weight, with the main families being *hsp 100, hsp 90 hsp 26, hsp 60, hsp 70*, HSPs are an essential part of every living organism's cellular apparatus. These are highly conserved proteins that, especially in times of stress, are crucial for preserving cellular homeostasis (Liu et al., 2024). There are a few explanations for why the body needs HSPs. Nearly every biological function depends on correctly folded proteins. Misfolded protein aggregates, which are toxic to cells, have been associated with several diseases, including neurodegenerative disorders like

Alzheimer's and Parkinson's disease. (Carpano et al., 2024). Stressful situations are regularly encountered by cells and can harm proteins and other biological constituents. HSPs offer a vital defense mechanism that helps cells endure and recover from these harsh circumstances (Tukja, 2024). For cells to survive under stress, it is essential to be able to stop needless or premature cell death. This phenomenon is especially crucial for tissues such as brain neurons, which are difficult to regenerate. Innate and adaptive immunity are both aided by HSPs, which enable the body to create a powerful defense against infections. Additionally, they support immunological homeostasis and reduce inflammation (Kunachowicz et al., 2024). Proteins that have been damaged or misfolded can accumulate and become hazardous to cells. HSPs assist in preserving cellular proteostasis and averting the negative consequences of protein aggregation by promoting protein breakdown (Rathinam et al. 2024). Significant alterations occur in cells throughout development and differentiation, necessitating the exact control of protein function. HSPs preserve the function and integrity of proteins, ensuring that these processes proceed without any obstacle (Skivaki et al., 2024). An organism's ability to adapt to changes in its environment is essential to its survival. This adaptive response, which enables organisms to endure and recover from environmental stresses, is largely dependent on HSPs (Amstrup et al., 2024).

Researchers are significantly troubled by the utilization of animals in biological testing and research, not only because of scrutiny from animal rights organizations but also because of the challenges posed by intraspecies variation, making data interpretation difficult, and the high costs associated with each experimental setup (Kauts et al., 2023). As a result, scientists are driven to investigate other small model organisms, including *Caenorhabditis elegans, Drosophila melanogaster, and Saccharomyces cerevisiae*. These organisms have been employed to model various diseases, successfully demonstrating disease characteristics and revealing numerous genes that influence the accumulation and lethality of disease-associated proteins (Kauts et al., 2023). In our research, we focused on determining the biological activity of *Drosophila melanogaster* after exposure to PET microplastics. *Drosophila melanogaster* serves as an excellent model for studying the effects of environmental pollutants, including microplastics, heavy metals, pesticides, and endocrine-disrupting chemicals**.** Due to its high genetic similarity to mammals and well-mapped genome, the fruit fly allows researchers to

investigate toxicity mechanisms, cellular stress responses, and genetic mutations caused by environmental contaminants. The utility of *Drosophila melanogaster* in environmental studies is expanding, particularly in the fields of toxicology, pollution assessment, and ecological health monitoring. Its genetic tractability, short life cycle, and physiological similarities to higher organisms make it an ideal model for studying the biological consequences of environmental pollutants. By leveraging *Drosophila* research, scientists can better understand the ecological and health risks posed by contaminants and contribute to the development of effective mitigation strategies. Oxidative damage, cellular toxicity and heat shock proteins expression are the main focuses of our study. In addition to observing the direct impacts on development, reproduction, longevity, and behavior, researchers investigating the toxicity of microplastics in *Drosophila* can also investigate the underlying biological pathways (Richard et al., 2024). Understanding the potential effects of microplastics on more sophisticated creatures, such as humans, can be aided by these results.

CHAPTER 2

REVIEW OF LITERATURE

The increasing prevalence of microplastics in the environment has become an urgent worldwide issue, which calls for a thorough examination of the elements that contribute to their widespread distribution (Zeng, 2023). Human-induced activities, including insufficient waste management, industrial processes, and the accumulation of larger plastic objects, have significantly contributed to the proliferation of micro plastics in diverse ecosystems (Nikpay et al., 2024). The persistence of microplastics is exacerbated by their durability and resistance to degradation, which endangers both terrestrial and aquatic habitats. In addition, physical, chemical, and biological influences the fragment of macro plastics into smaller particulates accelerates the dispersion of microplastics throughout a variety of environments (Huang et al., 2021). Furthermore, their capacity to transport and absorb contaminants has ecological and environmental ramifications. It is critical to conduct an extensive literature review to understand the complex interplay of elements that contribute to the increase in microplastic concentrations (Binda et al., 2021). This will facilitate the development of efficient mitigation strategies and policies aimed at reducing the detrimental effects of microplastics. In the early 20th century, there was substantial acceleration in the creation of synthetic polymers, which are the fundamental components of plastics (Cai et al., 2023). The year 1907 marked the beginning of the age of plastic, since it was the year when Bakelite, the first totally synthetic material, was created. During the following decades, there was a significant increase in the manufacturing of plastic, which was driven by the adaptability, durability, and cost-effectiveness of plastic (Al Mahmud et al., 2024). The era immediately after World War II saw a spike in the manufacture of plastic that had never been seen before. This surge was driven by technical breakthroughs as well as an increase in the demand for consumer products. Plastics have become an important component of our daily lives, as they find uses in a large range of sectors, including manufacturing and packaging (Dey et al., 2024). On the other hand, the fact that plastics are so long-lasting has turned out to be a double-edged sword since the fact that they remain in the environment has become an increasing source of concern. It was not until the 21st century that the word "microplastics" became widely used; nonetheless, the existence of such minute plastic particles in the environment had been recognized for quite some time (Courtene- Jones et al., 2022). In the 1970s, researchers started looking at the effects that plastic garbage had on marine life, including birds and animals that live in the ocean. The first research

on microplastics focused on the existence of these particles in seas and the possible damage they may cause to marine species (Chatterjee and Sharma, 2019). The dissociation of larger plastic material, the use of microbeads in personal care products, and industrial processes are all potential origins MPs (Patil et al., 2024). MPs may also come from other sources. In addition, the washing process causes synthetic fibers to be shed from clothes, which contributes to the pollution caused by microplastics (Fernandes et al., 2024).

Beyond their inherent ecological risks, microplastics can interact with various pollutants, leading to synergistic effects that exacerbate environmental and health challenges. Microplastics can adsorb a range of chemical pollutants, including heavy metals, persistent organic pollutants (POPs), and pharmaceuticals, due to their large surface area and hydrophobic properties. This adsorption can enhance the bioavailability and toxicity of these pollutants. For instance, a study (Adeleye et al., 2024) found that pollutants like chemical solvents significantly increase the mechanical stretching effects of microplastics, suggesting that microplastics can act as vectors for harmful pollutants, facilitating their penetration through lipid membranes and strongly affecting their biophysical properties. The combination of microplastics and heavy metals poses significant threats to aquatic life and human health. The study emphasizes the need for further investigation into these synergistic effects to better understand the compounded risks. Persistent organic pollutants, such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), can adhere to microplastic surfaces, leading to increased toxicity when ingested by organisms. Furthermore, floating debris can absorb these pollutants from seawater, making plastic far more deadly in the ocean than it would be on land. Hydrophobic contaminants are also known to bioaccumulate in fatty tissues, biomagnifying up the food chain and putting pressure on apex predators and humans (Zevkic, 2024). Microplastics can also interact with pharmaceuticals and personal care products (PPCPs), leading to complex combined toxic effects on aquatic organisms. A study (Du et al., 2024) found that the adsorption of antibiotics on microplastics induces complex combined toxic effects on aquatic organisms. The study emphasizes the need for further research to understand the mechanisms of compound toxicity caused by the adsorption of antibiotics on microplastics. In real-world

environments, organisms are exposed to mixtures of microplastics and various pollutants simultaneously. For example, a study (Li et al., 2024) introduces the concept of the "microplastome," defined as the entirety of various plastic particles and their associated matters, highlighting the complex interactions between microplastics and environmental pollutants.

2.1 Types of microplastics and their characteristics

Additionally, microplastics may be manufactured from a wide variety of polymer types, and they can be found in a wide range of forms and sizes (Rochman, 2019). Plastics made of polyethylene, polystyrene, polyamide, polyester, acrylic (AC), and polypropylene (PP), are the types of microplastics that are detected in the environment most often and have different characteristics (Xu et al., 2020), as shown in Table 2.1. In general, microplastics are characterized by surfaces that are rough, porous, and uneven. However, microplastics may sometimes exhibit surface topographic properties that are complicated. It is also possible for them to have a variety of colors and morphological characteristics, such as fibers, films, foams, and pieces (Yang et al., 2021). After being released into the environment, microplastics may include a variety of chemical additives, including phthalates, PBDEs, and TBBPA (Godswill et al., 2019). These compounds have the potential to leach out of polymers and into the environment. Marine life, soil contamination, human health, and environmental problems are some of the potential dangers that might be posed by microplastics (Ghosh et al., 2023). Microplastics can also represent major threats to human health. (Senathirajah et al., 2021).

Table 2.1: Characteristics of different microplastics and their applications

S.No.	Type of polymer	Density in (g cm^{-3})	Main applications	References
1.	Polyethylene (PE) PE with a high density PE with a low density	Non-biodegradable, density is 0.94, which is considered to be low. Both nonbiodegradable, high density of 0.96	The most common applications for polyethylene are packaging film, shopping bags, toys, wires and cables, garbage envelope, agricultural mulch, drinking bottles, and decorative items for the home. Global production rate = 38 million tonnes /year	(Hung et al., 2022)
2.	Polyethylene terephthalate	High density (1.36-1.39)	Polythene terephthalate is often considered to be among the most significant polymers that are used in everyday life. Pillows, textile fibers, peanut butter and jam jars, plastic drink bottles, processed meat packaging, sleeping bags, and electronic devices are just some of the many industrial goods that make use of the polymer Global production rate = 36 million tonnes /year	Benyathiar et al., 2022)
3.	Polypropylene	Low density (0.84-0.93)	Variety of products, including but not limited to: packaging, reusable food containers, bottle caps,	

			automobile batteries, interior components, and laboratory equipment, are all manufactured using polypropylene. Global production rate = 46 million tonnes /year	(Sternschuss et al., 2012)
4.	Polyvinyl chloride	High density non biodegradabe (1.37-1.5)	PVC is a highly adaptable material with the capability to serve a wide range of applications. Some examples of its possible applications include window frames, resilient flooring, drainage pipes, cladding, paperwork, medical gadgets, blood storage bags, raincoats, bottles, cable and wire insulation, and electrical pipes. Global production rate = 38 million tonnes /year	(Salimi et al., 2022)
5.	Polystyrene	Density ranges from 1.03-1.07	Disposable plastic cutlery, smoke detector housings, jewel cases, license plate frames, tableware, and electrical gadgets are all examples of products that are manufactured using polystyrene (PS), which is a synthetic polymer. Global production rate = 14 million tonnes /year	(Dong et al., 2020)

6.	Acrylonitrile-butadiene-styrene	Density ranges from 1.03–1.07	Acrylonitrile Butadiene Styrene, more often referred to as ABS, is a thermoplastic material that is extensively used for the purpose of forming lamps, enclosures, automobile bodywork, wheel covers, stiff items such as pipework, and safety equipment. Global production rate = 06 million tonnes /year	(Tse et al., 2021)
7.	Styrene acrylonitrile	Density ranges from 1.05–1.1	Resins made of styrene-acrylonitrile (SAN) are polymers that are transparent to the human eye and are used broad variety of applications, including packaging, medical applications, some automotive applications, home items, and a great number of compounded products. Global production rate = 0.6 million tonnes /year	(Gargas et al., 2008)
8.	Polyamide	high strength and durability, Density ranges between (1.11–1.14)	Synthetic polyamides are often utilized in a variety of applications due to their exceptional strength and durability. Some examples of these applications include water hose nozzles, sportswear, the automotive industry, carpets, helmets, kitchen utensils and windscreen wipers. The production of transportation is the	(Guo et al., 2012)

			major user of polyamide (PA), accounting for 36% of the total consumption of the material. Global production rate = 1.3 million tonnes /year	
9.	Polyurethane	Having very low density (0.41–0.6)	In the field of polyurethanes, the most common use is the production of rigid foams for use in thermal insulation systems for refrigerators and freezers. For consumer refrigerators and freezers to meet the energy ratings that have been needed, rigid polyurethane foam is an essential and cost-effective material of choice. Global production rate = 10 million tonnes /year	(Naureen et al., 2021)
10.	Polycarbonate	Density ranges from 1.21–1.20	Common applications for personal computers include the production of vandal-resistant glass, riot shields, infant feeding bottles, safety helmets, electrical components, and headlight lenses, among other commonly used applications. Global production rate = 2.2 million tonnes /year	(Flak et al., 2021)

2.2 Microplastic Distribution in the Environment

2.2.1 Impact of Microplastics on Aquatic and Terrestrial ecosystem

When exposed to certain microbes or parasites in laboratory settings, polyethylene (PE) and polypropylene (PP), which are the most prevalent plastics found in water, exhibit symptoms of breakdown. According to Pinto et al. (2020), these plastics are often present in water. However, it is yet unknown whether organisms are capable of degrading marine plastics under circumstances that occur naturally in the ocean. According to the explanation provided by (Thushari and Senevirathna, 2020), plastics, both primary and secondary, have the potential to introduce themselves into marine habitats. According to Diepens and Koelmans (2018), larger plastic objects may occasionally decompose into microplastics in the environment, and plastic debris can infiltrate marine ecosystems either directly or indirectly from a variety of sources, as illustrated in Figure 2.1. According to (Hurley et al., 2020), plastic buildup is more likely to take place in regions that have a high population density or where there is a diverse range of human activities, including those in close proximity to garbage disposal facilities. Studies have demonstrated that soils treated with sewage contain significantly higher concentrations of microplastics compared to untreated soils. Furthermore, soils have a remarkable capacity to retain microplastics over extended periods. (La Daana et al., 2022). According to (Machado et al., 2018), it is possible that some animal species, particularly those with short life spans, are already suffering from the consequences of this new stressor, which is directly caused by humans. According to (Atiwesh et al., 2021), terrestrial animals may be subjected to extensive exposure to plastic pollution, which has the ability to affect their biological and physiological systems both now and in the future. According to (Hopewell et al., 2009), the disposal of plastics in landfills results in the waste of both energy and materials, this is because plastics are utilized in a variety of applications to replace heavier materials such as glass and metals. According to Hossain et al. (2020), the persistence of these polymers causes vast amounts of abandoned plastics to accumulate as garbage in landfills and natural areas worldwide. Studies by (Cormier et al., 2021; Roch et al., 2020) highlighted that the harmful impact of microplastics on marine life often occurs when aquatic organisms mistake them for food, either by eating or inhaling. Microplastics are often confused with edible materials,

leading to their ingestion by marine animals. Despite their widespread presence, our understanding of how microplastics affect aquatic life is still in its early stages, as noted by De Haan et al. (2022). Depending on their thickness, microplastics can either float on the water surface or sink to the seabed, as discussed previously (Barbir et al., 2021). On the other hand, terrestrial animals are not immune to the detrimental effects of microplastics. Ingested microplastics can lead to physical blockages in the digestive systems of various species, causing malnutrition or death. For example, in Lamu, Kenya, donkeys have been reported to die from ingesting plastic waste, leading to fatal digestive blockages (Jeong et al., 2024). Moreover, laboratory studies have shown that microplastics and chemicals in plastics may delay an animal's development, cause problems with reproduction, and even make it difficult for them to fight off disease. Emerging evidence suggests that microplastics pose a significant health threat to terrestrial vertebrates (Hou et al., 2022). Studies on rodents have shown that ingestion of microplastics can lead to various health issues, including inflammation and disruption of gut microbiota. These findings raise concerns about the potential implications for other terrestrial vertebrates, including humans, especially considering the increasing prevalence of microplastics in the environment (Sun et al., 2021). Microplastics have the potential to adsorb and transport persistent organic pollutants (POPs) and emerging organic contaminants within terrestrial ecosystems. This "Trojan Horse" effect can facilitate the movement of these harmful substances through the food chain, posing additional risks to terrestrial organisms (de Souza et al., 2022). The sorption potential of microplastics is influenced by various factors, including water matrix, pH, ionic strength, and aging of microparticles. Furthermore, a study published by Trackic in (2024) revealed that conventional plastic glitter can severely reduce the reproduction of essential soil organisms like springtails, impacting soil health and plant growth. In contrast, a new type of glitter made from cellulose nanocrystals showed no such toxic effects, suggesting that alternative materials could mitigate the negative impacts of microplastics on the environment.

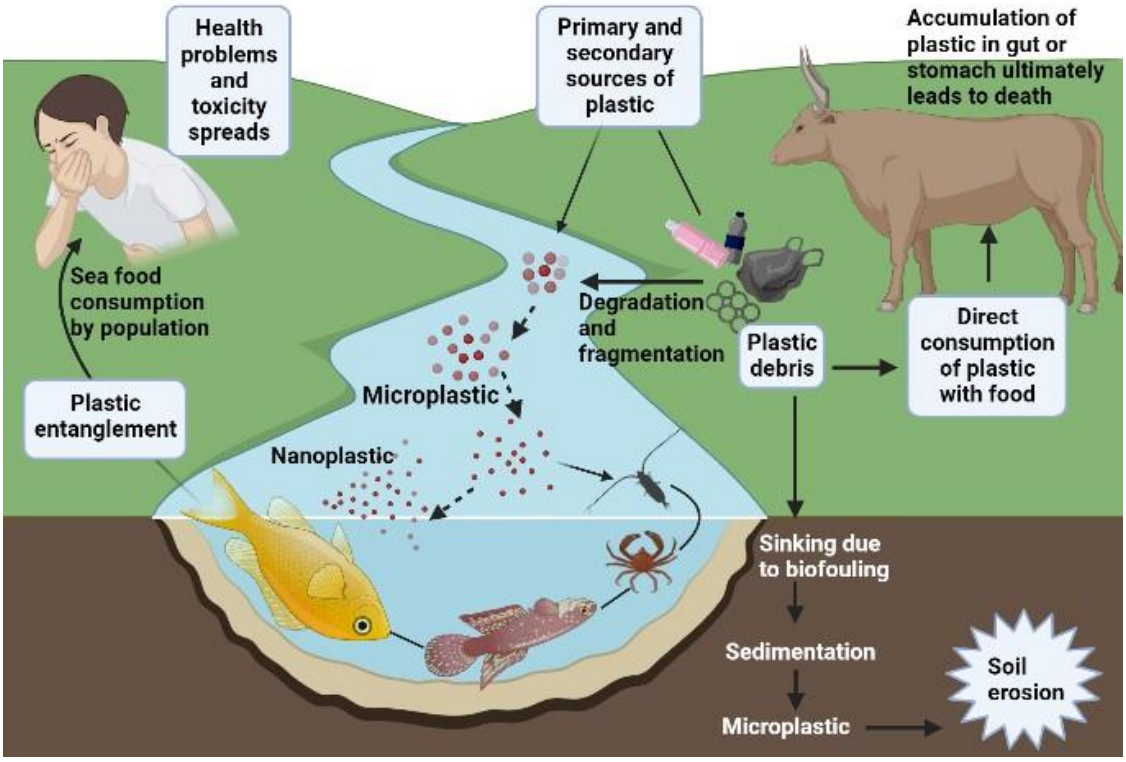

Figure 2.1: - The impact of plastic consumption in aquatic ecosystems on both terrestrial and aquatic organisms.

2.2.2 Microplastic deposition into soil

Garbage debris from urban areas, discharges from industrial facilities, and improper waste management are some of the factors that contribute to the prevalence of microplastics in these settings (Key et al., 2023). The sediments that are located at the depth of water bodies are often the locations where microplastics are eventually discovered (Kurniawan et al., 2023). This accumulation has the potential to have negative effects on benthic organisms and disrupt the ecosystems that are present in sediments throughout the course of time (Thanigaivel et al., 2023). MPs have been detected in substantial quantities in soils worldwide. This accumulation is primarily attributed to agricultural activities, the use of plastic mulches, and the breakdown of plastic waste in landfills. (Arif et al., 2024). The presence of microplastics in soil is becoming more widespread and presents a threat to the biodiversity of soil ecosystems (Chia et al., 2021). When MPs pollution is present in soil and groundwater, it poses a risk to human health, plants, nematodes, and earthworms. Additionally, the features of

soil might be altered as a result of this pollution. To solve this problem, many remediation strategies have been suggested (Kehinde et al., 2020). These tactics include sifting plastic, encouraging synthetic or biological breakdown, and replacing traditional plastics with biodegradable substitutes. Plastics that have been contaminated may bring potentially harmful compounds into the soil, which might thus contaminate subsurface water supplies as well as other water sources (Kumar et al., 2023). According to (Lozano and Rillig, 2020), improper disposal of plastic debris, whether by wind or domestic animals, adds to the pollution of terrestrial environments. The levels of soil moisture may be altered as a result of the potential of microplastic fibers to influence the rates of water retention and evaporation in the soil (Wu et al., 2020b). Microplastics, much like a variety of microorganisms, have the potential to affect the structure and function of soil, which may affect the quality of food and pose long-term dangers to human health (Helmberger et al., 2020). According to Wang et al. (2019), soil microorganisms play crucial roles in the elimination of microplastics and other hazardous compounds, as well as the decomposition of organic waste, which is beneficial to the development of plants. Additionally, microplastics have the potential to have a detrimental effect on the structure of the soil as well as earthworms, as shown in Figure 2.2 (Zhang and Liu, 2018). According to Zhang et al. (2020), the concentration of plastic particles in soil is often greater than the concentration observed in water and marine habitats.

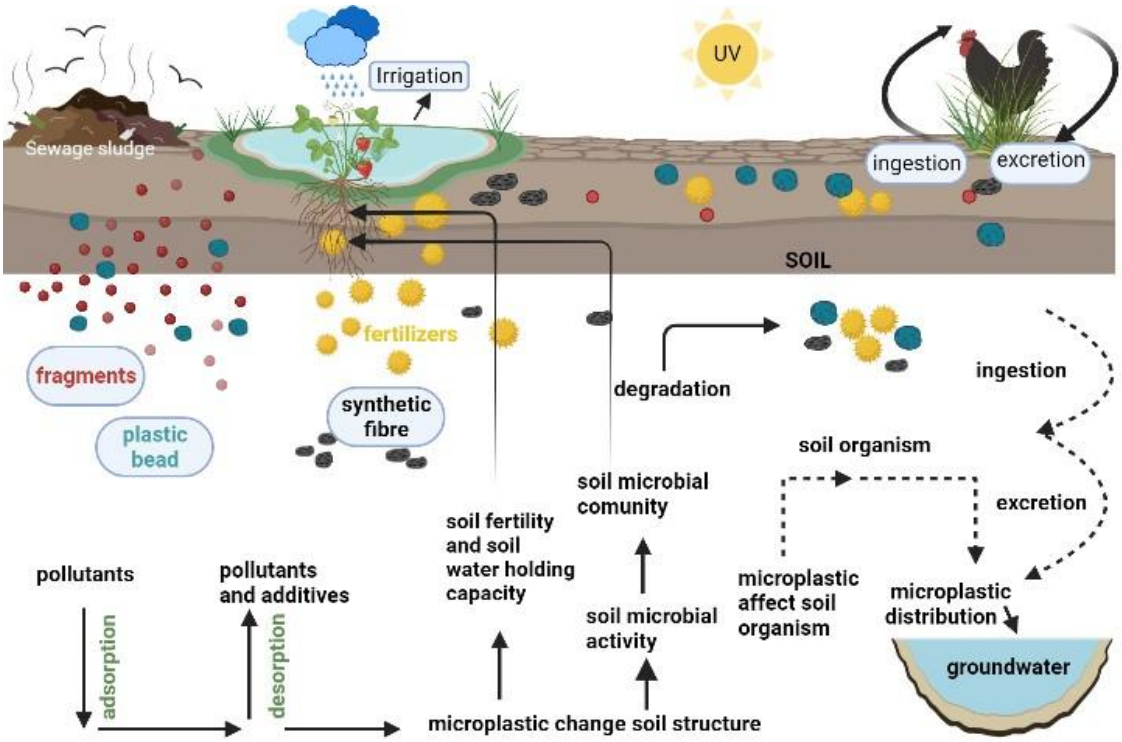

Figure 2.2: - Contamination of microplastics and their effects on soil components and organisms.

2.2.3 Effects on human beings

Although a variety of implications have been discovered for aquatic organisms, the prospective impacts that they may have on conditions on earth are completely unknown. Furthermore, the bulk of the plastics that wind up in the ocean are made, used, and discarded. They are abandoned on land (Teuten et al., 2009) and then come into contact with people and other species that live on land (Harris, 1953). A wide range of biological pathways are responsible for the consumption of microplastics and nano plastics, which are approximately 2.5 μm in size, by humans through inhalation and digestion (Wright and Kelly, 2017). In addition to causing disruptions in receptors and signaling, MPs and NPs can cause damage to the plasma membrane (Liu et la., 2024). In addition, the expression of genes may be influenced by stress caused by the aforementioned factors, which potentially increases the immunological response of host cells by activating the toll-like receptors (TLRs) that are responsible for mediating the immune response of the host via natural and secreted (DAMP) substances. The production of reactive oxygen

species (ROS) by NADP oxidases may be triggered by stressors. An increase in ROS may be the consequence of mitochondrial malfunction caused by micro- and nano plastics originating from endosomes or in response to stressors. Additionally, an increase in ROS may be the result of a loss in the efficiency of activities occurring inside the electron transport chains of mitochondria. Micro- and nano plastics have the potential to enter the blood circulation and can be transferred to other organs via transcytosis (Das, 2023; Khan and Jia, 2023). The most well-known of these pathways include ingestion, dermal sorption, and breathing (Schwabl et al., 2019). If the barrier that protects the gastrointestinal system is breached, micro- and nanoplastic might enter the bloodstream. According to (Parashar and Hait, 2020), even if microplastics are too tiny to penetrate through the skin, they are still able to enter the body via nasal passages, sweat glands, and hair follicles. Research by Talsness et al. (2009) revealed that a total of 10 grams of human feces included microplastics 50–500 μm in size, which were composed of polypropylene and polyethylene. Among the pollutants that are found in high amounts in the human body are polybrominated diphenyl ethers (PBDEs), and tetra bromo bisphenol A (TB-BPA), bisphenol A (BPA). According to (Smith et al., 2018), these pollutants are also found in high concentrations in the environment. In young people, who are more susceptible to exogenous exposure, the levels are often greater, which indicates that there is a need to minimize exposure to these drugs (Yee et al., 2021). Hazards of MPs based on their chemical and physical properties are still undetermined (Fucic et al., 2018). This is attributed to their association with synthetic chemicals used during production and their ability to absorb contaminants from the air. According to Tong et al. (2020), plastic has various degrees of toxicity and an array of effects on various organs inside the human body.

2.3 Available studies on the effects of microplastics on model organisms

An increasing number of studies on MP contamination is prevalent in terrestrial ecosystems. In the field of microplastic contamination research, invertebrates are considered potentially useful model animals for examining different kinds of contamination (Demir et al., 2023). In terms of the pollution caused by MPs, their potential is contingent upon the various species. Key features such as the ability to

withstand environmental stress, feeding habit, behavioral flexibility, ecological niche and life cycle strategies, along with the characteristics of MPs, are taken into consideration (Prokic et al., 2021). Research on invertebrates has predominantly focused on the taxonomic group's studies with Mollusca (106), and Arthropoda (100). (Elgarahy et al., 2021). Within Arthropoda, 84 studies have focused on various species of Crustacea, particularly those in the Branchiopoda and Malacostraca groups (Trestrail, 2021). Additionally, 95 studies have examined species from the order Bivalvia. Bivalvia and Crustacea, due to their filter-feeding behaviors, widespread distribution, key roles in the food chain (mainly as primary consumers), and importance in the human diet, are highly vulnerable to microplastics. As a result, they are a major focus of scientific research. (Kibria, 2023). These are the key qualities that make them interesting for research. Microplastics (MPs) interact with a wide variety of animals after they are released into the environment. The most common way in which they do this is by ingestion, since many organisms are unable to discern between plastic and food (Eze et al., 2024). It is possible for MPs to travel from the intestines to other areas of the body once they have been consumed (Jabeen et al., 2018). Studying the ingestion and accumulation of microplastics (MPs) is crucial for understanding the potential harm caused by exposure to these particles. The absorption, retention, and migration of MPs into tissues are influenced not only by their characteristics, but also by factors like the species, feeding behaviors, and habitats of the exposed organisms (Feng et al., 2023). Evidence of MPs being ingested or accumulating in the digestive tract has been documented in 253 studies, representing 51% of the total. On the other hand, 182 studies, accounting for 28% of the total, confirmed bioaccumulation into other tissues (Pedriza and Jaumot, 2020). Two hundred and eighty-two of these investigations concentrated on invertebrates, whereas one hundred and fourteen were conducted on vertebrates. This research investigated only ingestion and bioaccumulation without evaluating any further impacts of MPs at the subcellular, cellular, individual, or population level (Sun et al., 2023). MPs have been reported to be ingested and bioaccumulated across various invertebrate and vertebrate taxa, with 99 and 110 studies on invertebrates and 154 and 72 studies on vertebrates, respectively. Among invertebrates, Mollusca (33 studies on ingestion, 66 on bioaccumulation) and Arthropoda (42 studies on ingestion, 25 on bioaccumulation) have been the most extensively researched groups. Xu et al. (2020)

detected microplastics (MPs) in 32 out of 38 invertebrate species, with gastropods exhibiting the highest concentrations of MPs. Similarly, Dioses-Salinas et al. (2020) highlighted that although data on terrestrial species remain sparse, MPs are likely to interact with soil-dwelling invertebrates. Prendergast-Miller et al. (2019) reported that earthworms, particularly *Eisenia fetida*, face significant exposure to MPs due to their burrowing and ingestion behaviors. This observation is reinforced by fluorescence imaging conducted by Wang et al. (2019b), which revealed earthworms consuming polyethylene and polystyrene particles. Furthermore, Silva et al. (2020) found that *Hediste diversicolor* exposed to polystyrene exhibited prolonged burrowing activity, a behavior with potential ecological ramifications given the critical role of polychaetas in bioturbation of soil and sediment.

Microplastics can enter organisms through direct ingestion or trophic transfer, the latter occurring when prey containing MPs is eaten by a predator. Trophic transfer is the main route for MP uptake in predators (Nelms et al., 2018). MPs from marine crustaceans to various fish species, suggesting that fish at higher trophic levels could act as indicators of MP contamination (Zhang et al., 2019). While all vertebrate groups are vulnerable to MP ingestion, research has largely concentrated on fish, with significantly fewer studies examining amphibians (4), birds (9), reptiles (5), and mammals (15). Carlin et al. (2020) reported the presence of MPs in the gastrointestinal tracts of 55% of freshwater birds and 56% of seabirds. Evidence of MP bioaccumulation has been observed in fish, amphibians, mammals and birds (58 ,6 ,6 and 1 report respectively), although no data currently exists for reptiles.

The scientific literature shows that while much research has focused on the ingestion and digestive accumulation of MPs, there is limited investigation into their translocation from the digestive tract to other organs (Wen et al., 2024). Due to their smaller size, invertebrates are often studied for whole-body distribution patterns, resulting in more frequent reports of bioaccumulation relative to ingestion. In vertebrates, studies on MP ingestion are twice as numerous as those on tissue accumulation (Garcia et al., 2024). The study of tissue accumulation is vital for understanding the physiological impacts and other adverse effects resulting from the ingestion of plastic debris (Dey et al., 2024). Concerns about human health are mounting, as microplastics (MPs) ingested by species

frequently included in human diets could accumulate in their tissues (Ali et al., 2024). Despite this, a significant knowledge gap persists, particularly regarding MP levels in marine muscle, the most commonly consumed part of fish. Typically, biological samples are analyzed for MPs through tissue digestion, followed by filtrate analysis, with the KOH digestion method proving effective for various tissue types (Malafaia et al., 2022; Thiele et al., 2019).

In studies on microplastic (MP) accumulation and its effects, invertebrates are often examined either as whole organisms (82 studies) or through targeted analyses of soft tissues in groups such as Bivalvia, Gastropoda, and Crustacea (53 studies). The primary pathways for MP assimilation in these organisms include the gastrointestinal system (73 studies) and gills (23 studies) (de Sá et al., 2018; Schirinzi et al., 2020). Other tissues that have been studied include the hepatopancreas, digestive glands, and liver (26 studies), hemolymph (20 studies), gonads (5 studies), kidneys (1 study), mantle (6 studies), muscle (11 studies), and coelomic fluid or epidermis (1 study each) (Mancia et al., 2020 The most often investigated tissue for MP presence in vertebrates is the gastrointestinal tract (161 studies), followed by the liver, entire body, gills, muscles, blood, brain. kidney and gonads, gills, muscle, blood, brain, kidneys, and gonads following (45, 32, 30, 20,16, 16, 14 and 9 studies respectively). (Batel et al., 2020; Park et al., 2020). However, fewer studies have focused on other tissues such as the skin, spleen, heart, bladder, lungs, nerve tissue, uterus, and thymus. These relatively unexplored areas are valuable points to determine health impacts of MP exposure/accumulation on vertebrates.

In light of this, there is an urgent need to improve our knowledge of how microplastics (MPs) are distributed and accumulate across a variety of tissues to discover the possible detrimental consequences that they may have. According to Bour et al. (2018), there is a frequently held belief that MPs tend to collect in the gastrointestinal system. This is because the gastrointestinal tract is relatively simple to dissect and minimizes the amount of tissue that has to be analyzed. This is especially true in studies that include larger animals. According to (Lusher et al., 2017), investigations that concentrate on the stomach and intestines are especially pertinent for microplastics that are much larger

than 0.5 mm. This is because microplastics of this size often do not pass through the gut wall readily. In addition, fecal analysis has been used not only to identify the ingestion of MPs but also to understand their destiny inside organisms. Research has been undertaken on both invertebrates and vertebrates, with five studies being conducted on invertebrates and seven studies being conducted on vertebrates.

2.4 Polyethylene terephthalate microplastics

PET (polyethylene terephthalate) is a common polymer with a wide range of uses. Many difficult challenges arise from the science of production and the architecture of the fabrication of this plastic (Guajardo and Andler, 2024). PET fibers are widely used in the formation of films, synthetic fibers, and other commercial materials. PET is colorless and has crystalline resin with great durability, elastic modulus, and damage tolerance. It is used in storage units such as boxes, thermoplastic items, fabrics, and technology/construction (Chan and Zinchenko, 2023). Owing to its outstanding liquid, gas, and vapor barrier properties, as well as its poor permeability, PET is widely used in the packaging industry as a jar or film. PET consists of four stages, as shown in Figure 2.3: (I) transesterification or direct esterification, (II) prepolymerization, (III) polycondensation in the molten state, and (IV) polycondensation in the solid-state (Ravindernath and Mashelkar, 1984).

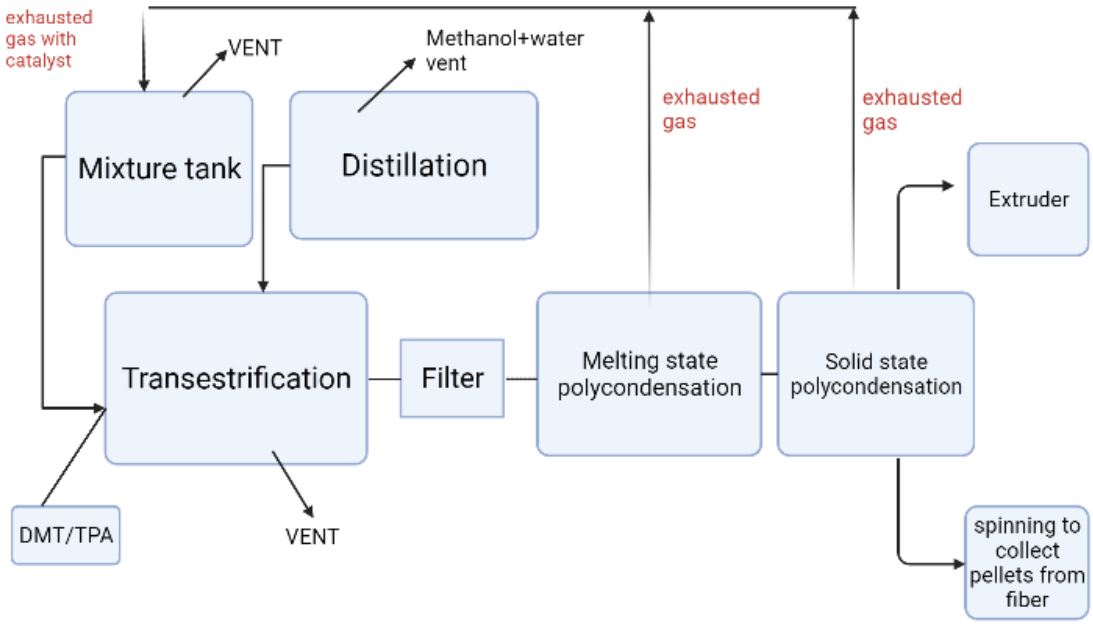

Figure 2.3: Process of polyethylene terephthalate microplastic formation

Polyethylene terephthalate, sometimes known as PET, is a form of plastic that stands out among the many other kinds of plastics because of its widespread use in single-use plastic items, materials for packaging, textiles, and other commercial products (Pilapitiya and Ratnayake, 2024). PET microplastics have become an increasing concern over the presence and possible toxicity of PET microplastics. As a result, more research is required to understand the environmental implications of these microplastics (Adeleye et al., 2024). The fragmentation and degradation of PET objects, including drinkables, containers, and textiles, is the source of PET microplastics. This degradation may occur as a result of activities that include physical, chemical, and biological processes. The accumulation of PET microplastics into the environment is caused by a combination of factors, including industrial operations, urban runoff, and inefficient waste management efforts (Yang et al., 2024). A broad variety of environmental matrices, such as freshwater and marine ecosystems, sediments, soils, and air fallout, have been shown to include microplastics made of PET. PET microplastics are capable of undergoing a wide range of physical and chemical changes, which ultimately results in their dispersion across extensive distances (Arif et al., 2024). These microplastics display complicated transport paths. In both aquatic and terrestrial ecosystems, the sorption of pollutants onto PET microplastics has the potential to change the destiny of these microplastics and their bioavailability (Cui et al., 2023). Because of their ability to bioaccumulate and bio magnify, PET microplastics and the pollutants that are connected with them present potential dangers to both aquatic and terrestrial species (Pencik et al., 2023). PET microplastics can have a negative impact on both aquatic and terrestrial biota via the processes of physical ingestion, entanglement, and leaching of additives and contaminants that have been adsorbed (Kurniawan et al., 2023). Microplastics made of polyethylene terephthalate (PET) have been shown to have sublethal effects on organisms, including reduced nutrition, immunity, reproduction, and growth (Franzellitti et al., 2019). It is possible that the trophic transmission of PET microplastics would result in cascade impacts within ecosystems, which would change the dynamics of communities and the functioning of ecosystems (Zhai et al., 2024). Only a few toxicological studies have been conducted on the harmful impact that PET microplastics have on organisms, especially with respect to chronic exposure and sublethal endpoints (Tavakolpournegari et al., 2024). In the case of PET microplastics, the release of

chemical additives and pollutants that have been adsorbed raises concerns about the possible toxicity of these microplastics as well as their ecological ramifications (Kadacczapska and Grembecka, 2024). To evaluate the impacts of PET microplastics across a variety of environmental compartments and biological taxa, standardized toxicity tests and biomonitoring methodologies are needed (Papac et al., 2023). The ways in which PET microplastics interact with other environmental stressors, including pollution and climate change, are not well known and need more research.

2.5 PET microplastics and their effects on organisms

2.5.1 Effects on aquatic organisms

Ingesting PET is one of the most straightforward ways to harm fish. Microplastics, which are small pieces of plastic waste measuring less than 5 mm, are widely found in aquatic settings. These microplastics are commonly mistaken for food by fish (Lehel and Murphy, 2021). The consumption of PET microplastics can have a number of negative impacts; for example, fish digestive tracts may become physically blocked as a result of microplastic accumulation. This may cause the fish to feel satiated longer than necessary, which may decrease its appetite and increase the risk of malnutrition and stunted growth (Mallik et al., 2021). Microplastics in the digestive tract might increase the difficulty of nutrient absorption, which exacerbates nutritional inadequacies. Fish that waste more energy and breakdown indigestible plastic particles have less energy left over for growth, reproduction, and other essential processes. This phenomenon is known as energy depletion (Jahan et al., 2024). In addition to potential health dangers, the ingestion of PET microplastics may also pose toxicological hazards. PET is able to absorb dangerous substances from its surroundings. When fish are exposed to a range of hazardous compounds when they consume these contaminated microplastics, they may develop a number of health problems (Bamigboye et al., 2024). Heavy metals and organic pollutants can travel via PET microplastics. These compounds have the potential to seep into fish tissues and have harmful consequences, such as immunological dysfunction, endocrine disturbance, and liver damage. Fish that are exposed to substances linked to PET may produce ROS (Amponsah et al., 2024). This oxidative stress can harm DNA, proteins, and cells, lowering longevity and affecting general health. The hormone balance of fish can be disrupted by toxic compounds leached from PET, which can result in reproductive problems such as decreased fertility, aberrant

offspring development, and skewed sex ratios (Muhib et al., 2024). Studies have shown that fish behavior can be impacted by exposure to PET microplastics. Fish exposed to PET microplastics may show signs of changed eating habits, such as less effective foraging or a shift in the kind of food they choose to eat (Harmon et al., 2024). Their survival and growth may be impacted by this. According to some studies, fish exposed to microplastics may exhibit reduced predator avoidance behaviors, increasing their vulnerability to predators. Variations in social behaviors, including mating or schooling rituals, might affect fish populations' resilience and disturb population dynamics (Saeed, 2024).

2.5.2 Effects on terrestrial organisms

Small plastic particles called PET microplastics, which are produced when PET goods breakdown, are becoming increasingly acknowledged as contaminants in terrestrial settings (Talukdar et al., 2024). Numerous terrestrial creatures are impacted by their existence, including plants, invertebrates that live in the soil, and possibly even mammals (Huang et al., 2024)**.** Earthworms, nematodes, and arthropods are examples of soil invertebrates that are essential to the health of the soil and the operation of ecosystems. These creatures may be negatively impacted by PET microplastics. PET microplastics can be consumed by soil invertebrates, who may mistake them for food (Priya et al., 2024). Blockages in the digestive tract caused by microplastics might hinder the absorption of nutrients and lower caloric intake. Growth rates and reproductive success may be lower if energy is devoted to the digestion of indigestible microplastics. Chemical exposure can have harmful consequences, such as oxidative stress and tissue and cell damage (Thacharodi et al., 2024). Microplastics and their related substances have the potential to trigger an immunological reaction, which might result in inflammation and worsen general health. Although research on the direct absorption of microplastics by plants is ongoing, there is evidence to support possible negative effects on plant health. PET microplastics can change the porosity and structure of soil, which can impact aeration and water retention (Dey et al., 2024). Thus, plant root development and nutrient absorption may be affected by this process. Microplastics may be absorbed by plant roots and moved to shoots, according to some data. Plant tissues containing microplastics may disrupt physiological functions, which might lower yield and growth. Microplastics that collect on plants have the potential to enter food chains when ingested

by herbivores (Sridharan and Gayathri, 2024). Although there is not much research on how PET microplastics affect land mammals, there may be hazards, particularly for species that feed in polluted areas. Microplastics that are found in soil, water, or air can be consumed or inhaled by terrestrial animals (Gayathri et al., 2024). Microplastics that are consumed can obstruct or erode the digestive tract, making it more difficult for nutrients to be absorbed and increasing the risk of pain or damage. Microplastics that are inhaled can accumulate in the respiratory system, which may result in long-term harm or respiratory distress (Megha et al., 2024). Microplastic-related chemicals have the potential to cause harmful reactions, such as immune system dysfunction, liver damage, and endocrine disruption. In terrestrial food webs, microplastics can ascend when organisms ingest polluted prey (Toha et al., 2024). Predators and animals at relatively high trophic levels, including humans, may be impacted by this bioaccumulation. By affecting the well-being and ability of different species to reproduce, long-term exposure to PET microplastics can lower biodiversity (Rose et al., 2023). Ecosystem resilience and stability may be affected by this (Ahmed et al., 2024). The harm posed by PET microplastics to terrestrial creatures in the environment is increasing. Their presence in the soil and air has wider ecological ramifications and impacts a broad spectrum of animals, from mammals to invertebrates (Sucharitakul et al., 2024). By making these efforts, we can protect the diversity and health of terrestrial habitats while lessening the harmful consequences of microplastics (Ziani et al., 2023).

2.6 Heat shock proteins

Heat shock proteins, often known as HSPs, are latent proteins that do not become active until the usual development circumstances are interrupted (Story and Story, 2023). These specialized proteins are synthesized only when there is even a minor increase in temperature over the usual range. They are never produced under normal conditions (Gouda et al., 2024). There is a broad incidence of the creation of HSPs that has been documented in all of the species that have been examined, ranging from bacteria and plants to mammals, including humans (Jeyachandran et al., 2023). The fact that HSPs are found in such a broad variety of animals shows that they existed at an early stage in evolution and perform important biological roles (Singh et al., 2024).

As a result, cells manufacture a family of proteins called heat shock proteins, or HSPs, in response to stressful events. In their salivary gland polytene chromosomes,

Drosophila melanogaster larvae exhibit an odd puffing pattern when they are inadvertently exposed to relatively high temperatures (Chowdhuri and Lakhotia, 1986). This pattern indicated that there were significant changes in gene expression as a result of increased temperature (Ritossa, 1962). This was the first time that their existence was discovered. It was first thought that these proteins are known as heat shock proteins, but it is now known that they are produced in response to a wide variety of stressors. These stressors include exposure to ultraviolet radiation, low temperatures, the process of wound healing or tissue remodeling, and situations in which nutrients or oxygen are deficient, among other factors (Kregel, 2002). In addition, the activation of heat shock genes may be triggered by a wide variety of factors, including transition metal ions, amino acid analogs, hypoxia, sulfydryl reagents, and glucose deprivation, among others. According to Santoro (2000), the proteins that are created in response to such stressful situations are referred to as stress proteins, and the reaction that they demonstrate is usually referred to as upregulation. It is predominantly the heat shock factor (HSF) that is responsible for inducing the overexpression of stress proteins, which is primarily controlled at the transcriptional level (Westerheide et al., 2012). Additionally, the activation of HSPs is triggered when there is a surge in proteins that are unhealthy or damaged. According to Himalian and Singh (2019), these proteins play a significant role in protecting cells from damage by promoting the production of a variety of key cellular processes and ensuring that other functions are maintained.

The "puffing" pattern was first observed in *Drosophila* chromosomes when the metabolic uncoupler 2,4-dinitrophenol and heat were applied in 1962 by the Italian geneticist Ferruccio Ritossa (Ritossa, 1962). The discovery of stress proteins, now called HSPs, was prompted by this puffing phenomenon. In 1974, it was first noted that certain stimuli, such as heat shock, might induce *Drosophila* cells to produce more proteins (Schlesinger, 1990). *hsp 100, hsp 90, hsp 70, hsp 60*, and the tiny HSP families are the main families in which HSPs are categorized according to their molecular weight, functions, and homologies in the amino acid (AA) sequence (Yakubu et al., 2023).

2.6.1 HSP 90

Several members of the *hsp 90* family are present in the cytoplasm of eukaryotic cells. Their molecular weights are between 82 and 90 kDa (Karmazin, 2023). Tubulin, calmodulin, actin, kinases, and other receptor proteins have been shown to be associated

with it. The endoplasmic reticulum (ER) is the home of endoplasmin homologs belonging to the *hsp 90* family in higher eukaryotic organisms (Youssef t al., 2023). There are homologs of *hsp 90* in prokaryotes as well. The *hsp 83* gene, which is a member of the *hsp 90 f*amily (Nover, 2022), is located at the 63BC locus in *Drosophila*. While heat and other environmental factors are known to promote it, it may also manifest itself under normal development circumstances. The only HSP -coding gene known to include an intron in *Drosophila melanogaster* is *hsp 83*. The cytoplasmic localization of *Drosophila melanogaster Hsp 83* is consistent with that of mammalian *Hsp* 90; however, upon heat shock, *hsp 83* is also observed at the heat-induced 93D puff site (Morcillo et al., 1993). *hsp 83* has been shown to be present in the chromosomes of both vertebrates (*Hsp* 90) and *Drosophila melanogaster* (Lange et al., 2000). A simple molecular mechanism for evolution may be provided by this protein family (Himalian and Singh, 2019). Histone *hsp* 90 is composed of three separate domains. First, the dimerization domain at the N-terminus is essential for binding ATP. The middle domain is connected to this domain by means of an unstructured charged linker. The second function of the middle domain is to connect the N- and C-terminal ends of the protein. Finally, the protein's intrinsic dimerization is mostly due to the C-terminal domain (Craig and Schlesinger, 1985). The C-terminal domain maintains its dimerization activity indefinitely, in contrast to the N-terminal domains, which undergo transient dimerization upon ATP binding, as shown in Figure 2.4.

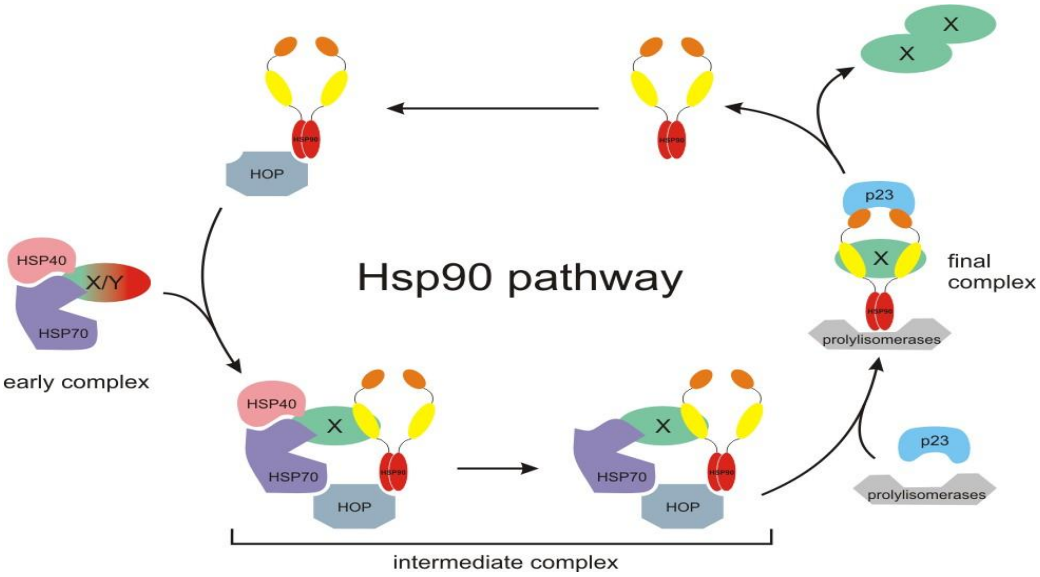

Figure 2.4: The *hsp* 90 chaperone cycle involves the folding of immature, partially folded proteins, such as steroid receptors, denoted as X/Y. In this process, *hsp 40*, *hsp 70*, and p23 function as partner chaperones, while Hop serves as a co-chaperone. Furthermore, X-X represents a fully mature, correctly folded protein dimer. (Prodromou et al., 2000).

2.6.2 HSP 70

One of the most numerous and earliest proteins to be induced in response to different stresses is the *hsp 70* family, which has a molecular weight between 68 and 75 kDa. It is also the most conserved class of HSPs (Lachapelle et al., 2007). The *hsp 70* family HSPs, which includes both constitutive and inducible isoforms, is rather large and has been the subject of much research. *hsp 70*, or its functional analog, is present from the most basic microbes to the most complex creatures. *hsp 70* proteins are either constitutively expressed or tightly confined to the endoplasmic reticulum, mitochondria, and cytoplasm (Rosenzweig et al., 2019). Several studies have shown that the *hsp 70* gene may be used as a bioindicator for risk assessment by predicting cytotoxicity in response to various environmental variables. Heat shock cognate (hsc70) genes, on the one hand, and *hsp 70*, on the other hand, are constitutively expressed. The eleven genes that make up this human gene family include *hsc70, hsp 70, grp78,* and *grp75* (Kampinga and Criag, 2010). *hsp 70* protein activity is controlled by cofactor chaperone-like nucleotide exchange factors (Moro and Muga, 2006). A hydrophobic domain that can bind denatured proteins and an ATPase domain make up the structure of *hsp* 70. Like hexokinase and actin, *hsp 70* has an ATPase region. Hydrolysis of ATP by the ATPase domain provides the energy necessary to systematically relax the surface-exposed hydrophobic regions of denatured proteins, enabling their renaturation to their original shape (Belenichev et al., 2023). To avoid nonspecific intermolecular aggregation, the substrate-binding domain attaches to denatured proteins on its own. *D. melanogaster* has two members of the *hsp 70* family: heat-inducible *hsp 70* and constitutively expressed Hsc70 (Zhao et al., 2023). The temporal and geographical patterns of expression of five distinct hsc70 genes in *D. melanogaster* are characterized by normal growth conditions. The heat-inducible *hsp 70* is encoded by two sets of genes in *D. melanogaster*, located on the right side of chromosome 3 (87C1 and 87A7) (Sha

et al., 2023). Two copies of the heat-inducible *hsp 70* genes are located at the 87A7 site, whereas three copies are present at the 87C1 site. The distance between the two groups is approximately 500 kilobase pairs. There is a high degree of sequence similarity (97%) among the protein-coding regions of these five *hsp 70* genes (Gouda et al., 2024). There is 48% sequence identity between dnak (the bacterial *hsp 70*) in *E. coli* and *hsp70* in *Drosophila*, 85% identity between *hsp 70* in mice, and 74% identity between *hsp70* in yeast. Heat shock triggers the transcription of all five *hsp* 70 genes. The *hsp 68* gene, which encodes a 68 kDa polypeptide, is located on the right arm of chromosome 3 at 95D locus, in addition to the five copies of the *hsp* 70 gene (Himalian and Singh, 2019). To decrease activity, the *hsp 70* system causes protein aggregation in critical pathways by interacting with both fully folded and extended peptide regions of proteins. *hsp 70* typically remains coupled to ATP when it does not interact with a substrate peptide. When *hsp 70* is isolated, it's very low ATPase activity means that it takes quite a while for the protein to undergo spontaneous hydrolysis (Castelli et al., 2024), as shown in Figure 2.5

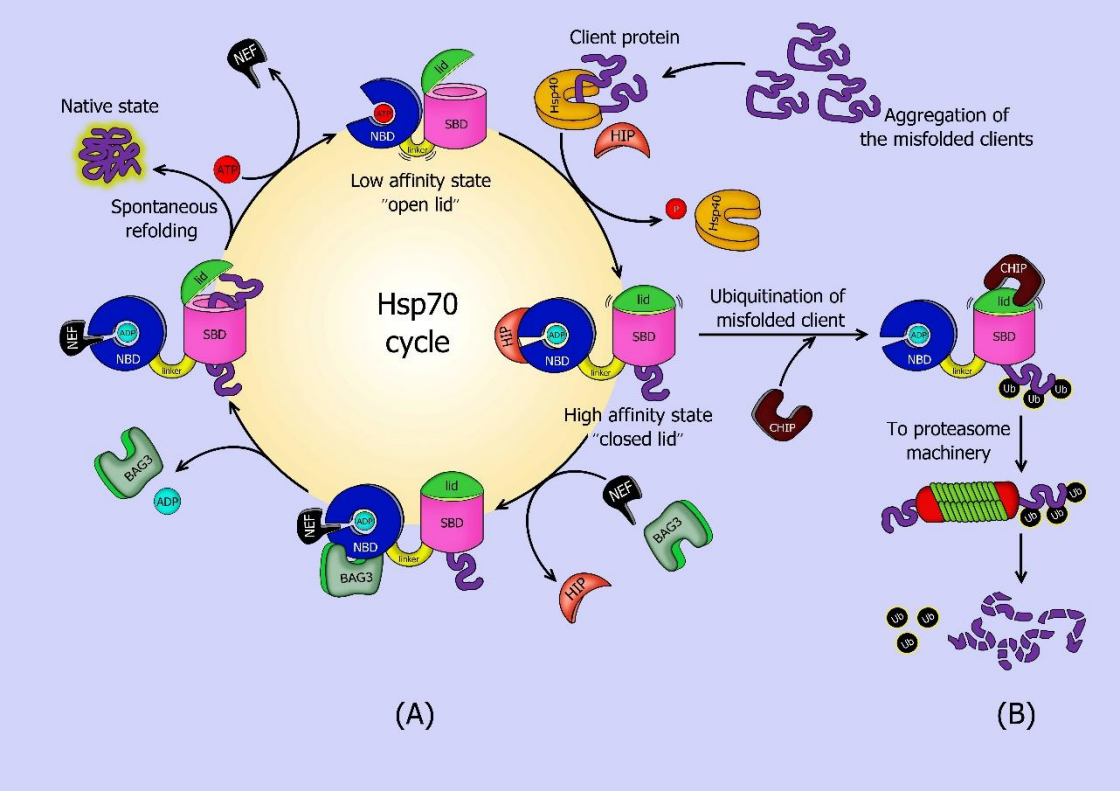

Figure 2.5: The role of *hsp 70* in the (re)folding and degradation of misfolded client proteins is depicted as follows: (a) The *hsp 70* ATP–ADP cycle for client protein (re)folding involves a conformational shift in the chaperone triggered by ATP hydrolysis and the subsequent exchange of nucleotides. (b) The *hsp 70*–CHIP complex promotes the ubiquitination of client proteins, leading to their degradation via the proteasome. CHIP, which binds to the TPR domain of *hsp 70*, functions as a ubiquitin ligase for the client proteins. Here, CHIP stands for C-terminus of *hsp 70*-interacting protein, *hsp 70* refers to heat shock protein 70 kDa, and TPR denotes the tetratricopeptide-repeat domain. (Vostakolaei et al., 2021).

2.6.3 HSP 60

The *hsp 60* family of proteins is involved in polypeptide folding and protein translocation to a lesser extent; its molecular weights are 58–65 kDa (Singh et al., 2024). All bacterial cells, eukaryotic mitochondria, and chloroplasts include these proteins. The genome of the nucleus contains the genes that encode them. *hsp 10* and *hsp 60* are eukaryotic analogs of GroEL and GroES and of *E. coli*, respectively (Yadav et al., 2024). Specifically, they refold heat-denatured proteins in response to proteotoxic stress and high-temperature shock. People often call them "chaperonins," and they help with protein folding in the same way as the *hsp 60* family does. For chaperonins to bind and fold proteins efficiently, a cochaperonin such as GroES or *hsp* 10 must be present (Bhakta et al., 2024). In contrast to the *hsp* 60/GroEL proteins, which have a stronger affinity for partly folded structures, the *hsp* 70/DnaJ complex mostly interacts with extended conformations of polypeptides or short peptides. When freshly produced polypeptides fold, these two protein families work in sequence. Members of the *hsp* 60 family were recently discovered in *Drosophila* (Kandil et al., 2024). The original *D. melanogaster hsp* 60 gene was cloned from the X chromosome 10A locus. The 'Berkeley *Drosophila* Genome Project' has identified two more *hsp* 60 genes in *D. melanogaster* at the 21D and 25E loci. Approximately 80% identity is observed in the protein sequences of the genes at 25E and 10A; however, only approximately 60% homology is shown with the 21D *hsp* 60 gene (Cao et al., 2024). The 10A gene encodes a developmentally regulated *hsp 64* protein that is expressed in most cell types, but the 21D gene encodes a particular *hsp 64* that is expressed in male germ cells and has a

specialized function. It seems that heat shock may induce the *hsp 64* gene at the 25E locus in the larval intestine, Malpighian tubules, and fat bodies (Macario and De macario, 2023). Molecular studies suggest that *hsp60* plays a pro-carcinogenic role by promoting the survival of cancer cells. This is achieved through its interaction with and inhibition of the intracellular clusterin isoform in neuroblastoma cells (Alimardan et al., 2023). According to Deocaris et al. (2006), *hsp 60* enhances cell survival by upregulating anti-apoptotic proteins such as survivin, Bcl-2, and Bcl-xL, maintaining mitochondrial transmembrane potential, and suppressing caspase-3 activation. Moreover, *hsp60* supports cytosolic cell survival by inhibiting the translocation of the pro-apoptotic protein Bax to mitochondria (Lianos et al., 2015). Its anti-apoptotic properties are further strengthened through interactions with molecules like survivin, p53, and mitochondrial *hsp70*. Mitochondrial permeability is controlled by a multichaperone complex that includes *hsp60, hsp90*, and tumour necrosis factor receptor-associated protein-1 (TNFRP1), with *hsp60* being particularly critical in tumour cells compared to normal cells (Ghosh et al., 2010; Rodríguez et al., 2016). The mitochondrial permeability transition pore, where *hsp 60* interacts with cyclophilin D in tumor cells, plays a critical role. Disruption of this interaction can inhibit tumor cell growth, induce caspase-dependent apoptosis, and alter mitochondrial permeability (Ghosh et al., 2010).

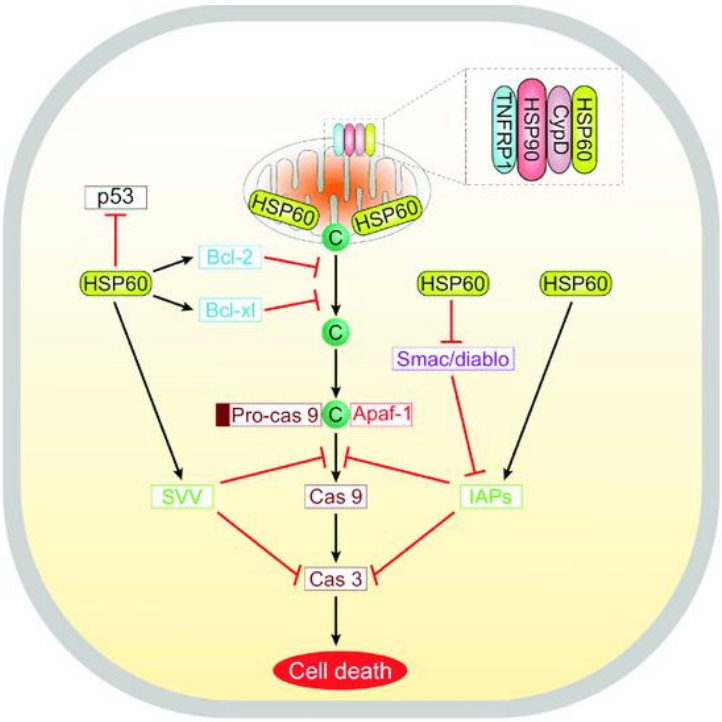

Figure 2.6: The function of *hsp 60* in controlling tumour cell apoptosis is depicted in this figure. It demonstrates how *hsp 60* suppresses apoptosis by upregulating the production of anti-apoptotic proteins that stop cytochrome c from leaving mitochondria, including Bcl-2, Bcl-xL, and survivin. To maintain mitochondrial permeability in tumour cells, *hsp 60* combines with cyclophilin D (Cyp D), *hsp 90*, and tumour necrosis factor receptor-associated protein 1 (TNFRP1) to create a multichaperone complex. Additionally, oncogenic *hsp 60* interacts with p53 to decrease its activity. Additionally, by regulating the release of smac/diablo from the mitochondria and affecting the activity of inhibitor of apoptosis (IAP) proteins, which prevent caspase activation, *hsp 60* regulates apoptosis.

2.6.4 Small HSPs

Their shared α-crystalline domain, which is located at the C-terminal region, normally has between 80 and 100 amino acid residues. Five primary types of tiny HSPs are thought to be synthesized by vertebrates: A- and B-crystallins, along with small heat shock proteins (sHsps) of molecular weights 17 kDa (*hsp B3*), 20 kDa (*hsp 20*), and 25–27 kDa (*hsp* 25/27), have been identified (Sugiyama et al., 2000). Notably, among these smaller HSPs, HSP 27 acts as a substrate for stress-regulating enzymes such as the p38 MAP kinase. Four different small HSPs in *Drosophila*, *hsp 22, hsp 23, hsp 26, and hsp 28*, are encoded by genes found at the 67B locus on chromosome 3's left arm. The total homology of these tiny HSPs in *Drosophila* is approximately 50% (Turan, 2023). Most of the HSPs that have been identified are peptidyl-prolyl isomerases. Furthermore, ubiquitin is a heat-induced eukaryotic protein that is classified as an HSP. Histone H2b in *Drosophila*, glyceraldehyde-3-phosphate dehydrogenase, enolase, and albumin in the rat liver, and α-interferon in mammalian cells are among the other eukaryotic proteins that react to heat. In addition, prokaryotes have ATP-dependent and heat-inducible ion proteases, lysU and rpoD, which are RNA polymerases (Himalian and Singh, 2019).

2.6.5 Functions of HSPs

HSPs are crucial in facilitating the folding of newly synthesized proteins and the refolding of misfolded or denatured proteins, especially during stress conditions. As

molecular chaperones, HSPs bind to exposed hydrophobic regions on unfolded or partially folded proteins, preventing aggregation and ensuring proper folding (Jeyachandran et al., 2023). This process is vital for maintaining cellular proteostasis by ensuring proteins acquire the correct structure and function, thus preventing the formation of harmful protein aggregates. HSPs play key roles in the cellular stress response, helping cells cope with various stressors, including oxidative stress, heat shock, heavy metals, toxins, and infections. Stress causes rapid induction of HSP expression, which is a cell adaptive response to proteotoxic assaults (Chan and Groisman, 2024). By increasing their HSP levels, cells improve their ability to withstand and repair damage caused by stress, which helps to preserve tissue integrity and encourage cell survival (Balakrishnan et al., 2023). In addition to their function in protein folding, heat shock proteins (HSPs), which are characteristic elements of several neurodegenerative illnesses, including Alzheimer's, Parkinson's, and Huntington's disorders, are essential for preventing protein misfolding and aggregation (Gouda et al., 2024). HSPs attach to misfolded or aggregated proteins, blocking harmful interactions and promoting their removal via cellular degradation processes, including autophagy and the ubiquitin–proteasome system. HSP*s* support the lifespan and health of neurons by preventing the buildup of misfolded proteins, which helps reduce the harmful consequences of protein aggregation (Wu et al., 2023). Moreover, HSPs alter a number of signaling pathways linked to immunological responses, apoptosis, differentiation, and cell proliferation. They affect the stability, activity, and subcellular localization of important regulatory proteins, including kinases, apoptotic proteins, and transcription factors (Gu et al., 2023). Highlighting their wider importance beyond protein folding and stress response, HSPs may control cellular processes essential for development, tissue repair, and immunological defense by modifying signaling pathways. HSPs are involved in the transport and assembly of protein complexes within cells in addition to their function as chaperones (Cabaud-Gibouin et al., 2023). They aid in the movement of proteins across cellular membranes so that they may be directed toward certain organelles or subcellular spaces. In addition, HSPs aid in the formation of multiprotein complexes, including proteasomes, spliceosomes, and ribosomes, which guarantee the effective synthesis, processing, and breakdown of proteins necessary for cellular survival, as shown in Table 3.1. (Pomella et al., 2023).

Table 3.1: - Proposed functions of heat shock proteins.

HSP family	Molecular weight	Cellular location	Proposed function
hsp 100	100 to 104 kDa	Cytosol	Protein folding
hsp 90	82 to 90 kDa	Cytosol, ER, and nucleus	Regulation of steroid hormone receptors and protein translocation.
hsp 70	68 to 75 kDa	Cytosol, ER, nucleus, and mitochondria	Antiapoptotic
hsp 60	58 to 65 kDa	Mitochondria	Refolds protein and prevent aggregation of denatured proteins
hsp 26	15–30 kDa	Cytosol, nucleus	Microfilament stabilization, antiapoptotic

2.7 *Drosophila melanogaster* (Oregon R^+)

The classification of *Drosophila* encompasses a diverse group of species within the family Drosophilidae, which belongs to the order Diptera (flies) (Markow and O'Grady, 2005). While *Drosophila melanogaster* is perhaps the most well-known and extensively studied species within this genus, thousands of other *Drosophila* species are distributed worldwide, each with its own unique characteristics and ecological niches (Skevington and Dang, 2002). Taxonomically, the genus *Drosophila* is part of the subfamily Drosophilinae, which is further subdivided into several tribes and subtribes. Within the genus *Drosophila*, species are classified on the basis of morphological features such as body size, coloration, wing venation, and genital morphology (Fischer et al., 2023). Additionally, molecular techniques, particularly DNA sequencing, are increasingly used to elucidate evolutionary relationships and refine species classification. One notable

feature of *Drosophila* classification is the subdivision into species groups, which are clusters of closely related species that share morphological and genetic similarities (Clancy et al., 2023). For example, the melanogaster species group includes *Drosophila melanogaster* and several closely related species, whereas the virilis species group comprises species such as *Drosophila virilis* and its relatives. In addition to species groups, *Drosophila* species are often classified on the basis of their ecological preferences and habitat specialization. Some species are associated with specific types of fruit or plant material, whereas others are found in more diverse habitats, such as decaying vegetation, fungi, or even human-made environments, such as garbage dumps and fermenting fruit (Sharma et al., 2023). Furthermore, *Drosophila* classification is dynamic, with ongoing taxonomic revisions and the discovery of new species contributing to our understanding of the genus's diversity and evolutionary history. Recent advancements in molecular biology and phylogenetics have facilitated the identification of cryptic species and the delineation of species boundaries, shedding light on the intricate patterns of speciation and adaptation within the genus (Fischer et al., 2023).

Overall, the classification of *Drosophila* is complex and evolving, encompassing a wide range of species with diverse morphological, ecological, and genetic characteristics. Through continued research and exploration, scientists have aimed to elucidate the evolutionary relationships, ecological interactions, and genetic mechanisms that underpin the remarkable diversity of *Drosophila* flies.

2.7.1 *Drosophila* morphology

Wild type fruit flies have brown-yellow bodies with black bands running diagonally across the abdomen, and red eyes. The mature fly has eight or nine abdominal segments, three thoracic segments, and a head. Its thoracic segments are as follows: the first has wings, the second has halters, and the third has a pair of legs on each of the three (Landgraf et al., 2006). *Drosophila* are known to display sexual differences in both male and female; males are different from females by a characteristic of dark coloured lower abdomen, presence of sex combs. In order to aid in mating with females, males also

develop claspers, which are spikey hairs around the anus and genital area. (Helfrich & colleagues, 2007).

2.7.2 *Drosophila* as a model organism

The utilization of invertebrates, specifically the *Drosophila* model system, has seen a rise as a model for studying human diseases (Lopez-Ortiz et al., 2023). The effects of mutated *Drosophila* genes on various diseases have been thoroughly investigated, with a particular emphasis on cellular processes such as regulation of gene expression, synaptic transmission, cell death, and subcellular trafficking (Gartz and Wildonger, 2023). *Drosophila*, with its modest genetic makeup of 4 chromosomes and approximately 12,000 genes, stands in contrast to humans with 23 chromosomes and around 20,000 genes (Demir, 2021). Despite the simplicity of its nervous system, consisting of about 300,000 neurons compared to the human brain's 100 billion neurons, *Drosophila* proves to be an excellent model organism due to its amenability to genetic manipulations and large-scale genetic screening (Cotterill and Yamaguchi, 2023). Furthermore, the conservation of *Drosophila* cell biology throughout evolution in humans enhances its value as a model organism (Yamamoto et al., 2024). Interestingly, almost 75% genes are linked to human diseases have homology in *Drosophila*, indicating a high degree of conservation in the fruit fly. (Jaiswal et al., 2012). The examination of developmental processes in *Drosophila* has unveiled a remarkable degree of functional conservation in genes, exemplified by the encoding of a cell-cell communication molecule through a hedgehog gene (Chain, 2011). *Drosophila melanogaster* serves as a widely accepted animal model for studying developmental biology, genetics, and molecular biology (Mirzoyan et al., 2019). Its ease of cultivation in a laboratory setting, short lifespan as shown in Figure 2.7, and absence of any reported vectoring of known diseases contribute to its popularity.

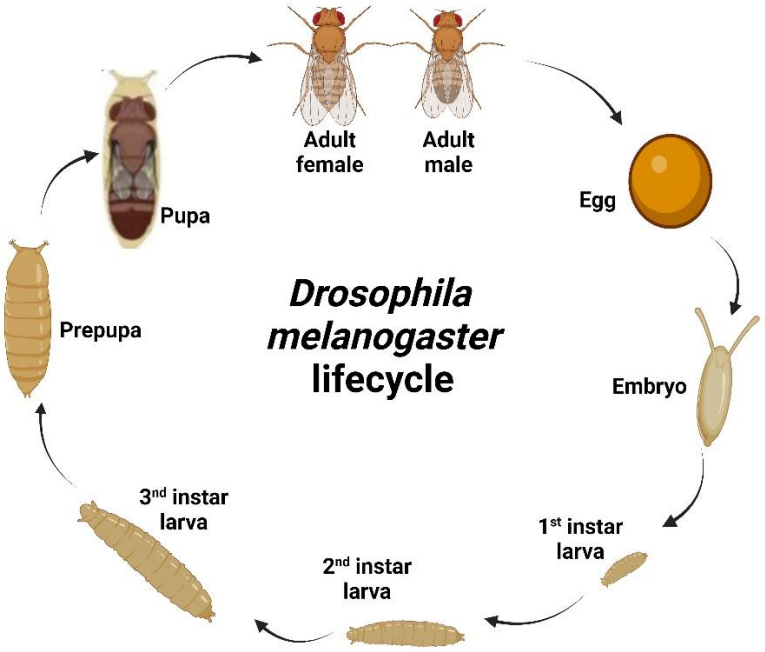

Figure 2.7: Lifecycle of *Drosophila melanogaster*

There is a notable degree of similarity between the genes of *Drosophila melanogaster* and those of higher animals. For instance, there is 73% homology between the human and *Drosophila hsp*70 genes (Bier, 2005). Using *D. melanogaster* also complies with the European Centre for Validation of Alternative Methods recommendations, which support the adoption of alternative bioscience techniques by regulators and scientists as a means of reducing, improving, or replacing the use of animals in laboratory research. (Chen et al., 2020). Notably, in the years 1999-2000, *Drosophila* was instrumental in elucidating aspects of human diseases. These all points make *Drosophila* as a suitable model organism to conduct our research further (Ugur et al., 2016).

CHAPTER 3

HYPOTHESIS

The exponential expansion of plastic pollution is one of the most urgent concerns that society is facing. Currently, this is a concern that affects all individuals, and a significant number of individuals are conducting research on this topic. A substantial amount of research has demonstrated that plastic has detrimental effects on aquatic life. Numerous studies have examined various types of microplastics; however, we have focused on polyethylene terephthalate (PET) because of its exceptional durability and difficulty in decomposition, which results in its disposal in the environment. Studies on the toxicity of PET microplastics have identified inflammation, cellular injury, and genotoxicity as potential adverse effects on human health. This study employed the fruit fly *Drosophila melanogaster* as a model for cellular, biochemical, behavioral, and molecular investigations.

The toxicity of PET microplastics was initially assessed at various concentrations and exposure durations, as the concentration at which it had the greatest impact was not obvious in previous studies. This is necessary because of the ongoing lack of conclusive evidence regarding the effects of PET microplastics on terrestrial model organisms. In summary, certain compounds, such as microplastics, may have adverse effects; however, research on the manner in which organisms react to these substances is substantially inadequate. What is the precise mechanism by which microplastics induce this toxicity, and how does the body's primary defense system react to stress? Consequently, to achieve this objective, our research examined the immune system's response to a nontarget organism, *D. melanogaster*, via heat shock proteins. The study hypothesizes that exposure to microplastic will induce a differential heat shock protein (HSP) response, wherein hsp70 and hsp83 may exhibits a rapid, transient and a more sustained expression pattern. The magnitude of HSP induction is expected to be dose-dependent, with higher stress levels leading to amplified HSP expression.

The expression of HSPs is crucial for the identification of diseases, as cells are frequently exposed to a variety of adverse conditions, including nutrient deprivation, hypoxia, and the accumulation of damaged proteins. These cells respond by increasing the production of HSPs to survive and proliferate. The presence of high levels of specific HSPs in tissues or blood can indicate the presence and aggressiveness of tumors, which is valuable information for disease management. Furthermore, it is hypothesized that

these responses are not only species-specific but may also provide insights into the adaptive mechanisms of other microbial and aquatic and terrestrial ecosystems, offering potential indicators for environmental stress monitoring and biotechnological applications.

CHAPTER 4

OBJECTIVE

1. To study the role of Polyethylene terephthalate (PET) of microplastic (2-100 µm) on *Drosophila* through behavioral and development assays.
2. To investigate biochemical and cellular toxicity in *Drosophila* exposed to PET microplastics.
3. To examine heat shock proteins and oxidative stress markers in PET microplastic-exposed *Drosophila* via biochemical, cellular, and molecular assays.
4. To identify suitable HSP biomarker following PET microplastic exposure based on molecular parameter.

CHAPTER 5

MATERIAL AND METHODOLOGY

5.1 *Drosophila* Strain

This study employed the *Drosophila melanogaster* (Oregon R^+) strain. Dr. Anurag Sharma from NITTE University in Mangalore, India, provided the *Drosophila* stock for research work, and was originally sourced from the Bloomington *Drosophila* Stock Centre (BDSC) at Indiana University Bloomington, USA.

5.1.1 Rearing of *Drosophila melanogaster*

Under controlled conditions, *Drosophila melanogaster* (Oregon R^+, wild-type strain) was fed a conventional *Drosophila* diet of maize/corn powder, agar, yeast, propionic acid, sodium benzoate or sucrose (Singh et al., 2010). The flies were maintained under specific environmental conditions, including a dark-light cycle of 12 h and a temperature of 24 ± 1 °C, in a sterile laboratory setting at Lovely Professional University in Phagwara, Punjab, India.

5.1.2 Preparation of a standard *Drosophila* diet

For 2 units of food (760 ml; 8 bottles and 2 Petri plates),

Maize powder	35 g
Sugar (Sulfur free)	30 g
Agar Agar	04 g
Yeast	12 g
Sodium benzoate	02 g (mixed in 6-8 ml of 80% ethanol)
Propionic acid	02 ml

5.2 PET Microplastic Formation

The PET plastic pellets were purchased from Sigma–Aldrich and then ground into a fine powder with the help of a grinder. The PET plastic powder was then sieved with a 0.02 mm sieve to obtain 2–100 µm PET particles.

5.3 Characterization of PET microplastics

5.3.1 FTIR (Fourier transform infrared spectroscopy)

FTIR difference spectroscopy has become a key tool in studying photosynthesis and related fields, providing valuable insights that complement the structural data obtained from techniques like X-ray diffraction and nuclear magnetic resonance (NMR). By enabling reaction-induced FTIR difference spectroscopy, researchers can detect subtle

structural changes, hydrogen-bond interactions, and proton transfer processes within proteins—details that may be too fine for X-ray diffraction to reveal (Berthomieu and Hienerwadel, 2009). For the FTIR (Fourier transform infrared spectroscopy) study, PET microplastics were used as samples for investigation. Sample discs with a clear appearance were made by carefully placing 10 mg of the material within a KBr pellet of 100 mg in weight. An FTIR spectrophotometer from Perkin-Elmer was subsequently used to analyze the samples (Villacorta et al., 2022).

5.3.2 FE-SEM (Field emission scanning electron microscopy) and EDX (energy dispersive X-ray spectroscopy)

Scanning electron microscopy (SEM) employs a concentrated beam of high-energy electrons to produce a range of signals from the surface of solid samples. These signals, resulting from the interaction between the electrons and the sample, provide detailed information regarding the surface morphology, chemical composition, and the crystalline structure and orientation of the sample's components (Ural, 2021). The FE-SEM (field emission scanning electron microscopy, JEOL model) image provided important information regarding the surface characteristics, structure, and dimensions of the particles. PET microplastics were examined at various magnifications in this study. The EDX (energy dispersive X-ray spectroscopy) spectrum clearly revealed particles, which allowed the composition and purity of the PET microplastics to be determined. The energy levels of these X-rays were then visualized as peaks in the spectrum, providing vital information regarding the composition and elemental composition of the PET microplastic (Singh et al., 2010).

5.3 Treatment schedule for *Drosophila* flies and larvae

In the experimental design, third-stage *Drosophila melanogaster* larvae and adult flies were separated into five distinct groups, as shown in Table 4.1. Group I served as the control and was cultured under standard *Drosophila* feed. Another group (Group II) was exposed to a mixture of ethanol and distilled water (vehicle control). Food containing PET was administered to Groups III, IV, and V according to previous methods (Liang et al., 2022), with some modifications: 10 g/L, 20 g/L, and 40 g/L PET microplastic were mixed with a 1:1 ratio of ethanol and distilled water (DW), respectively. Each group of larvae was permitted to consume their respective food for 2, 24 and 48 h. The flies were

then subjected to a 15-day treatment to evaluate their behavioral and locomotory activity (Shen et al., 2021).

Table 4.1: Groups according to treatment schedule and duration of exposure.

Group	Treatment	Duration of Larvae Feeding	Duration of Fly Intervention
Group I	Control (normal *Drosophila* diet)	2, 24, and 48 hours	15 days
Group II	Vehicular control (ethanol and distilled water)	2, 24, and 48 hours	15 days
Group III	PET microplastic (10 g/L) + ethanol and DW (1:1)	2, 24, and 48 hours	15 days
Group IV	PET microplastic (20 g/L) + ethanol and DW (1:1)	2, 24, and 48 hours	15 days
Group V	PET microplastic (40 g/L) + ethanol and DW (1:1)	2, 24, and 48 hours	15 days

5.4 PET microplastic uptake/accumulation by *Drosophila melanogaster*

5.4.1 Fluorescence microscopy

Accurate risk assessment in future investigations depends on identifying the accumulation of PET microplastics in *Drosophila*. A targeted approach was used to accomplish this objective. Staining with Nile red dye has become an easy-to-use, low-cost method for assessing the detrimental impact of a broad variety of micro and nano plastics on the environment (Shruti et al., 2022). To stain the PET microplastics, the protocol was followed as previously described (Villacorta et al., 2022). One milliliter of Nile red solution (0.50% in DMSO) was added. The stained pellet was washed several times with ethanol in a 0.10 M PBS solution at pH 7.40 to remove any remaining stain. Nile red-stained PET microplastics in the midgut and hindgut of *Drosophila* larvae are easily visible to the naked eye (Alarby et al., 2023). The accumulation of PET microplastics in the living tissues of the groups treated with 10 g/L, 20 g/L, or 40 g/L PET is shown in Figure 5.1. The presence of microplastics was observed under a stereomicroscope and fluorescence microscope by observing dissected and whole larvae. This method provides insight into the paths taken by PET microplastics after ingestion.

Figure 5.1: Demonstration of groups receiving the control diet or diets supplemented with different concentrations of plastic and dyed with Nile red dye.

5.4.2 Confocal microscopy

The ability to perform precise risk assessments in related research is contingent upon the identification of PET MP accumulation in *Drosophila*. The aim was achieved by the use of a focused strategy. The use of Nile red dye has emerged as a cost-effective and user-

friendly technique for evaluating the adverse ecological effects associated with a wide range of microplastics (Shruti et al., 2022). The PET MP was stained according to a previously described methodology (Villacorta et al., 2022). A volume of Nile red (Hi media, DJ Corporation, Jalandhar, Punjab, India) solution (1 mL) in 0.50% dimethyl sulfoxide was added. The pellet was stained and subjected to several washes with 0.10 M phosphate-buffered saline (PBS) at pH 7.40 in ethanol solution to eliminate any residual staining. The presence of stained PET MP in the digestive tract of *Drosophila* larvae may be readily observed without any magnification. PET MP particles were detected by confocal microscopy (CLSM, Olympus, FV1200, Bhatinda, India), wherein observations were made on dissected larvae, which were suspended in a 1% solution of PBS and subsequently affixed on a microscopic slide with a single cavity. To perform confocal visualization of Nile red-stained PET MP and confirm their presence, an excitation wavelength of 514 nm was used, and the emitted light was collected within the range of 546–628 nm. With the help of previous publications, we confirmed the green fluorescence of the PET MP (Alarby et al., 2023).

5.5 Nutrition value (food intake) of flies

A capillary feeder assay (CAFE) was used to assess the potential impact of PET microplastic exposure on food intake in adult flies. The experimental setup involved standard fly vials containing 5 mL of 1% agar, sealed with a sponge bung. Capillaries filled with 10 μL of either a 10% sucrose solution (for the control group) or sucrose solutions mixed with varying concentrations of PET microplastics (10, 20, and 40 g/L) were placed in the vials. To confirm ingestion, a drop of blue food coloring was added to the sugar syrup for both the control and treated flies. Following a four-hour fasting period, the flies (n = 3) were transferred to CAFE chambers (Kholy and Naggar et al., 2023), with identical chambers maintained without flies to serve as evaporation controls. After 24 hours, the food capillaries were removed, and the distance between the initial and final marks on the capillary tubes was measured using a digital Vernier caliper. This measurement allowed the calculation of the food consumed by each fly during the 24-hour period. To account for evaporation, the average evaporative loss from at least three control vials without flies was calculated and subtracted from the measured food

consumption of the flies. The total consumption per fly was determined using the formula: Food uptake (μL) = measured distance (mm)/1.57 mm

Food consumption per fly (μL) = (Food uptake [μL] - Evaporative loss [μL])/total number of flies in the vial (Diegelmann et al., 2017).

5.6 Behavioral and developmental analysis

5.6.1 Climbing activity

In accordance with a previous study, specific modifications were made to the ascent evaluation (Sharma et al., 2012). Twenty flies were placed in a plastic cylinder that was 20 cm in length and 2 cm in breadth. Any fly that made it over a 15 cm line after being lightly tapped at the bottom for 30 s was recorded unless it had fallen into one of the vials. The percentage of inspected flies that were able to ascend 15 cm above the surface was the climbing count after 15 days of exposure. The data are presented as a percentage of the overall fly count (ntotal), which included the number of flies above (nabove) and below (nbelow) 15 cm. Standard deviations were provided with reported findings on the basis of counts from three independent assessments.

$$1/2[(ntot + n^{above} - n^{below})/n^{tot}]$$

5.6.2. Jumping activity

The activation of neuromuscular functions was evaluated by jumping activity (Singh et al., 2022). The frequency of locomotor activity appeared to influence the jumping response threshold. To conduct the experiment, newly hatched flies were placed in a cylindrical container marked from 1 to 10 cm, and their individual leap distances from the bottom of the container were measured. The total/average number of jumps performed over five repetitions was used to quantify the leaping behavior after 15 days of exposure. To ensure reliable results, each cohort of 100 flies was subjected to the experiment five times.

5.6.3. Crawling activity of larvae

The larval crawling experiment was conducted via a protocol published in earlier papers. Nine third-instar larvae were placed on a glass Petri dish covered with 2% agarose from the control and treatment groups that had been rinsed with PBS (pH 7.4) to eliminate any residues of food. Three separate 1-minute durations were used to observe larvae

crawling on an agar surface set on a graph sheet after 24 and 48 h of exposure. To determine how far each treatment group's larvae traveled in 1 min, we measured how many grid lines (cm) they traveled in 60 s and then determined the group's mean (Parimi et al., 2019).

5.6.4. Emergence of Flies

The female flies were observed laying eggs synchronously for one hour, after which these eggs were collected on Petri dishes containing regular food. After approximately 24 h of egg laying and newly emerged Oregon R^+ strain first-instar larvae were transferred to various experimental groups. These groups included controls, a vehicular control, and three groups treated with 10 g/L, 20 g/L, and 40 g/L PET, respectively. Each group consisted of three replicates, each containing fifty larvae. On the first day, the first adult fly emerged and continued until each and every fly had been enclosed in the control group; the total number of flies emerging from each group was counted and then compared to the control. Using the methodology described previously with slight modifications, the development of the flies in each of the categories was assessed (Gayatri and Krishnamurthi, 1981; Singh et al., 2022).

5.6.5. Survival Assay

The effects of prolonged exposure of *Drosophila* to PET microplastics were evaluated via a modified version of a previously established survival experiment (Mohideen et al., 2015; Dan et al., 2019). The lifespans of 50 adult male and female flies were separately monitored as they were fed from vials containing control, ethanol + DW, or PET microplastic feed (10, 20, or 40 g/L) until their death. The vials of food were stored horizontally and replenished every week. The number of dead flies in the food vials was counted every day, excluding those that had fled or adhered to the food. After the flies had all perished, a survival curve was produced via GraphPad Prism 6. The log-rank Mantel–Cox test was subsequently run to determine statistically significant differences in outcomes between the various treatment groups.

5.7 Determination of Cellular Toxicity in the Gut Region (Trypan Blue Staining)

The evaluation of cell viability was conducted according to the protocol mentioned in the publication (Krebs, 1997), with some modifications (Singh et al., 2010). This expeditious and straightforward technique enables differentiation between viable and nonviable cells. The evaluation of cell death involves a comprehensive examination of the gastrointestinal tract, and the principle behind this method relies on the impermeability of the cell membrane to blue dye. Living cells possess an intact cell membrane, preventing the passage of trypan blue into the cytoplasm (Himalian et al., 2022). The cytotoxicity of the PET MP was assessed via trypan blue dye in the tissues of *Drosophila* subjected to treatment. Following the completion of the treatment, a series of washes were performed on a total of 10–12 larvae using a phosphate-buffered saline solution at a concentration of 0.1 M and a pH of 7.4. The midguts that had been dissected were subsequently submerged in a solution of trypan blue dye (Hi media, Jalandhar, Punjab, India) (0.4%) for 15 min. The larvae were subsequently examined via a stereomicroscope (Quasmo, Kwality Scientific, 220 V AC, 50 Hz, Jalandhar, India), and photographs were captured to facilitate trypan blue staining and meticulous analysis. Using ImageJ software version 5.0, we calculated the percentage of stained cells.

5.8 Biochemical assay

5.8.1 Total protein content

5.8.1.1 Principle of methodology

The Lowry technique, often referred to as the Biuret test, is based on a chemical interaction between peptide bonds and copper ions in an alkaline environment. In order to function as a potent reducing agent, the larval homogenate is combined with an alkaline solution that contains $CuSO_4$. $CuSO_4$ interacts with the protein's peptide bonds to generate Cu^+ ions. Folin-Ciocalteau's reagent is then introduced. Molybdenum blue, a complex of Mo (IV) and Mo (V) ions, is produced when Cu^+ ions react with Mo (VI) ions in this mixture. The protein concentration is indicated by the blue colour that results, which is measured at a wavelength of 600 nm. Protein concentrations are quantified through the utilisation of standards.

5.8.1.2 Procedure

1. BSA (bovine serum albumin) stock mixture, 1 mg/ml

2. A total of 50 ml of 20% Na_2CO_3 (sodium carbonate) was combined with 50 ml of 0.1 N sodium hydroxide solution (0.4 g was dissolved in 100 ml of distilled water).

3. A total of 10 ml of 1.56% $CuSO_4$ solution was combined with 10 ml of 2.37% sodium potassium tartrate solution. To create the analytical reagent, 100 ml of reagent I was mixed with 2 ml of reagent II.

4. Folin–Ciocalteu's reagent solution (1 N): On the day of use, commercial 2 N reagent was diluted with an equal volume of water (2 ml of distilled water was mixed with 2 ml of reagent).

A variety of dilutions of the BSA stock solution were prepared. Incubate for 10 minutes after adding 0.2 ml of larval protein homogenate to 2 ml of the alkaline $CuSO_4$ solution. Gently combine the mixture. Next, add 0.2 ml of Folin-Ciocalteau's reagent and incubate in the dark for 30 minutes. Utilise a Perkin Elmer 2030 reader to determine the optical density (OD) at 600 nm. Create a graph to ascertain the protein concentration that is unknown.

The phenolic groups of tryptophan and tyrosine residues in the protein react with Folin-Ciocalteau's reagent to generate a blue complex, with a maximal absorption at approximately 660 nm. Protein estimation is conducted in accordance with the methodology established by (Lowry et al., 1951). In each test tube, varying dilutions of BSA were prepared to a final volume of 5 ml. Two milliliters of each dilution was pipetted into distinct test containers, alkaline copper sulfate reagent was added, and the mixture was incubated at the normal room temperature for 10 minutes. Then, Folin reagent was added, and the mixture was incubated for an additional 30 minutes. The optical density (OD) was measured at 660 nm. The absorbance was plotted against the protein concentration to generate a standard calibration curve. This curve was used to determine the concentration of the unknown sample.

5.9 Nonenzymatic studies

5.9.1 Protein carbonyl assay

5.9.1.1 Principle of methodology

The levels of oxidized proteins in plasma, tissues, and cell homogenates may be measured via this technique.

5.9.1.2 Procedure

The protein carbonyl (PC) content of both control and treated larvae was determined using a modified version of the method described by Levine et al. (1994). The supernatant fraction was split into two equal portions: one was treated with 2.0 M HCl to serve as a blank, while the other was treated with 10.0 mM 2,4-Dinitrophenyl hydrazine (DNPH) in 2.0 M HCl to serve as the test sample. After an hour of incubation, 20% TCA was added to precipitate the mixtures, and a 1:1 ethyl acetate mixture was used for extraction. The granules obtained were dissolved in 1.0 ml of 6.0 M guanidine hydrochloride. The absorbance of the DNPH-treated sample was measured at 370 nm, with HCl used as the baseline. The molar absorption coefficient for DNPH was 22,000 M^{-1} cm^{-1} (6.22×10^{3} M^{-1} cm^{-1}), and the carbonyl content was quantified as nanomoles of DNPH incorporated per milligram of protein.

5.9.1.3 Calculation

Protein carbonyl (nmol/ml) = [(CA)/ (*0.011 μM^{-1})] (500 µl/200 µl)

The DNPH extinction coefficient at 370 nm is 0.022 μM^{-1} cm^{-1}, or 22,000 M^{-1} cm^{-1}

5.9.2 Malondialdehyde content (MDA)

5.9.2.1 Principle of methodology

Lipid peroxidation is started when a hydrogen atom is extracted from an unsaturated fatty acid, resulting in the creation of lipid radicals. These radicals subsequently react with oxygen molecules to generate lipid peroxy radicals, which in turn triggers a chain reaction that results in the production of lipid peroxides. Despite the fact that malondialdehyde (MDA) is not the primary end product of polyunsaturated fatty acid oxidation, it is produced by the enzymatic degradation and auto-oxidation of polyunsaturated fatty acids in cells. A pinkish-red chromogen that absorbs at 532 nm is produced when MDA reacts with two molecules of thiobarbituric acid (TBA) in an acid-catalyzed nucleophilic addition reaction.

5.9.2.2 Procedure

The malondialdehyde (MDA) content was measured via the Okhawa et al. (1979) method to evaluate the damage caused by PET microplastics. To prepare the tissue homogenate, 1 g of tissue was mixed with 5 ml of 0.1% (w/v) trichloroacetic acid (TCA), and the resulting mixture was centrifuged at 5000 rpm. A 1 ml portion of the supernatant was then combined with 6 ml of 20% (w/v) TCA containing 0.5% (w/v) thiobarbituric acid (TBA). The mixture was heated at 95 °C for 30 minutes and then rapidly cooled on ice. Absorbance of the supernatant was measured at 532 nm, with nonspecific absorbance at 600 nm subtracted for correction. The MDA level was determined using an extinction coefficient of 155 mM cm^{-1}.

5.9.2.3 Calculations

$$\text{MDA} = \frac{\text{Obs. absorbance} \times \text{total volume} \times 1000}{\text{Extinction coefficient} \times \text{sample volume} \times \text{larvae weight}}$$

5.10 Determination of enzymatic studies

5.10.1 Superoxide dismutase (*Cu-Zn* SOD) activity

5.10.1.1 Principle of methodology

The transformation of superoxide anions (O2-) into oxygen (O2) and hydrogen peroxide (H_2O_2) is catalysed by superoxide dismutase (SOD). SOD activity is measured by the reoxidation of photo reduced flavin in air, which produces superoxide anions. For this, the Kono (1978) approach is used, which is based on the fact that SOD inhibits the reduction of Nitroblue tetrazolium (NBT) dye by superoxide radicals. Hydroxylamine hydrochloride autooxidation (NH_2OH HCl) generates these superoxide radicals. An increase in absorbance at 540 nm is observed as a consequence of the reduction in NBT, which is employed to quantify SOD activity.

5.10.1.2 Procedure

The reaction mixture was assembled in cuvettes by combining 1.3 ml of sodium carbonate buffer, 500 µl of NBT, and 100 µl of Triton X-100. The reaction was triggered by adding 100 µl of hydroxylamine hydrochloride. A total of 70 µl of the enzyme extract was added after 2 minutes. The increase in absorbance at 540 nm was used to determine the percentage inhibition of the NBT reduction rate.

5.10.1.3 Calculations

Nitrite is produced when superoxide radicals oxidize hydroxylamine hydrochloride. The addition of NBT results in the accumulation of blue formazan, which in turn increases the absorbance at 540 nm. The addition of the enzyme SOD to the reaction inhibits the reduction of NBT to blue formazan by trapping superoxide radicals.

$$\frac{\text{(Change in absorption/min (blank) - Change in abs./min (group)}}{\text{Change in abs./min (blank)}} \times 100 = y$$

y (%) of inhibition is produced by 70 µl of a sample.

Hence, 50% inhibition is produced by

$$\frac{50 \times 70}{Y} = z \text{ µl of sample}$$

5.10.2 Catalase activity (CAT)

5.10.2.1 Principle of methodology

The method described by Aebi (1983) was employed to evaluate catalase activity, which is essential for safeguarding cells from oxidative damage induced by ROS. Catalase enables the conversion of hydrogen peroxide (H_2O_2) into oxygen (O_2) and water (H_2O). This activity can be monitored by observing either the emission of O_2 or the reduction in H_2O_2. Importantly, the absorption of H_2O_2 in the ultraviolet region increases as the wavelength decreases. The degradation of H_2O_2 can be directly monitored by observing the decrease in absorbance at 240 nm over time. The catalase activity was subsequently assessed by measuring the change in absorbance per unit of time.

5.10.2.2 Procedure

Three hundred microliters of enzyme extract were added to the reaction mixture, which was composed of 1.5 ml of phosphate buffer and 1.2 ml of H_2O_2. In this mixture, the rate of H_2O_2 decomposition was monitored by measuring the decrease in absorbance at 240 nm.

5.10.2.3 Calculations

$$\text{Unit Activity (U min}^{-1}\text{ g}^{-1}\text{ FW)} = \frac{\text{(Change in absorption/minute} \times \text{Total volume (ml))}}{\text{Ext. coefficient} \times \text{Vol. of sample (ml)}}$$

$$\text{Extinction coefficient (EC)} = 6.93 \times 10^{-3}\ \text{Mm}^{-1}\text{cm}^{-1}$$

$$\text{Specific catalase Activity (nmol U mg}^{-1}\text{ protein)} = \frac{\text{Unit Activity (U min}^{-1}\text{ g}^{-1}\text{ FW)}}{\text{Total protein Content (mg g}^{-1}\text{ FW)}}$$

5.11 To examine the effects of PET microplastics on reproductive activities

5.11.1 Dye Exclusion Test of the Ovaries and Testes of *Drosophila* to Determine Reproductive Toxicity

To assess potential tissue damage to the reproductive organs of adult flies, similar to previous procedures (Shabir et al., 2022), the flies were treated with different PET MP concentrations for 15 days. Five to ten ovaries and testes of *Drosophila* from each group were dissected and subjected to staining with trypan blue dye according to the protocol (Krebs, 1997; Mukhopadhyay et al., 2006) for 15 min. Following the staining process, the samples were thoroughly rinsed with phosphate-buffered saline (PBS) two to three times. The organs of 10–15 flies were examined via a stereomicroscope (Quasmo, Kwality Scientific, 220 V AC, 50 Hz), and images were taken to confirm the results.

5.11.2 Fertility, Fecundity, and Reproductive Performance

The approach used in this study was based on methodology (Gayatri and Krishnamurthi, 1981), with several adjustments (Singh et al., 2009). First-instar larvae that hatched after synchronous egg laying for half an hour were subsequently placed in several types of feeding media. The groups included a standard food (control), a vehicle control, and with varying concentrations of PET MP mixed food at 10, 20, and 40 g/L. The larvae were allowed to nurture their surroundings as they progressed through their personal growth. Virgin female and male flies were observed upon emergence from control and treated food. The flies were then separated and paired in vials (1 male + 1 female)

containing normal food for mating purposes. Five pairs of flies were selected for each treatment group, and they were individually placed in five vials. Over the course of the following ten days, the flies were moved to new vials on a daily basis.

The total number of eggs deposited within this time frame was recorded. The total fecundity, which refers to the overall number of eggs laid down throughout a span of 10 days, was determined on the basis of the provided data. We also recorded the number of flies that emerged from the eggs laid over the course of the ten-day period. By calculating the average number of flies that emerged per pair during this ten-day interval, we obtained a metric to assess reproductive efficacy. Additionally, the fertility percentage was also determined. The results were calibrated against mortality data.

5.12 Reverse transcriptase polymerase chain reaction (*RT–PCR*) to examine the role of heat shock proteins

5.12.1 RNA isolation

The gastrointestinal regions of *Drosophila melanogaster* larvae were extracted from all experimental groups after 48 h of treatment. This was achieved by submerging third-instar larvae in Poels' salt solution (PSS). In accordance with previous methods (Singh et al., 2010), the extracted tissue was subsequently transferred to Eppendorf containers filled with TRIzol Reagent via an RNA extraction kit (Bioserve RNA Extraction Kit, Hyderabad, India) to facilitate total RNA extraction. To evaluate the concentration and purity of the isolated RNA, the absorbance ratios at 230/260 and 260/280 nm were measured with a Nano Drop spectrophotometer (Denovix, Bioserve, Hyderabad, India).

5.12.2 cDNA synthesis

The RNA that was acquired underwent reverse transcription by cDNA synthesis using superscript IV VILO master mix (Invitrogen by Thermo Fisher Scientific, Karnataka, India) in accordance with the guidelines provided by the manufacturer. Each reaction mixture consisted of total RNA (10 μL), RT buffer for M-MuLV (4 μL), 10x solution for M-MuLV (2 μL), M-MuLV reverse transcriptase (RNase H) (1 μL), ribonuclease inhibitor (0.5 μL), 10 mM dNTP mix (2 μL), and molecular grade water to make 20 μL (final volume). The synthesized cDNA was stored at −20 °C until further use.

5.12.3 Polymerase chain reaction (PCR)

Next, quantitative PCR (qPCR) was performed utilizing a Quant Studio 5 Real-time PCR system (Thermo Fisher Scientific, India) and the previously designed primers *hsp*

83, hsp70, hsp60, and hsp26, which are listed in Table 1. The PCR mixture (total 25 µL) consisted of 2X PCR Taq Mixture (12.5 µL), 10 µM each of forward and reverse primers, cDNA (2 µL), and molecular biology grade water. A denaturation cycle of 94 °C for 3 minutes preceded 35 cycles of (*hsp83, hsp70, hsp60, and hsp26*) of 95 °C for 30 seconds (denaturation), 55 °C for 30 seconds (annealing), 72 °C for 1 minute (extension), and a final step at 72 °C for 5 minutes (final extension) that made up the optimised PCR conditions (Singh et al., 2010). A Vilber gel doc imaging system model was used to see the amplicons after they were separated on a 2% agarose gel with ethidium bromide at 5 V/cm. (E-BOX CX5. TS, Marne-la-Vallée, France). The intensity of the bands and % of gene expression was quantified by ImageJ software. Relative quantification of gene expression was performed in each experimental group using three independent biological replicates, with the concurrent amplification of β-actin serving as an internal control.

Table 5.1: Forward and reverse heat shock protein primers

hsp 83 Forward	5′CCCGTGGCTTCGAGGTGGTCT3′
hsp 83 Reverse	5′TCTGGGCATCGTCGGTAGTCATAGG3′
hsp 70 Forward	5′GAACGGGCCAAGCGCACACTCTC3′
hsp 70 Reverse	5′TCCTGGATCTTGCCGCTCTGGTCTC3′
hsp 60 Forward	5′ CCTCCGGCGGCATTGTCTTC3′
hsp 60 Reverse	5′ AGCGCATCGTAGCCGTAGTCACC3′
hsp 26 Forward	5′CAAGCAGCTGAACAAGCTAACAATCTG3′
hsp 26 Reverse	5′GCATGATGTGACCATGGTCGTCCTGG3′
β actin Forward	5′CCTCCGGCGGCATTGTCTTC3′
β actin Reverse	5′GGGCGGTGATCTCCTTCTGC3′

This experiment suggests that, exposure to microplastic-induced stress triggers specific gene expression responses**,** with distinct regulatory patterns in key stress-related genes. Validation of these expression profiles has been conducted through multiple quantitative PCR normalization strategies and technical replicates to ensure data accuracy. While the study is conducted under controlled laboratory conditions**,** it is anticipated that the

observed gene expression trends could serve as predictive markers for stress responses in natural environments. The choice of molecular assay optimized for sensitivity and specificity, will enable precise quantification of gene expression, establishing a robust methodology for future research on environmental stressors and will also provide the information of gene expression pathways.

5.13 Statistical analysis

For statistical analysis, we used one-way ANOVA, Tukey's comparison test, and the log-rank Mantel–Cox test with the mean ± SEM (n = 3) to identify significant differences in the results via GraphPad Prism software (version 5.01).

CHAPTER 6

RESULTS

6.1 PET microplastic formation

PET microplastics were formed as shown in Figure 6.1

Figure 6.1: PET microplastic powder formation

6.2 Characterization of PET microplastics

6.2.1 FTIR (Fourier transform infrared spectroscopy)

The FTIR analysis of PET microplastics revealed several characteristic bands with specific peak wavenumbers in cm^{-1}. For instance, the peak at 2918.51 cm^{-1} was associated with CH stretching, while the band at 1713.39 cm^{-1} indicated C=O stretching of the ester group. Other significant peaks included 1407.25 cm^{-1}, corresponding to the presence of a benzyl ring, and 1243.9 cm^{-1}, attributed to the elongation of the ester C=O bond. Vibrations of the ester group were observed at 1095.88 cm^{-1}, and the in-plane vibration of the benzene ring was detected at 1016.02 cm^{-1}. Additionally, the peaks at 872.58 cm^{-1} and 724.43 cm^{-1} were linked to the bending of the glycol group and the out-of-plane bending of the benzene ring, respectively. The peak at 493.97 cm^{-1} was assigned to the out-of-plane vibration of =C-H in the benzene group. These FTIR findings, shown in Figure 6.2, confirmed that the PET plastic consists of repeating units of the monomer ethylene terephthalate, with ester carbonyls typically found in the 1710–1725 cm^{-1} range (Alarby et al., 2023).

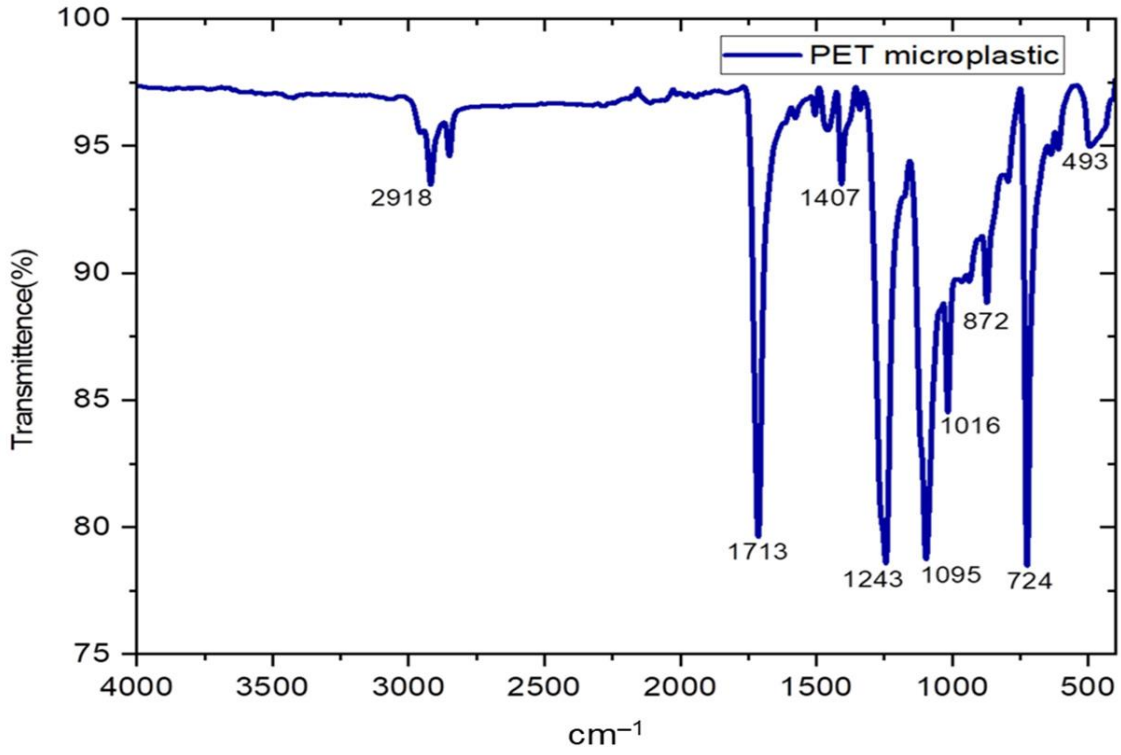

Figure 6.2: FTIR analysis of polyethylene terephthalate microplastics.

6.2.2 SEM (scanning electron microscopy) and EDX (energy dispersive X-ray spectroscopy)

SEM is extensively utilized for assessing the size and morphology of PET microplastics because of its ability to provide high-resolution images. The SEM images obtained revealed the irregular shapes of the PET microplastics, which included cylindrical, oval, circular, triangular, and even some indistinct shapes. The SEM images in Figure 6.3 A–C depict the various shapes and sizes observed. We determined the size % of the PET microplastics in the SEM images via ImageJ software (version 1.53), as shown in Figure 13 D. Additionally, Figure 6.4 presents the EDS analysis, revealing the elemental composition of the PET microplastic.

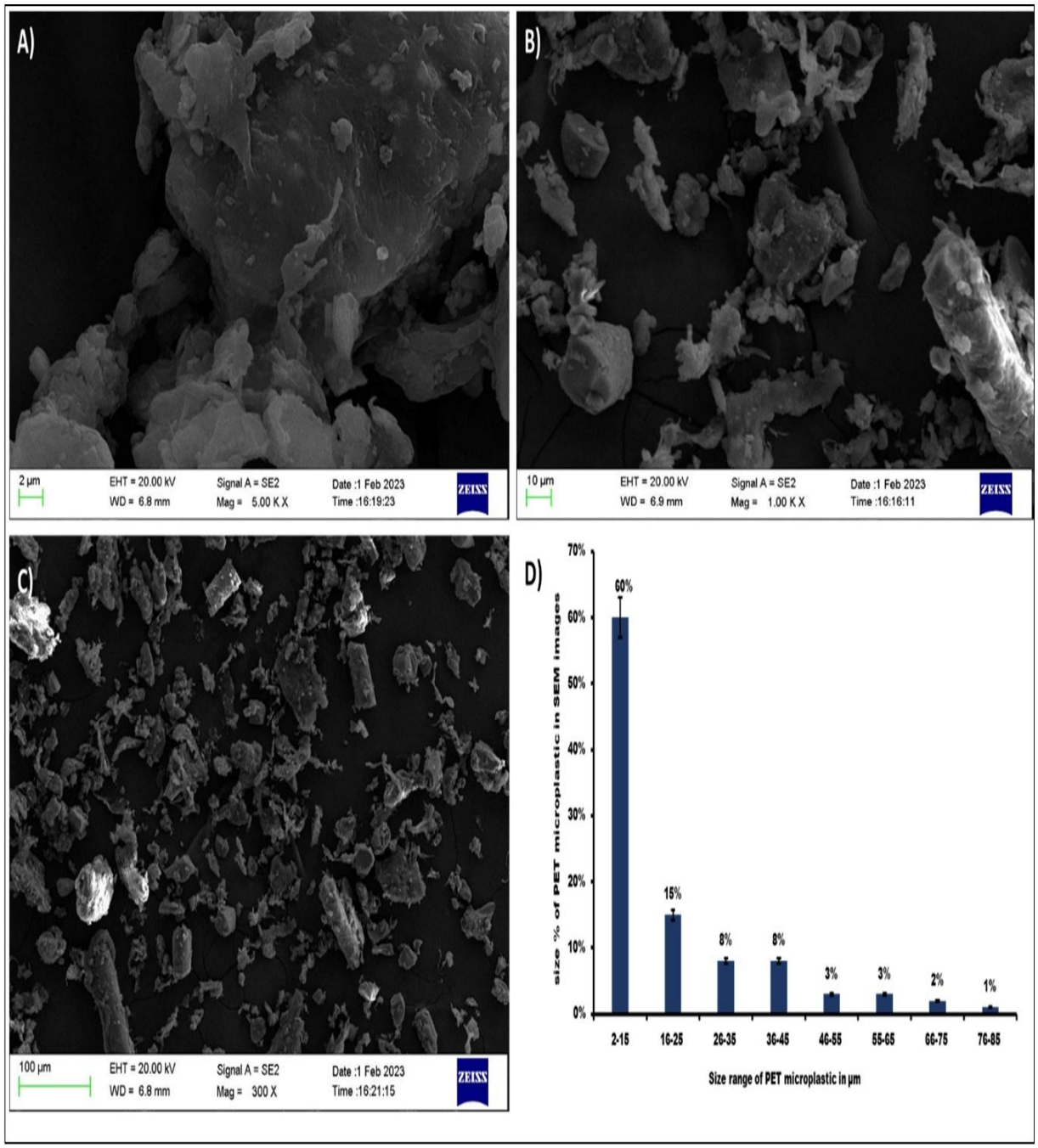

Figure 6.3: SEM analysis of PET microplastics. Images (**A**–**C**) of the PET microplastic demonstrated that the particles are irregular in shape and that their sizes vary from 2 to 100 µm, as determined by the ImageJ software. (**D**) Size % of PET microplastics via SEM.

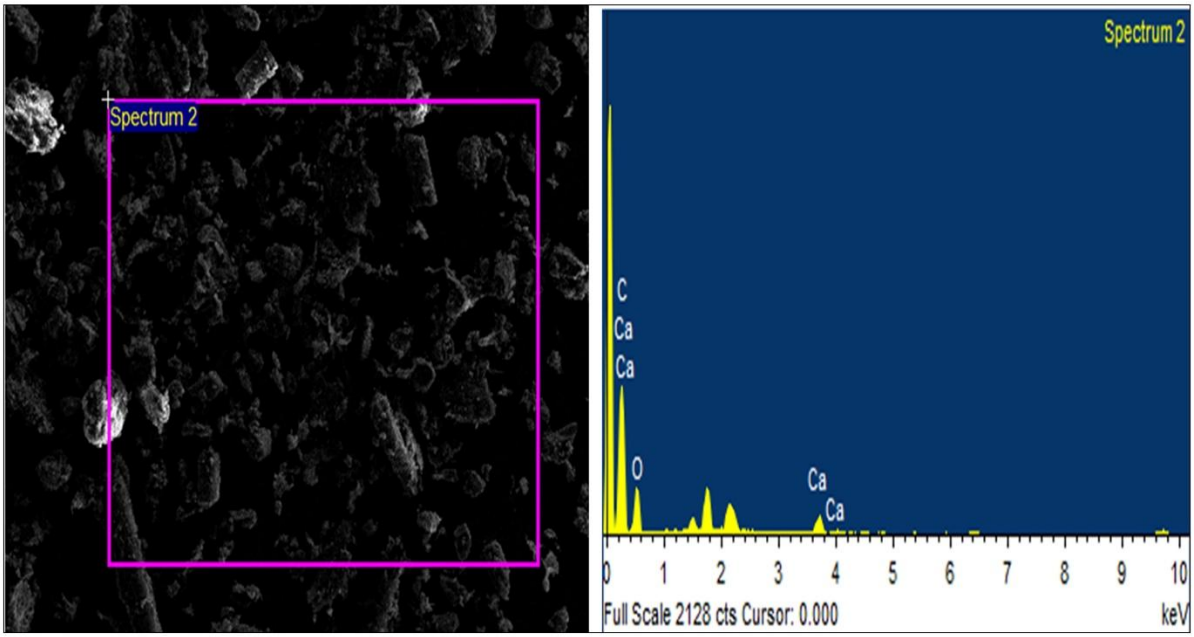

Figure 6.4: - EDX of the PET's elemental composition, indicating that it contains 53.65% carbon, 42.42% oxygen, and 3.93% wollastonite.

6.3 PET microplastic uptake/accumulation by *Drosophila melanogaster*

6.3.1 Fluorescence microscopy

Nile red dye is the most suitable stain for microplastic identification and detection because of its strong adsorption for plastics, enhanced fluorescence intensity, faster incubation time, and excellent affinity for a broad variety of plastic polymers. The results indicate the accumulation of PET microplastics according to the concentrations in the midgut and hindgut of the larvae under the stereomicroscope shown in Figure 6.5, and the fluorescence intensities of Nile red-stained PET microplastics were observed when excited within the range of 440–520 nm and emission was within the range of 445–545 nm at 10x magnification, as shown in Figure 6.6. This analysis provides a platform to ensure the accumulation of PET microplastics, which can be used in further investigations.

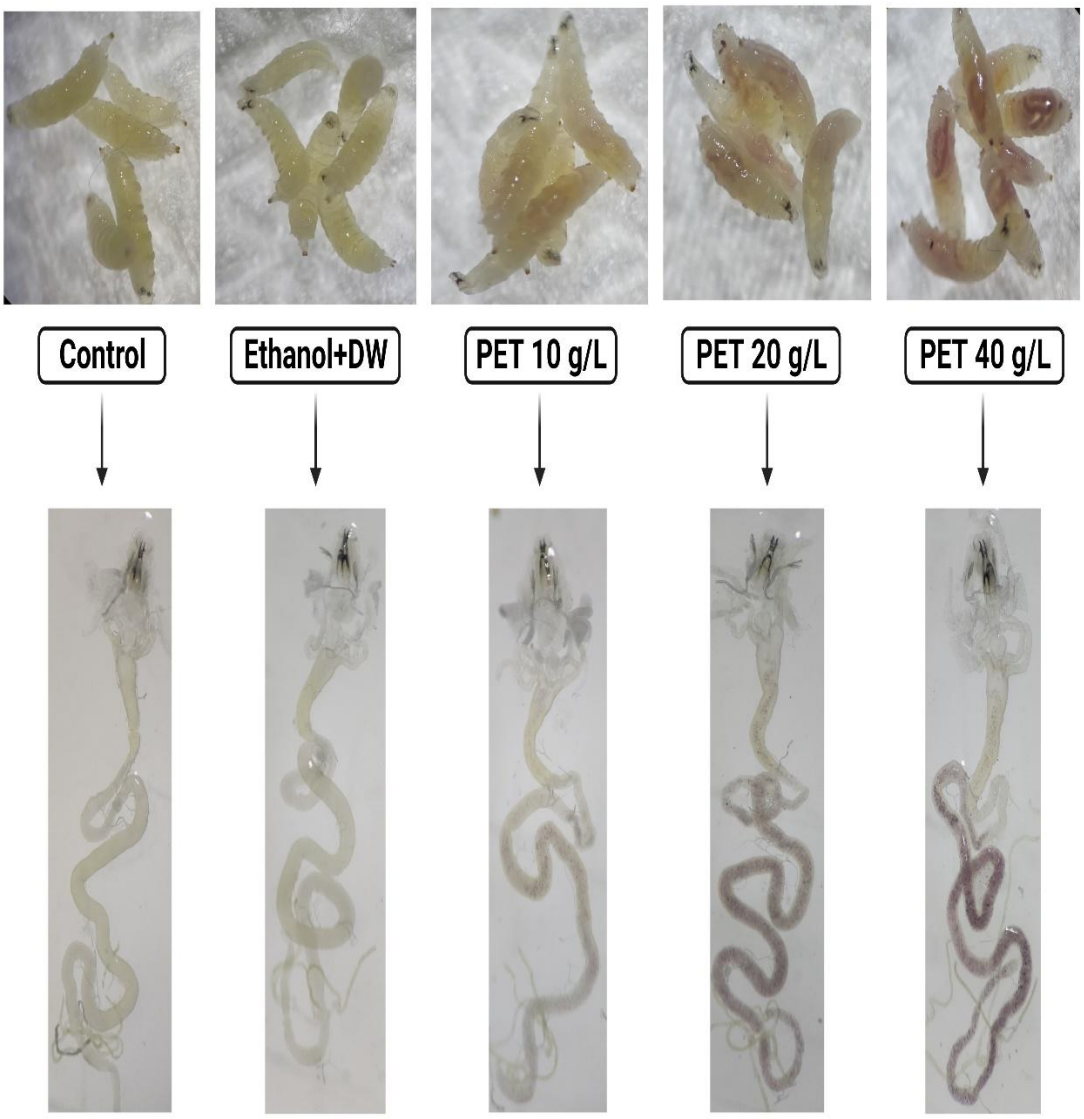

Figure 6.5: Accumulation of PET microplastics in the midgut and hindgut of whole and dissected larvae in the control, ethanol + DW, PET 10 g/L, PET 20 g/L, and PET 40 g/L groups. Plastic dyed with Nile red dye is clearly visible due to the translucent and membranous texture of the *Drosophila* larvae cuticle.

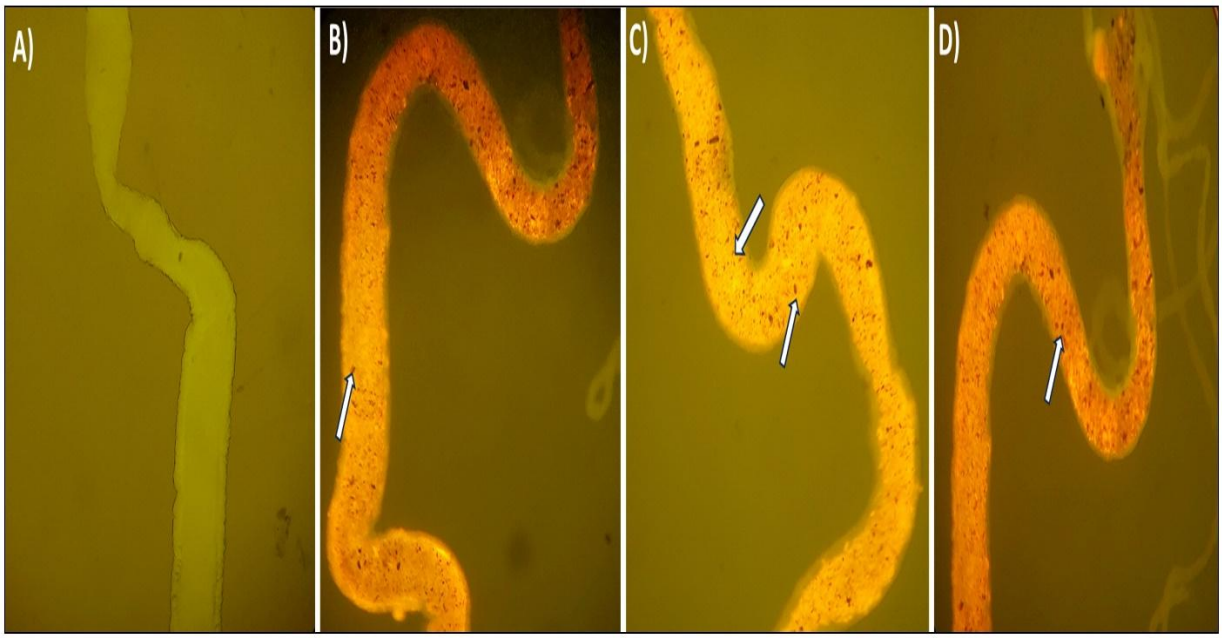

Figure 6.6. The accumulation of PET microplastics. (A) The *Drosophila* larval gut does not contain PET microplastics. (B-C) Nile red-stained PET microplastics. (D) Hindgut with Nile red-stained microplastics.

6.3.2 Confocal microscopy

To investigate this crucial step, confocal microscopy was used. Notably, the digestive tracts of the larvae presented distinctive green fluorescence attributed to PET, as illustrated in Figure 6.7. Subsequent analysis involved scrutinizing confocal microscopy images of the midgut portion of the dissected larvae shown in Figure 6.7 A2. The identification of microplastic particles within the midgut of *Drosophila* larvae provides compelling grounds.

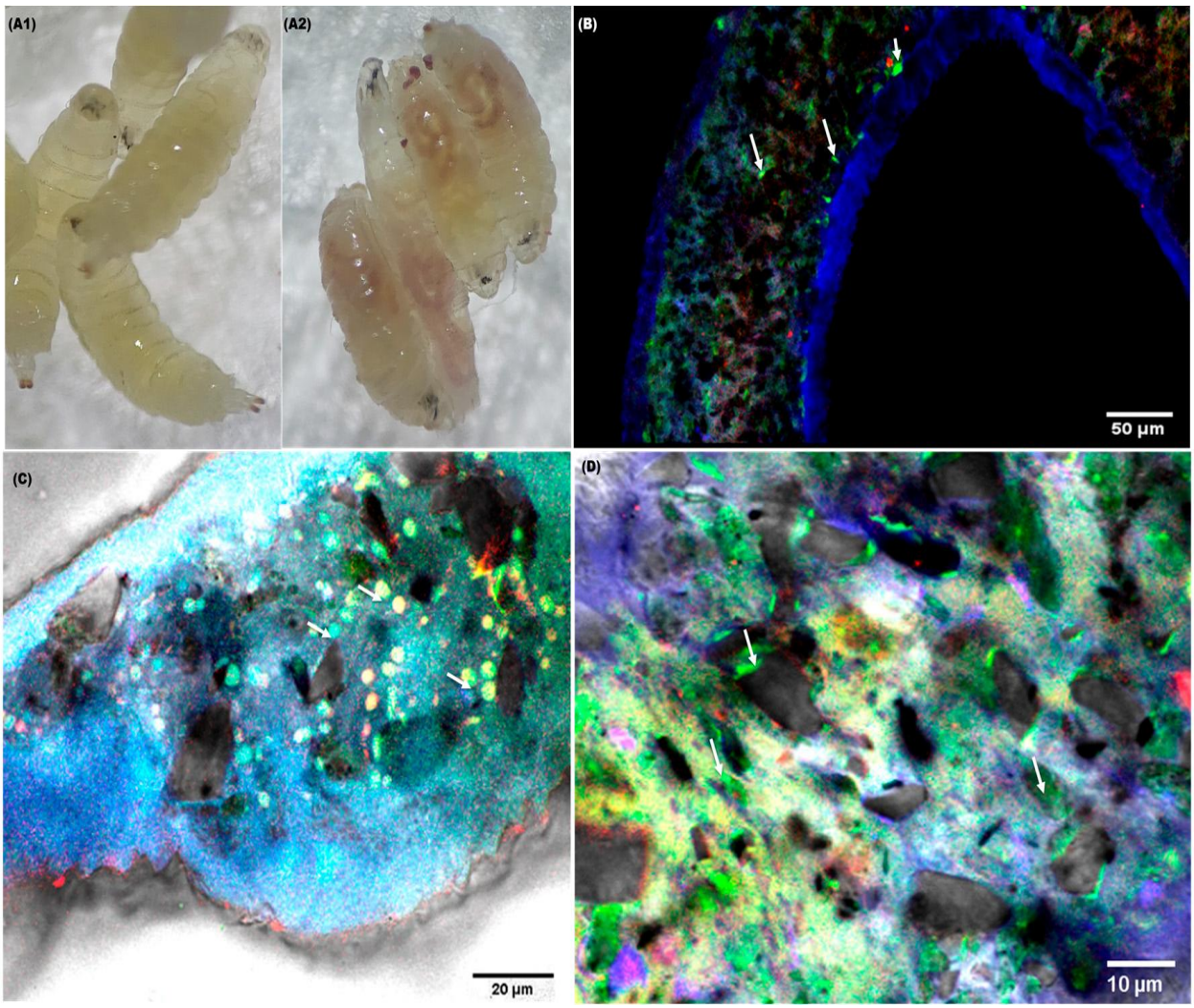

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Figure 6.7. Detection of PET MP via confocal microscopy. (A1) Larvae of the control group, (A2) larval gut treated with Nile red-stained PET MP, (B–D) confocal images of (A2) larval midgut showing the accumulation of PET MP with green fluorescence, as represented by white arrows. For (A1, A2), a stereomicroscope was used to capture images.

6.4 Nutrition value (food intake) of flies

Flies fed sucrose solution in the control and PET microplastic-treated food groups consumed almost equal amounts of food. Although the flies in the microplastic-treated groups consumed slightly less food, significant difference was observed neglegible , as shown in Figure 6.8. The consumption of food per fly per day in the control, 10, 20, and 40 g/L PET groups was 0.85, 0.87, 0.86, 0.81, and 0.85 µl/fly/day, respectively.

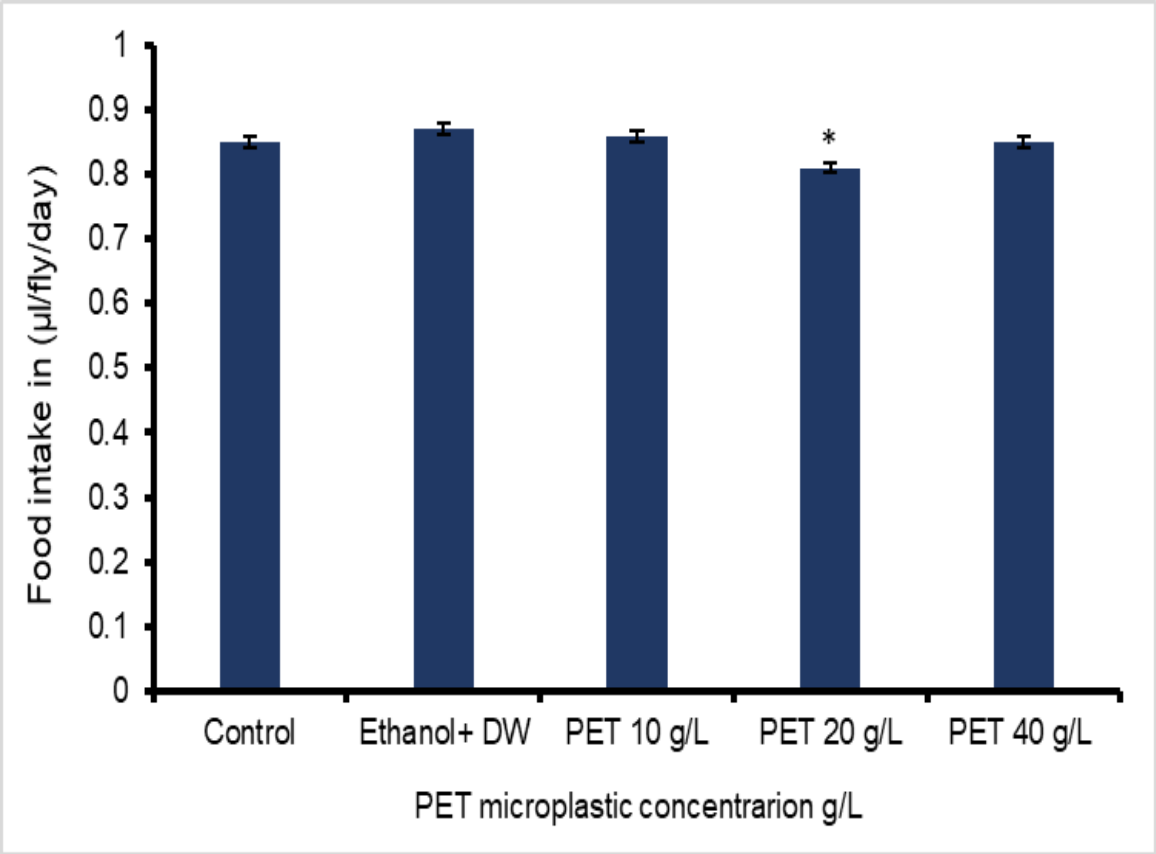

Figure 6.8: Food intake (µl/day/fly) in the control, vehicle control and PET microplastic-treated groups. A significant difference was detected at *p < 0.05 (n=3).

6.5 Behavioral and developmental analysis

6.5.1 Climbing activity

The full capacity of the control and ethanol +DW-treated flies climbed after 30 s (only a 10% decrease). As demonstrated in Figure 6.9, the climbing ability of the 20 g/L PET and 40 g/L PET groups significantly decreased, making it challenging for them to scale the plastic tube walls. However, there was no noticeable difference in the PET 10 g/L group; significance was determined via one-way ANOVA and Tukey's comparison test.

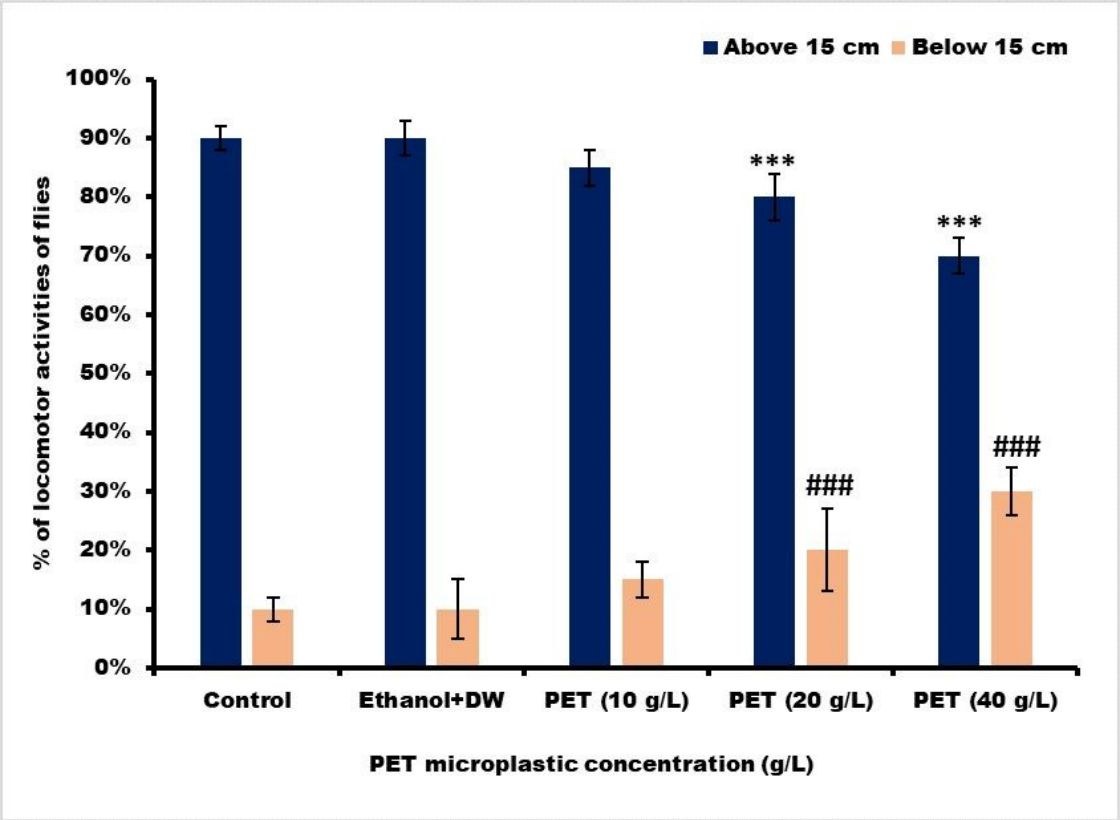

Figure 6.9. The climbing activity of *D. melanogaster* (Oregon R^{+}) flies was assessed after a 15-day period. Significance with the mean ± SEM is denoted as *** for $p < 0.001$ in the comparison of Groups I and II above 15 cm. Similarly, significance was denoted as ### for $p < 0.001$ in comparison with Group I below 15 cm. In this context, DW refers to distilled water, and PET represents polyethylene terephthalate**.**

6.5.2 Jumping activity

Compared with those in the control and ethanol + DW (5%) groups, the jumping behavior of the flies exposed to 20 g/L and 40 g/L PET was significantly lower, decreasing by 5% and 15%, respectively. As shown in Figure 6.10, the jumping ability of flies treated with g/L PET did not significantly decline. The means ± SEMs were compared via one-way ANOVA.

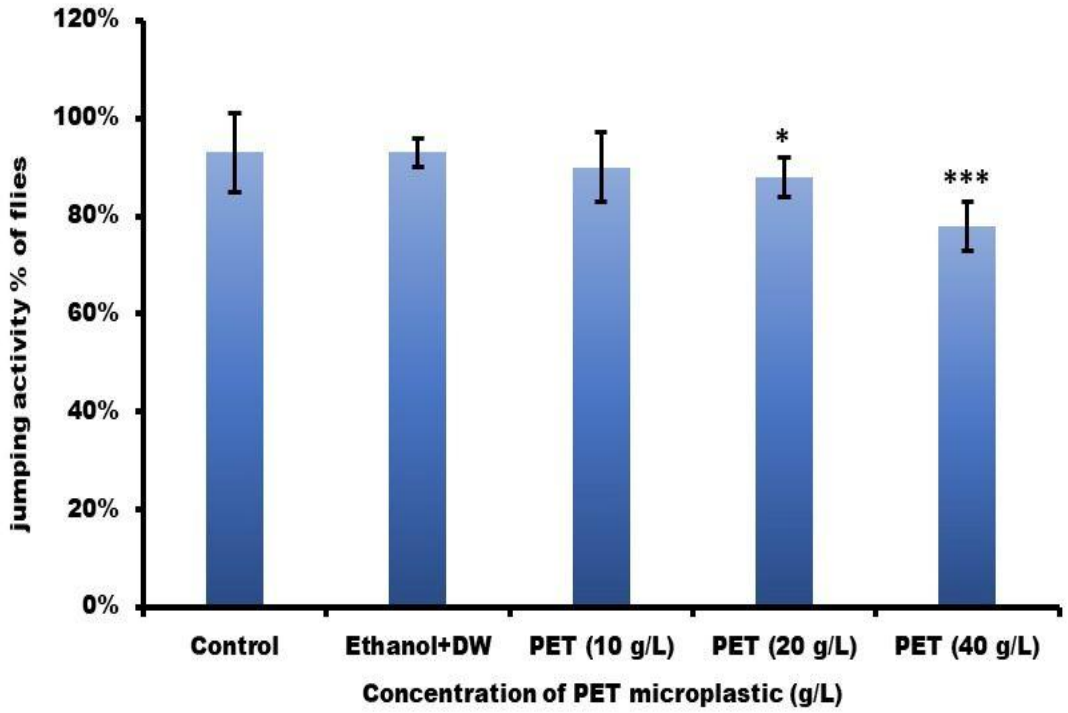

Figure 6.10: - Jumping activity of *D. melanogaster* (Oregon R^{+}) flies was examined after a 15-day exposure period. Significance is denoted as *** $p < 0.001$ and * $p < 0.5$ vs. Group I, with the means ± SEMs. In this context, DW refers to distilled water, and PET represents polyethylene terephthalate.

6.5.3 Crawling activity

As the larvae move by contracting their body wall musculature, any impairment in this locomotor function can be indicative of neuronal damage. To determine whether microplastics are hazardous to *Drosophila*, researchers may apply the larval crawling test, which measures how much it hinders the larva's ability to move about on its own. The average rates of crawling in Groups IV and V were slow as compare to group I, as shown in Figure 6.11.

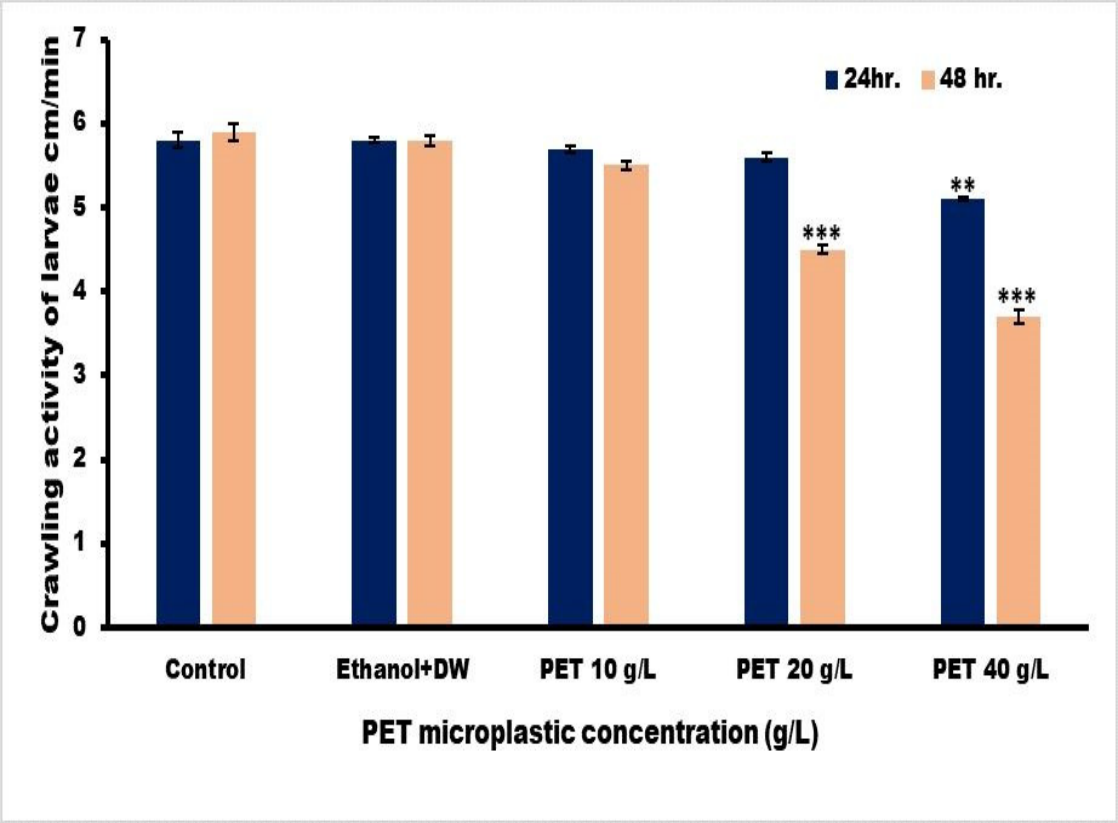

Figure 6.11: Graphical illustration of the path traveled by third-instar larvae in one minute. The statistical significance was determined as *** $p < 0.001$ and ** $p < 0.01$ vs. Group I.

6.5.4 Emergence of flies

In the control group, 98% of the flies emerged, and in the Ethanol+DW (vehicle control) group, 98% of the flies emerged within 12 days. For PET (10 g/L), PET (20 g/L), and PET (40 g/L), 94%, 71%, and 60%, respectively, of the flies emerged, as shown in Figure 6.12. Some of the flies were left to emerge in the PET (20 g/L) and PET (40 g/L) groups. Only the mentioned percentage of flies managed to emerge within 12 days.

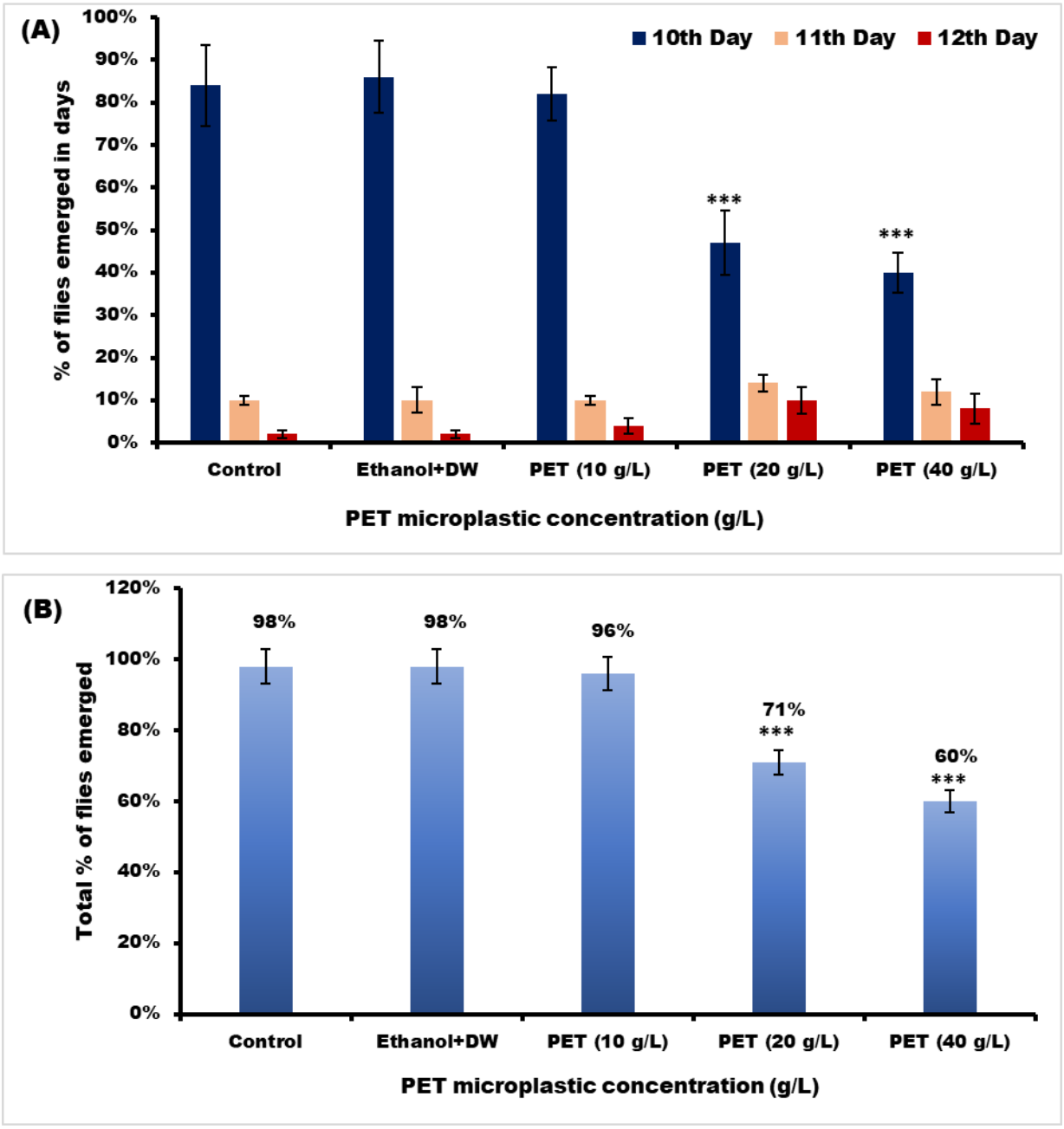

Figure 6.12: Emergence percentage of flies after exposure to PET microplastics. (**A**) The percentages of emerged flies were recorded for 3 consecutive days, specifically on the 10^{th}, 11^{th}, and 12^{th} days. Statistical significance with the mean ± SEM is denoted as *** $p < 0.001$ compared with Group I. (**B**) The total percentage of emerged flies across all groups up to 12 days was calculated. In this context, DW refers to distilled water, and PET represents polyethylene terephthalate.

6.5.5 Survival assay

The long-term toxicity of PET microplastics was tested via a survival assay. No statistically significant difference observed in the survival of the male flies, as shown in Figure 6.13A, but there was an observed decrease in the life span of female flies at PETs of 20 g/L and 40 g/L, as shown in Figure 6.13 B, according to statistical analysis via the log–rank Mantel–Cox test. The median survival rates of male flies in the control group, ethanol +DW, 10 g/L PET, 20 g/L PET, and 40 g/L PET were 47.5, 46, 46, 47, and 47 days, respectively, and those of female flies were 48, 47, 47, 43, and 41 days, respectively.

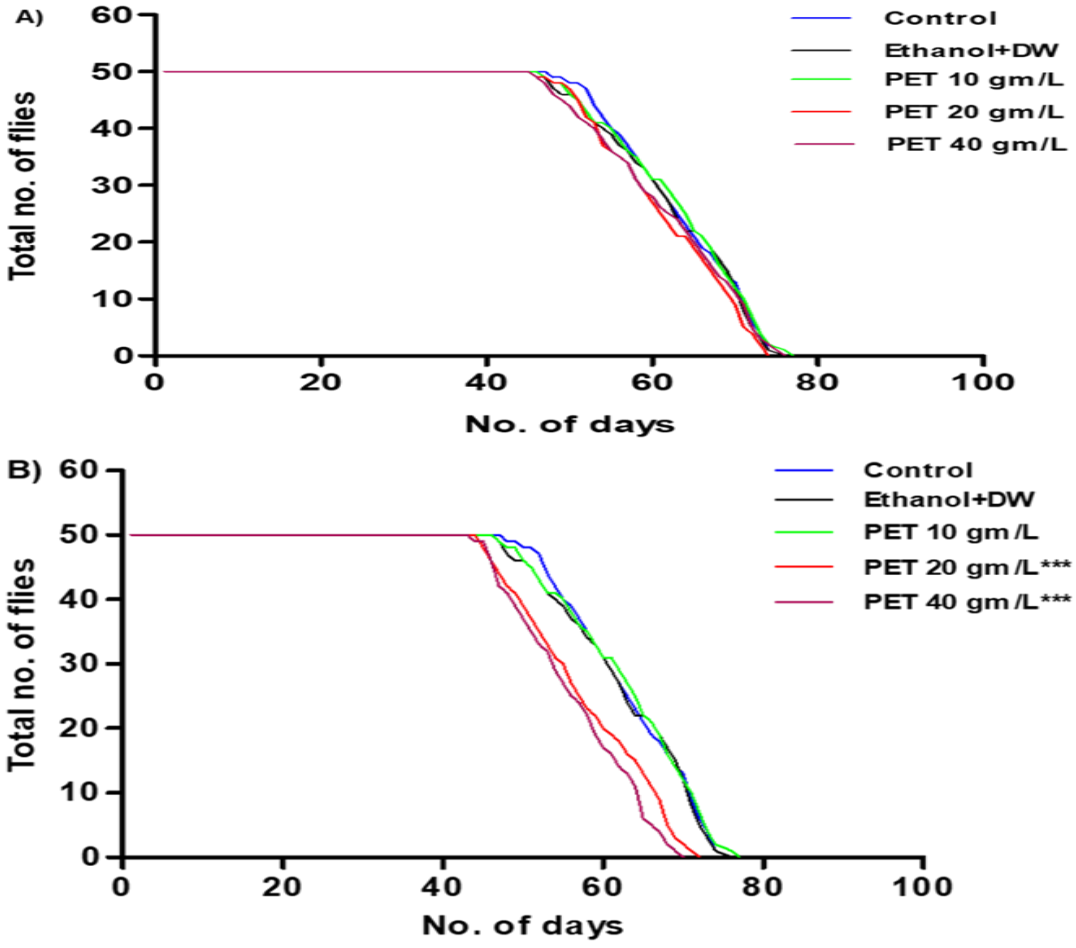

Figure 6.13: (A) The survival rate of male flies did not significantly differ in the life span of the PET microplastic-containing group vs. the control. (B) Significant decrease

in the life span of female flies in Groups IV and V, denoted as *** $p < 0.001$, in comparison with the control group.

6.6 Dye exclusion trypan blue assay for assessing cellular toxicity

The experimental findings are shown in Figure 6.14. Compared with those in the control group, the midgut tissues of larvae subjected to PET MP at concentrations of 20 and 40 g/L presented extensive blue staining at rates of 45% and 61%, respectively. No significant blue staining was observed in the untreated groups. The staining percentage was 1% in the control group, 2% in the ethanol + DW group (the vehicle control group), 12% in the 10 g/L group, 45% in the 20 g/L group, and 61% in the 40 g/L group, as calculated via ImageJ software.

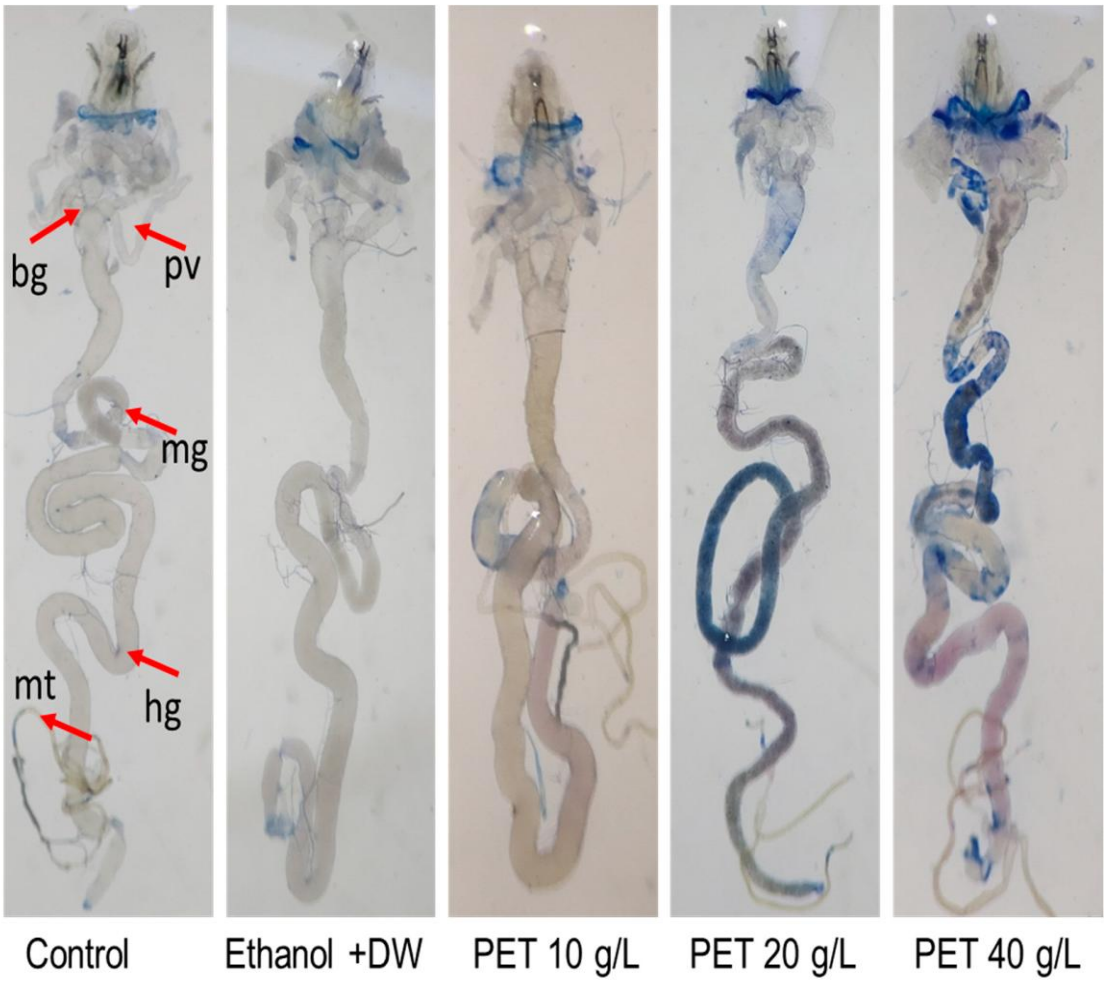

Figure 6.14. The dye exclusion test was conducted via trypan blue staining on dissected 72 h third instar larvae of *Drosophila melanogaster* (Oregon R^+) in the control or vehicle

control group and larvae exposed to 10 g/L, 20 g/L, or 40 g/L PET. bg = brain ganglia, pv = proventriculus, mg = midgut, hg = hindgut, mt = Malpighian tubules.

6.7 Total protein content (biochemical assay)

Following exposure to PET microplastics at concentrations of 20 g/L and 40 g/L, the total protein concentration *Drosophila* larvae significantly decreased, as shown in Figure 6.15 and Table 6.1. After 24 h of exposure, the 40 g/L PET group presented a lower protein content (8.81 ± 0.27 mg/ml) than did the control group (9.84 ± 0.09 mg/mL). Similarly, after 48 h, both the PET 20 g/L (6.32 ± 0.29 mg/mL) and PET 40 g/L (5.63 ± 0.09 mg/mL) groups presented lower protein concentrations than did the control/vehicular control group (9.56 ± 0.21 mg/mL). However, no significant decrease in protein concentration was observed in the 10 g/L PET group after exposure.

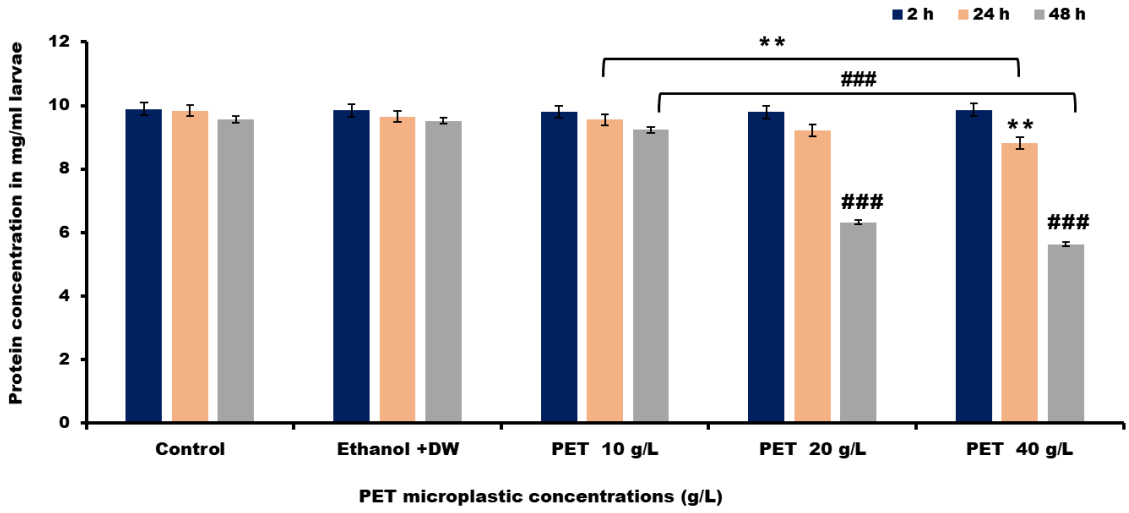

Figure 6.15: Total protein content of third-instar *Drosophila* larvae after 2, 24 and 48 h of treatment with PET MP. The average ± SD (n = 3) was calculated, and significant differences were considered at ** $p < 0.01$ after 24 h of treatment. Similarly, $^{###}$ $p < 0.001$ for 48 h. treatment group compared with the control group. **$p < 0.01$ indicates a significant difference after 24 h of treatment between the PET microplastic-treated groups; similarly, $^{###}$ $p < 0.001$ indicates a significant difference after 48 h of treatment between the PET microplastic-treated groups.

Table 6.1: Total protein concentration (mg/ml larvae) in the control and treated control groups

	2 hr.	24 hr.	48 hr.
Control	9.89 ± 0.05	9.84 ± 0.09	9.56 ± 0.21
Ethanol + DW	9.85 ± 0.09	9.65 ± 0.23	9.52 ± 0.21
PET 10 g/L	9.80 ± 0.17	9.55 ± 0.18	9.23 ± 0.14
PET 20 g/L	9.79 ± 0.20	9.21 ± 0.16	6.32 ± 0.29
PET 40 g/L	9.86 ± 0.22	8.81 ± 0.27	5.63 ± 0.09

6.8 Nonenzymatic studies

6.8.1 Protein carbonyl assay

Following exposure to PET microplastics at concentrations of 20 g/L and 40 g/L, the protein carbonyl content in *Drosophila* larvae significantly increased, as shown in Figure 6.16 and Table 6.2. After 24 hours of exposure, the 20 and 40 g/L PET groups presented higher protein carbonyl contents (36.87 ± 0.223 nmol PC/mg larval protein) and (37.76 ± 0.223 nmol PC/mg larval protein) than did the control group (28.64 ± 0.245 nmol PC/mg larval protein). Similarly, after 48 h, both the PET 20 g/L (38.08 ± 0.456 nmol PC/mg larval protein) and PET 40 g/L (41.07 ± 0.501 nmol PC/mg larval protein) groups presented increased protein carbonyl content in comparison with the control/vehicular control group (28.70 ± 0.287 nmol PC/mg larval protein). However, no significant increase in protein carbonyl content was observed in the 10 g/L PET group after exposure.

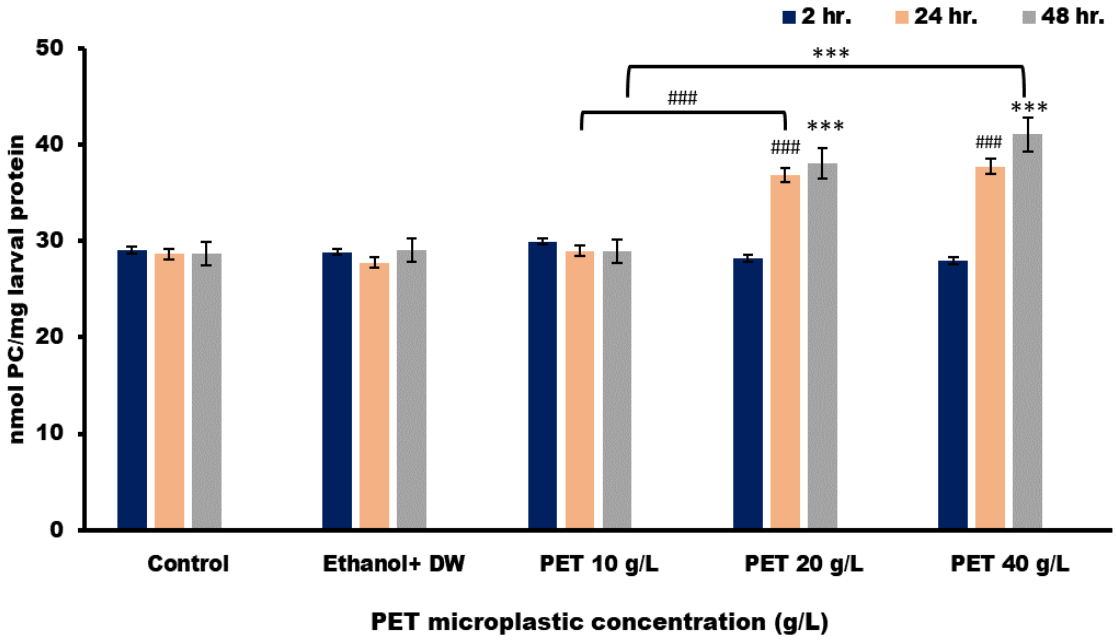

Figure 6.16: Protein carbonyl content of third-instar *Drosophila* larvae after 2 h, 24 h and 48 h of treatment with PET MP. The average ± SEM (n = 3) was calculated, and significant differences were considered at $^{###}$ $p < 0.001$ after 24 h of treatment. Similarly, *** $p < 0.001$ for the 48h treatment group compared with the control group.

Table 6.2: Protein carbonyl content (nmol PC/mg larval protein) between the control and PET-treated groups.

	2 hr.	24 hr.	48 hr.
Control	29.06 ± 0.051	28.64 ± 0.245	28.70 ± 0.287
Ethanol + DW	28.87 ± 0.102	27.78 ± 0.203	29.06 ± 0.198
PET 10 g/L	29.96 ± 0.031	28.98 ± 0.278	28.90 ± 0.278
PET 20 g/L	28.25 ± 0.167	36.87 ± 0.189	38.08 ± 0.456
PET 40 g/L	27.99 ± 0.145	37.76 ± 0.223	41.07 ± 0.501

6.8.2 MDA content (lipid peroxidation)

Following exposure to PET microplastics at concentrations of 20 g/L and 40 g/L, the MDA content in *Drosophila* larvae significantly increased, as shown in Figure 6.17 and Table 6.3. After 24 hours of exposure, the PET 20 and 40 g/L groups presented increased MDA contents (3.81 ± 0.101 n moles/h/mg larval protein) and (4.51 ± 0.109 n

moles/h/mg larval protein) compared with those of the control group (2.89 ± 0.021 n moles/h/mg larval protein). Similarly, after 48 h, both the PET 20 g/L (4.22 ± 0.014 n moles/h/mg larval protein) and PET 40 g/L (4.97 ± 0.100 n moles/h/mg larval protein) groups presented increased MDA contents in comparison with those of the control/vehicular control group (2.82 ± 0.111 n moles/h/mg larval protein). However, no significant increase in MDA content was observed in the 10 g/L PET group after exposure.

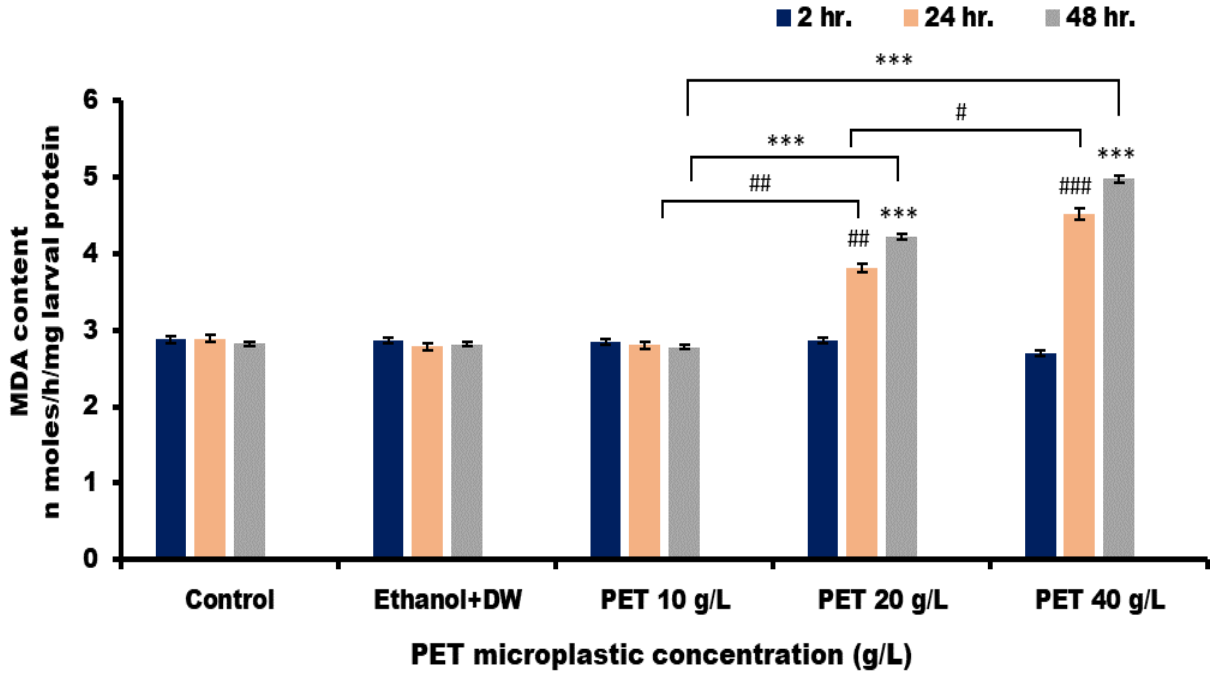

Figure 6.17: MDA content of third-instar *Drosophila* larvae after 2 h, 24 h, and 48 h of treatment with PET MP. The average ± SEM (n = 3) was calculated, and significant differences were considered at $^{\#\#}$ $p < 0.01$ and $^{\#\#\#}$ $p < 0.001$ after 24 h of treatment. Similarly, ***$p < 0.001$ for the 48-h treatment group compared with the control group. #$p < 0.05$ indicates a significant difference after 24 h of treatment between the PET microplastic-treated groups; similarly, *** $p < 0.01$ indicates a significant difference after 48 h of treatment between the PET microplastic-treated groups.

Table 6.3: MDA content (n moles/h/mg larval protein) between the control and PET-treated groups.

	2 hr.	24 hr.	48 hr.
Control	2.87 ± 0.051	2.89 ± 0.021	2.82 ± 0.111
Ethanol + DW	2.86 ± 0.107	2.78 ± 0.011	2.81 ± 0.021
PET 10 g/L	2.84 ± 0.041	2.80 ± 0.142	2.77 ± 0.132
PET 20 g/L	2.85 ± 0.109	3.81 ± 0.101	4.22 ± 0.014
PET 40 g/L	2.69 ± 0.100	4.51 ± 0.109	4.97 ± 0.100

6.9 Enzymatic studies

6.9.1 *Cu-Zn* superoxide dismutase (SOD) activity

Following exposure to PET microplastics at concentrations of 20 g/L and 40 g/L, the level of *Cu-Zn* SOD activity in *Drosophila* larvae significantly increased, as shown in Figure 6.18 and Table 6.4. After 24 hours of exposure, the PET 20 and 40 g/L groups presented increased SOD activity (6.116 ± 0.014 µmol/min/mg larval protein) and (7.495 ± 0.032 µmol/min/mg larval protein) compared with the control group (3.978 ± 0.112 µmol/min/mg larval protein). Similarly, after 48 h, both the PET 20 g/L (6.999 ± 0.121 µmol/min/mg larval protein) and PET 40 g/L (7.968 ± 0.196 µmol/min/mg larval protein) groups presented increased SOD activity in comparison with the control/vehicular control group (3.964 ± 0.125 µmol/min/mg larval protein). However, no significant increase in *Cu-Zn* SOD activity was observed in the 10 g/L PET group after exposure.

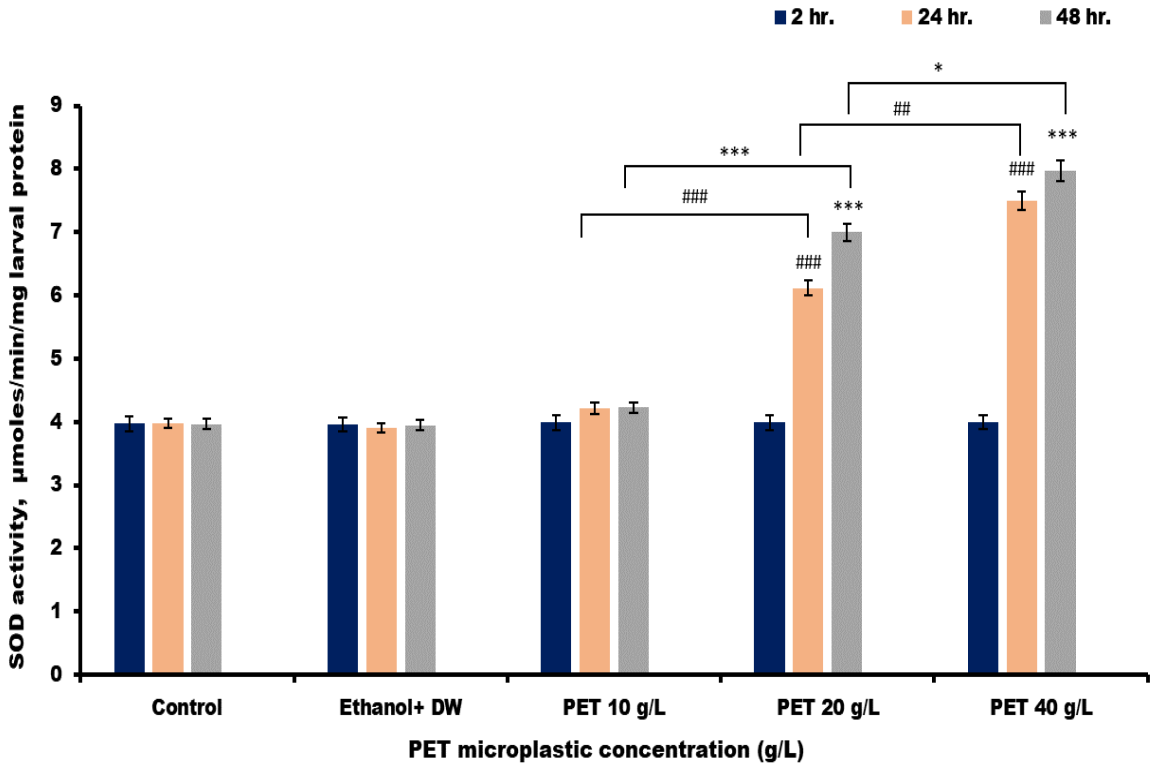

Figure 6.18: *Cu-Zn* SOD activity of third-instar *Drosophila* larvae after 2, 24 and 48 h of treatment with PET MP. The average ± SEM (n = 3) was calculated, and significant differences were considered at $^{###}p < 0.01$ and $^{###}p < 0.001$ after 24 h of treatment. Similarly, *** $p < 0.001$ for the 48h treatment group compared with the control group. $^{##}p < 0.01$ and $^{###}p < 0.001$ indicate significant differences after 24 h of treatment between the PET microplastic-treated groups; similarly, * $p < 0.05$ and *** $p < 0.001$ indicate significant differences after 48 h of treatment between the PET microplastic-treated groups.

Table 6.4: *Cu–Zn* SOD activity (µmoles/min/mg larval protein) in the control and PET-treated groups

	2 hr.	24 hr.	48 hr.
Control	3.967 ± 0.206	3.978 ± 0.112	3.964 ± 0.125
Ethanol + DW	3.960 ± 0.289	3.899 ± 0.013	3.945 ± 0.024
PET 10 g/L	3.986 ± 0.196	4.212 ± 0.021	4.223 ± 0.102
PET 20 g/L	3.985 ± 0.178	6.116 ± 0.014	6.999 ± 0.121
PET 40 g/L	3.996 ± 0.137	7.495 ± 0.032	7.968 ± 0.196

6.9.2 Specific catalase (CAT) activity

Following exposure to PET microplastics at concentrations of 20 g/L and 40 g/L, the specific catalase activity in the tissues of *Drosophila* larvae significantly increased, as shown in Figure 6.19 and Table 6.5. After 24 hours of exposure, the PET 20 and 40 g/L groups presented increased specific catalase activity (366.75 ± 0.322 µmol H_2O_2/min/mg larval protein) and (448.92 ± 0.502 µmol H_2O_2/min/mg larval protein) compared with the control group (226.21 ± 0.182 µmol H_2O_2/min/mg larval protein). Similarly, after 48 h, both the PET 20 g/L (396.26 ± 0.275 µmole H_2O_2/min/mg larval protein) and PET 40 g/L (495.26 ± 0.401 µmole H_2O_2/min/mg larval protein) groups presented increased SOD activity in comparison with the control/vehicular control group (224.14 ± 0.135 µmole H_2O_2/min/mg larval protein). However, no significant increased catalase activity was observed in the 10 g/L PET group after exposure.

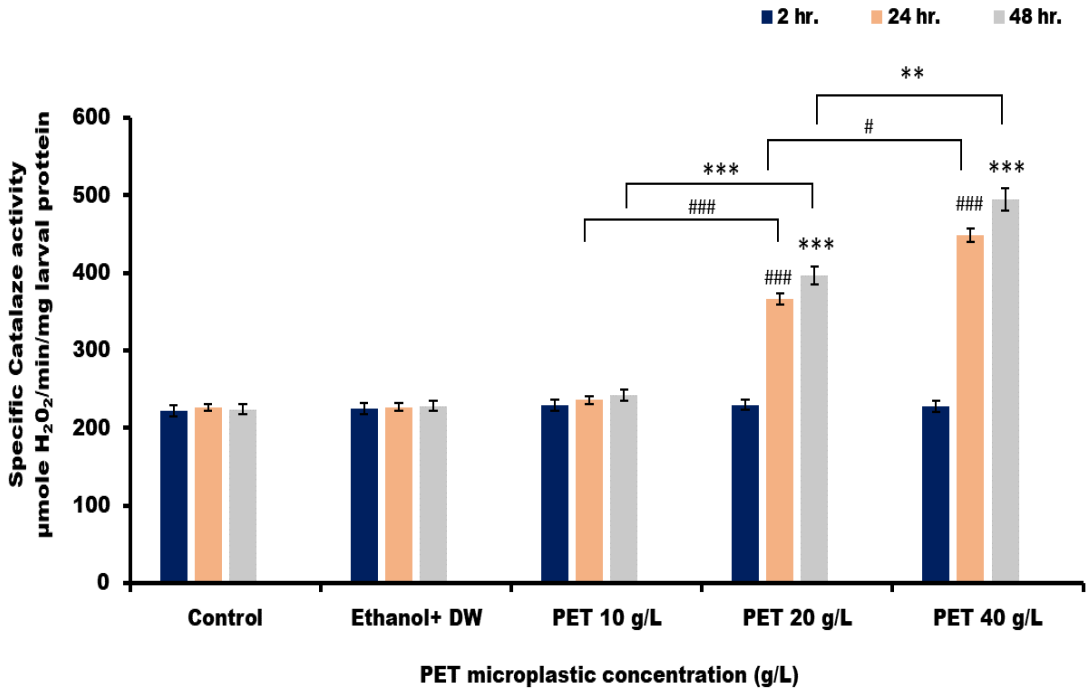

Figure 6.19: Catalase activity of third-instar *Drosophila* larvae after 24 h and 48 h of treatment with PET MP. The average ± SEM (n = 3) was calculated, and significant differences were considered at $^{###}$ $p < 0.001$ after 24 h of treatment. Similarly, *** $p <$ 0.001 for the 48h treatment group compared with the control group. #$p < 0.05$ indicates a significant difference after 24 h of treatment between the PET microplastic-treated

groups; similarly, ** $p < 0.01$ indicates a significant difference after 48 h of treatment between the PET microplastic-treated groups.

Table 6.5: Catalase-specific activity (µmole H_2O_2/min/mg larval protein) between the control and PET-treated groups

	2 hr.	24 hr.	48 hr.
Control	222.32 ± 0.101	226.21 ± 0.182	224.14 ± 0.135
Ethanol + DW	225.25 ± 0.126	227.15 ± 0.236	228.26 ± 0.101
PET 10 g/L	229.12 ± 0.134	236.21 ± 0.125	242.14 ± 0.156
PET 20 g/L	230.15 ± 0.110	366.75 ± 0.322	396.26 ± 0.275
PET 40 g/L	228.16 ± 0.121	448.92 ± 0.502	495.26 ± 0.401

6.10 Effects on reproductive health

6.10.1 Cytotoxicity test in the testes and ovaries of flies

The cytotoxicity of the PET MP was evaluated via a dye exclusion test (trypan blue) of testis and ovary tissues from treated *Drosophila* flies to determine whether PET MP exposure results in cytotoxic effects. Compared with those in the control group, the ovaries (mature follicles), male testes, and accessory glands of the flies exposed to PET MP at concentrations of 20 and 40 g/L after 15 days of treatment exhibited blue staining in the female ovaries, male testes, and accessory glands, as shown in Figure 6.20. There was no blue stain in the control or vehicle control groups. We also observed a reduced size of the left ovary in females, as shown in Figure 6.20 B5, during our analysis, but this finding is based only on visible observation and needs more scientific anatomical evidence.

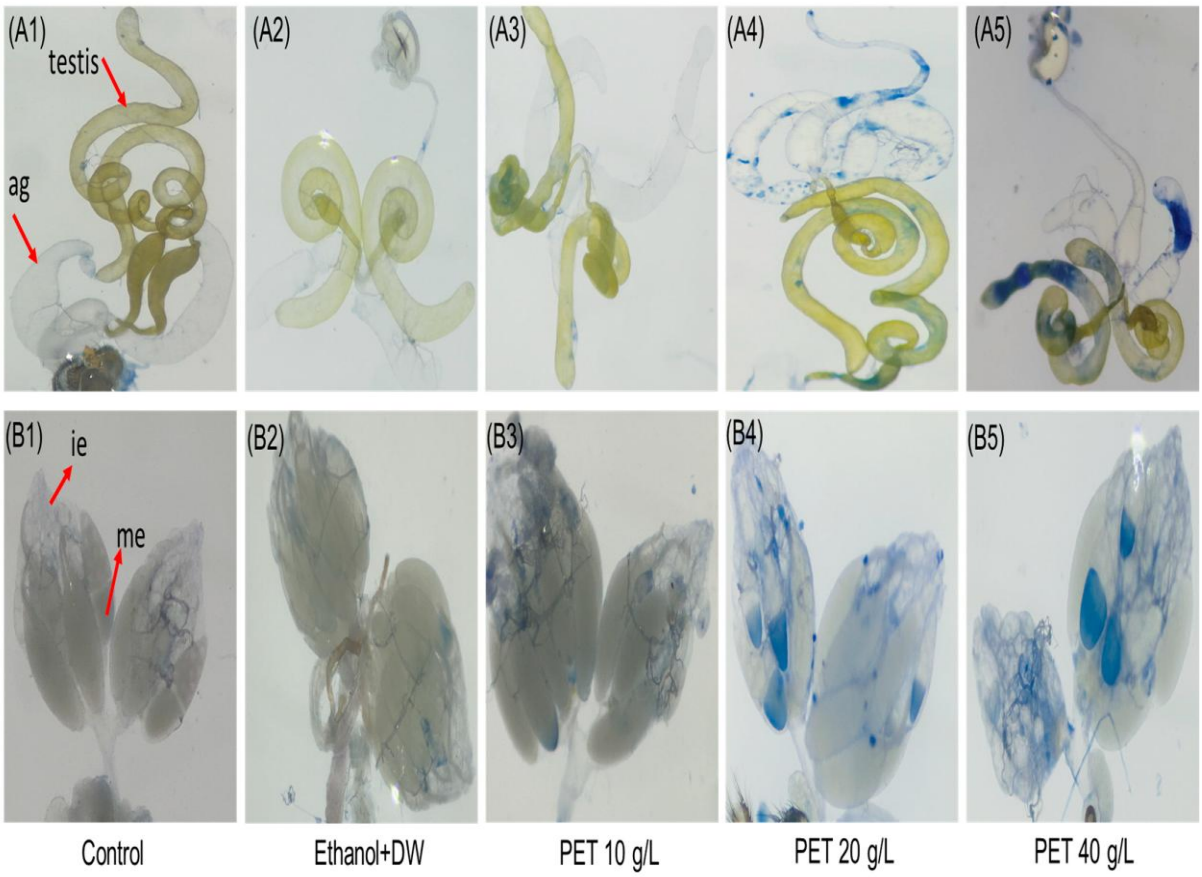

Figure 6.20: Cytotoxicity involving trypan blue staining of the reproductive organs of *Drosophila* flies subjected to 15 days of treatment. The groups included controls, vehicle controls, and those with PET concentrations of 10, 20, and 40 g/L. (**A1**–**A5**) Male reproductive organs across all test groups, whereas (**B1**–**B5**) female reproductive organs. ag = accessory glands, i.e., immature eggs; me = mature eggs.

6.10.2 Fecundity, Fertility, and Reproductive Performance

A significant decrease in the fecundity of *Drosophila melanogaster* at 20 and 40 g/L groups were compared with those in the control or vehicle control groups. The number of flies from egg laying also decreased in the 20 and 40 g/L PET groups, as depicted in Figure 6.21 A, compared with that in the control group. The fertility percentage shown in Figure 6.21 B indicates that the overall reproductive health of *Drosophila* flies deteriorated after the consumption of high dosages of PET MP. There was no significant effect of PET MP in the 10 g/L PET MP group.

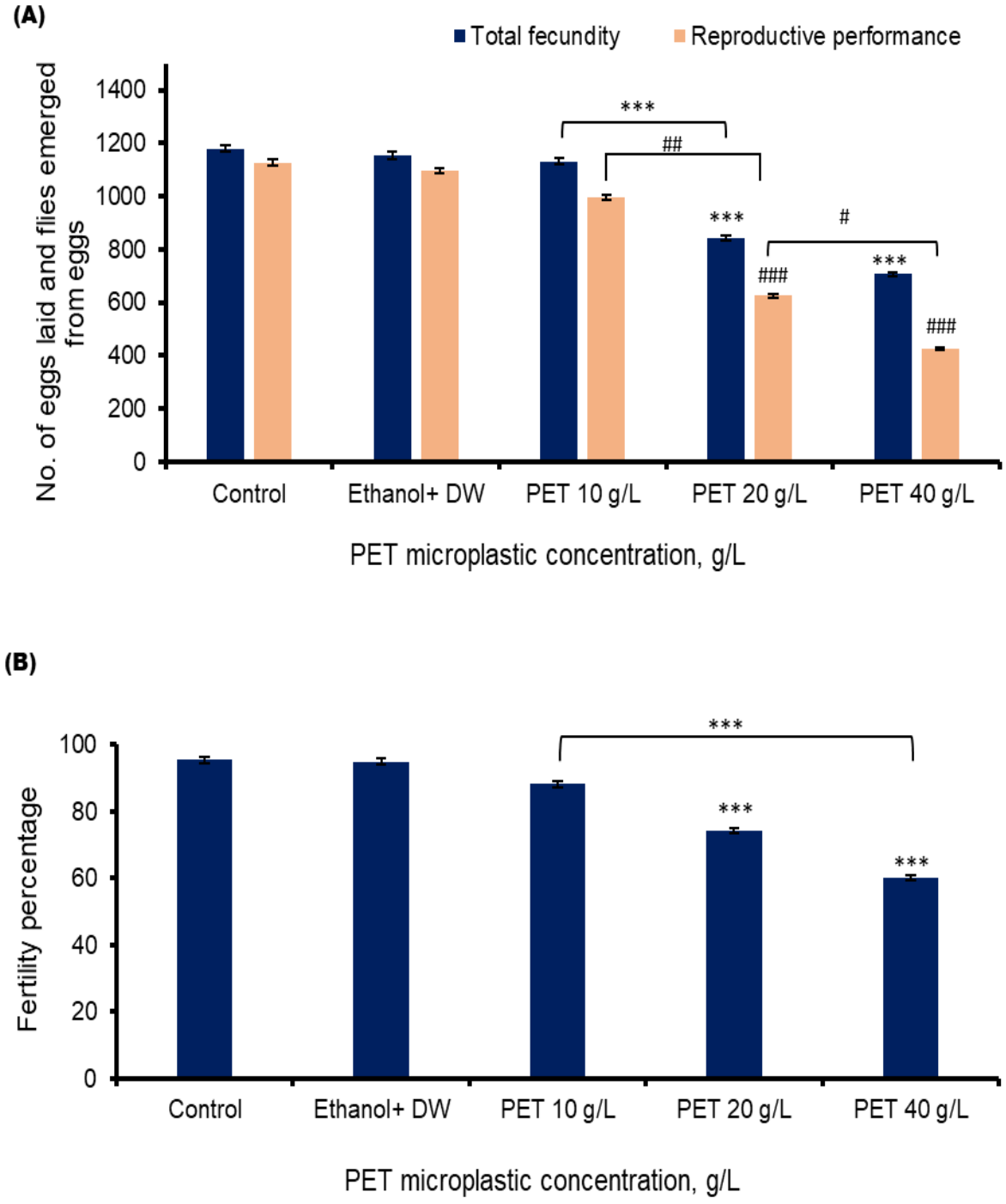

Figure 6.21. The reproductive health of *Drosophila melanogaster* is depicted in (A), which illustrates fecundity (number of eggs) and reproductive performance (total number of emerged flies), presented as the mean values with standard error of the mean (SEM) (n = 3). Statistical significance is denoted by *** $p < 0.001$ for fecundity compared with the control group and $^{###}$ $p < 0.001$ for reproductive performance relative to the control group. $^{#}$ $p < 0.05$ and $^{##}$ $p < 0.01$ indicate significant differences between

the PET microplastic-treated groups. (B) Fertility percentage, with statistical significance indicated by *** for $p < 0.001$ compared with the control.

6.11 Reverse transcriptase polymerase chain reaction (RT–PCR)

Figure 6.22 shows gel agarose band images and the fold changes in gene expression in *Drosophila* larvae after 48 h of exposure to PET microplastics. Notably, *hsp*83 exhibited more pronounced overexpression, and these findings collectively identify *hsp*83 as a prominent and responsive biomarker by emphasizing its role as a key component in the initial defense mechanism against microplastic-induced stress. Moreover, we observed that, compared with that in the control group, *hsp* 60 activity was downregulated, whereas *hsp*26 activity did not significantly change.

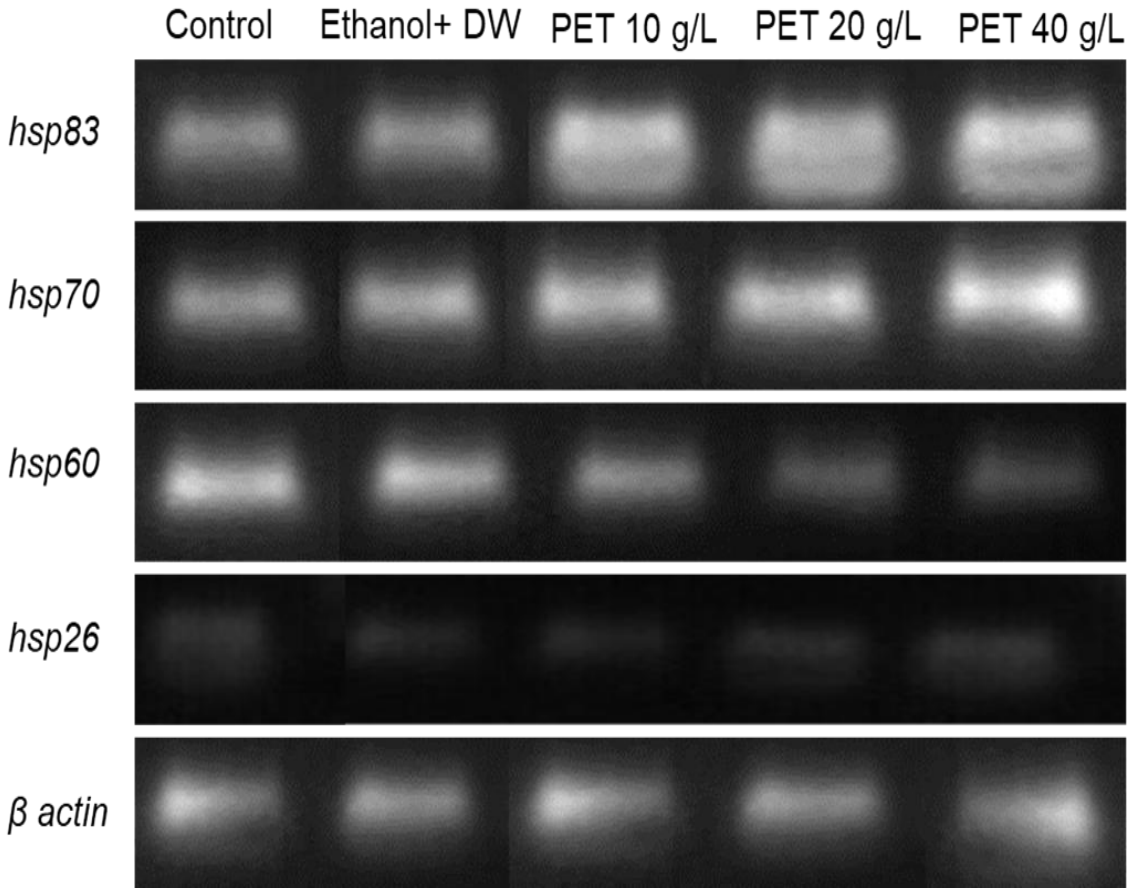

Figure 6.22: RT–PCR gel bands for the specific genes *hsp83, hsp70, hsp60,* and *hsp26* on agarose gels.

6.11.1 Role of *hsp* 83 in *Drosophila* after PET microplastic exposure

hsp 83 was significantly overexpressed in the 10, 20 and 40 g/L groups compared with the control group. The percentages of genes expressed were 13.4%, 13.8%, 16.3%, 20.43%, and 35.7%, respectively. The fold change normalized to β-actin was determined, as shown in Figure 6.23, for the control, vehicle control, and 10, 20, and 40 g/L PET groups (0.8 ± 0.02, 0.9 ± 0.01, 1.38 ± 0.03, 1.88 ± 0.04, and 2.88 ± 0.01, respectively).

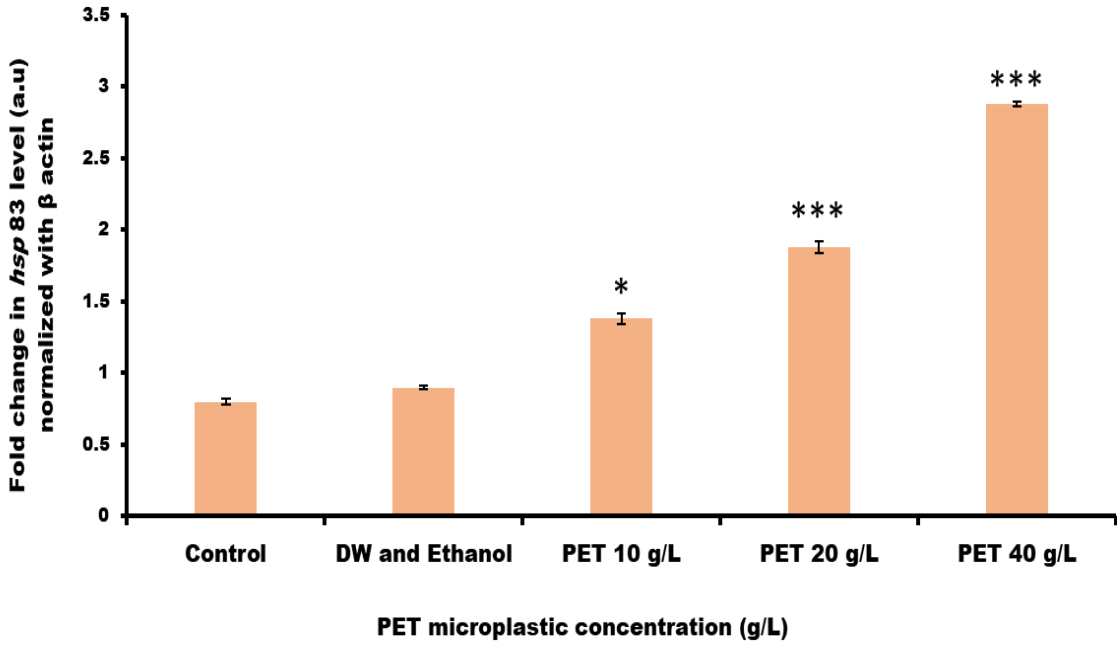

Figure 6.23: The graph shows the fold change in the gene expression level of *hsp 83* normalized to that of β actin, and statistical significance was assigned as * $p < 0.05$ and *** $p < 0.001$ for *hsp83* in comparison to the group I.

6.11.2 Role of *hsp 70* in *Drosophila* after PET microplastic exposure

Compared with that in the control group, *hsp 70* was significantly overexpressed at 20 and 40 g/L. The percentages of genes expressed were 8.7%, 10%, 18.41%, 20.5%, and 42.5%, respectively. The fold change normalized to β-actin was determined, as shown in Figure 6.24, for the control, vehicle control, and 10, 20, and 40 g/L PET groups (0.77 ± 0.02, 0.8 ± 0.01, 1.00 ± 0.02, 1.03 ± 0.01, and 1.92 ± 0.01, respectively).

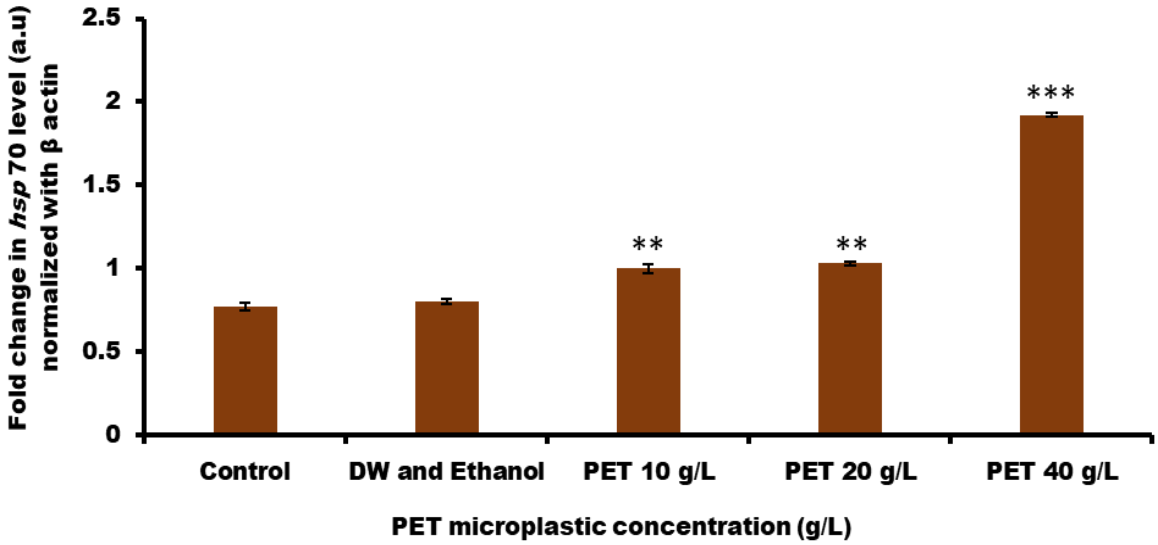

Figure 6.24: The graph shows the fold change in the gene expression level of *hsp 70* normalized to that of β actin, and statistical significance was assigned as *** $p < 0.001$ and ** $p < 0.01$ for *hsp 70*.

6.11.3 Role of *hsp 60* in *Drosophila* after PET microplastic exposure

Compared with that in the control group, *hsp* 60 was significantly downregulated at 20 and 40 g/L. The percentages of genes expressed were 39.0%, 25.4%, 13.19%, 12.53%, and 9.9%, respectively. The fold change normalized to β-actin was determined, as shown in Figure 6.25, for the control, vehicle control, and 10, 20, and 40 g/L PET groups (1.28 ± 0.03, 1.23 ± 0.01, 0.63 ± 0.02, 0.45 ± 0.02, and 0.49 ± 0.01, respectively).

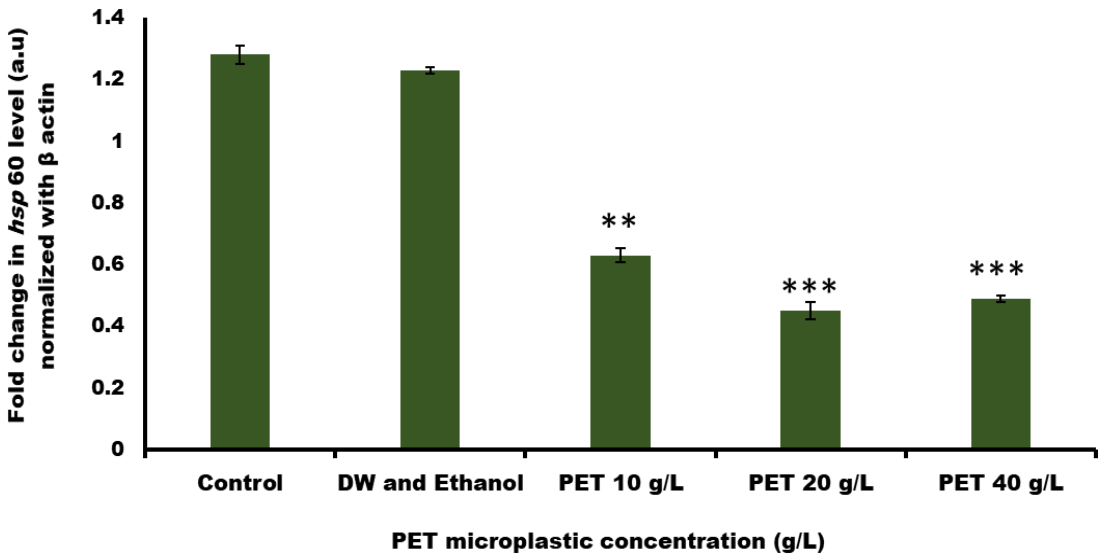

Figure 6.25: The graph shows the fold change in the gene expression level of *hsp 60* normalized to that of β actin, and statistical significance was assigned as *** $p < 0.001$ and ** $p < 0.01$ for *hsp 60*.

6.11.4 Role of *hsp 26* in *Drosophila* after PET microplastic exposure

hsp 26 did not significantly differ between the control and PET-treated groups. The percentages of gene expression were 20%, 19%, 21%, 20%, and 20%. The fold change normalized to β-actin was determined, as shown in Figure 6.26, for the control, vehicle control, and 10, 20, and 40 g/L PET groups (0.42 ± 0.01, 0.44 ± 0.03, 0.42 ± 0.02, 0.42 ± 0.01, and 0.44 ± 0.02, respectively).

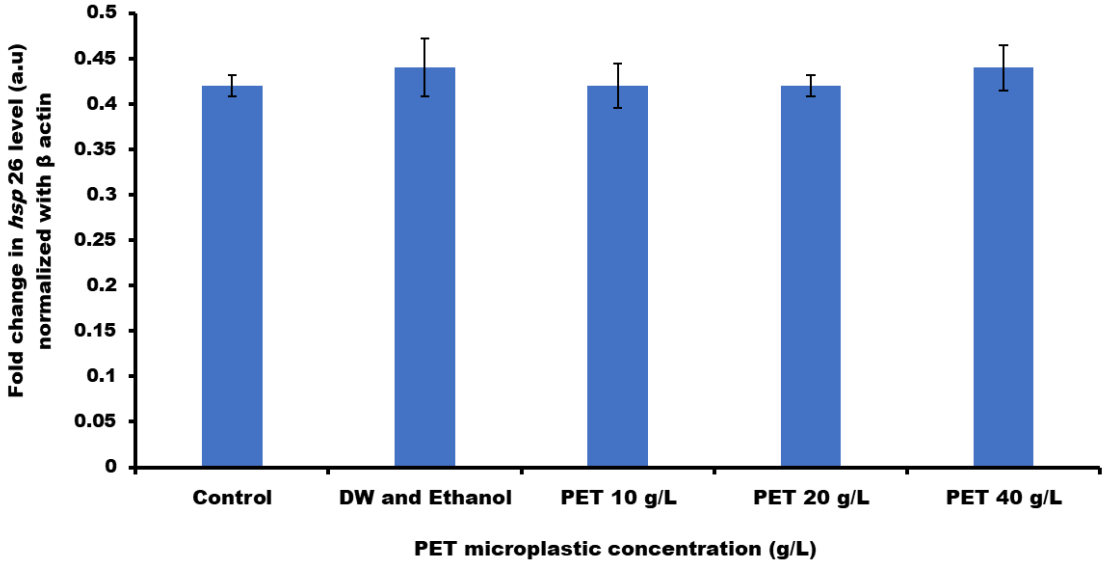

Figure 6.26: The graph shows the fold change in the gene expression level of *hsp* 26 normalized to that of *β* actin.

CHAPTER 7

DISCUSSION AND SUMMARY

Microplastics may enter the human body through various different channels, such as contaminated food and drink intake, inhalation of airborne particles, and contact consumer objects containing microplastics (Yee et al., 2021). Although the effects of microplastics on human health are yet unknown, there are serious worries. When consumed, hazardous compounds carried by microplastics might be dangerous (Horton et al., 2018). These substances may promote inflammation, interfere with endocrine function, or have other negative health consequences. Furthermore, the actual presence of microplastics in human tissues may exacerbate health problems by causing localized inflammation or damage (Anik et al., 2021). There may be financial repercussions from the pervasive existence of microplastics. Microplastic pollution of water bodies and ecosystems can have detrimental effects on industries, including tourism, aquaculture, and fisheries (Huang et al., 2021). Economic systems and lives reliant on these resources may be impacted by declining fish populations and impaired seafood safety. Furthermore, governments and communities may face increased financial hardship as a result of the high expense of removing pollutants from the environment and putting preventative measures in place (Hale et al., 2020). Knowing how microplastics affect the environment and human health can help advance the creation of safer, more sustainable substitute materials (Borriello et al., 2023). Additionally, it may promote the development of waste management technology that can collect and recycle plastics more successfully, limiting their discharge into the environment. Critical issues in contemporary biology and environmental science are addressed by research on heat shock proteins and microplastic toxicity (Sharma et al., 2022). The study of HSPs has advanced our knowledge of disease processes, cellular stress responses, and possible treatment modalities. The widespread and detrimental effects of plastic pollution on ecosystems and human health are highlighted by microplastic studies (Ribeiro et al., 2019). When combined, these academic disciplines offer perspectives that are critical for tackling environmental health issues and creating long-term solutions for upcoming problems.

Different aquatic model organisms have been used to investigate the effects of microplastics, such as the freshwater crustacean *Daphnia magna,* which is frequently employed in ecotoxicology because of its easy culture and sensitivity to contaminants (Mondellini et al., 2022). Microplastics are easily consumed by *Daphnia* and can

accumulate in their digestive tract, impairing development and feeding efficiency (Renzi et al., 2019). Reproductive success has been associated with microplastic exposure; populations exposed to plastic have been shown to have fewer offspring and to reproduce later. The swimming behavior of *Daphnia* may be altered by microplastics, increasing their susceptibility to predators (An et al., 2024). The soil-dwelling nematode *C. elegans* is employed in developmental and genetic biology. Because of its short lifespan and well-mapped genome, it is a great model for researching the molecular impacts of toxicology (Schopfer et al., 2020). Microplastic exposure causes oxidative stress, which escalates the generation of (ROS) and causes related harm. Research has demonstrated that microplastics can alter stress-related gene expression and damage DNA (Bhagat et al., 2021). Microplastics exposure has been linked to shorter lifespans, altered development, and decreased fertility. Because of their transparent embryos and quick growth, *Danio rerio* (zebrafish) are frequently utilized because of their toxicity and developmental biology (Singh et al., 2021). Microplastics can disrupt embryonic development, resulting in abnormalities, decreased hatching success rates, and altered growth patterns (Yin et al., 2021). Neurological development and function are impacted by microplastic exposure, which may result in behavioral abnormalities and cognitive impairments. Research on zebrafish has shown that microplastics may accumulate in their tissues and perhaps make their way into the food chain (Rojoni et al., 2024).

For our research, we selected a terrestrial nontargeted model organism to correlate the effects of PET microplastics on terrestrial organisms at the genetic level. To investigate the effects of PET microplastics on brain-related activities, studies were conducted using pure PET samples. Characterizing the size, shape, elements, and chemical structure of PET microplastics is crucial for understanding their properties. The FTIR technique was employed to analyze the characteristics of the PET microplastics and to identify the functional groups present. We also explored the catalytic interaction between enzymes and substrates, as well as the bioactive substances covalently bound to microplastics. Our FTIR analysis, which was supported by a previous study (Alaraby et al., 2023), confirmed the affiliation of these particles with PET. SEM and EDS analyses were conducted to examine the morphological texture and element configuration of the PET microplastics. SEM revealed irregular particles of microplastics with varying diameters, with some particles adhering to one another. The size of the particles was measured via

ImageJ software and was found to be between 2 and 100 µm. EDS analysis revealed that the PET microplastics contained 53.65% carbon, 42.42% oxygen, and 3.93% wollastonite. Considering that ingestion is the primary exposure route for microplastics, we conducted further experiments after determining their accumulation in the body. Our study focused on the behavioral activity of *Drosophila* after exposure to PET microplastics at different concentrations for different durations. Behavior can shed light on an organism's physiological processes (Singh et al., 2022), and in the case of *Drosophila*, their climbing and jumping abilities provide insights into their overall health. We observed significant impairments in locomotor activity in *Drosophila* exposed to PET microplastics at concentrations of 20 g/L and 40 g/L. These impairments were characterized by an inability to synchronize leg motions, resulting in the insects being stuck at the bottom of the plastic cylinder. The observed locomotor deficits could also be associated with increased energy demands in the mitochondria-rich muscles required for locomotion and flight. Uncoupled mitochondrial mechanisms and severe complex I inhibition have been linked to such locomotor difficulties, which could also be a reason for behavioral deformities (Shabir et al., 2022). Our results also support previous studies (Sharma and Hasan, 2020) that associated dopamine deficits with mobility disabilities because dopaminergic neurons play a major role in the behavior and locomotion of flies, as shown in Figure 7.1. This schematic representation suggests that disruption of neurons may be the cause of their impaired locomotion. Additional research is needed to determine whether the accumulation of PET microplastics can disturb dopaminergic neurons, thereby altering the physiological processes of flies. Another study, which was conducted on zebrafish, reported that the function of dopaminergic neurons was associated with behavior and locomotion activities (Xi et al., 2010).

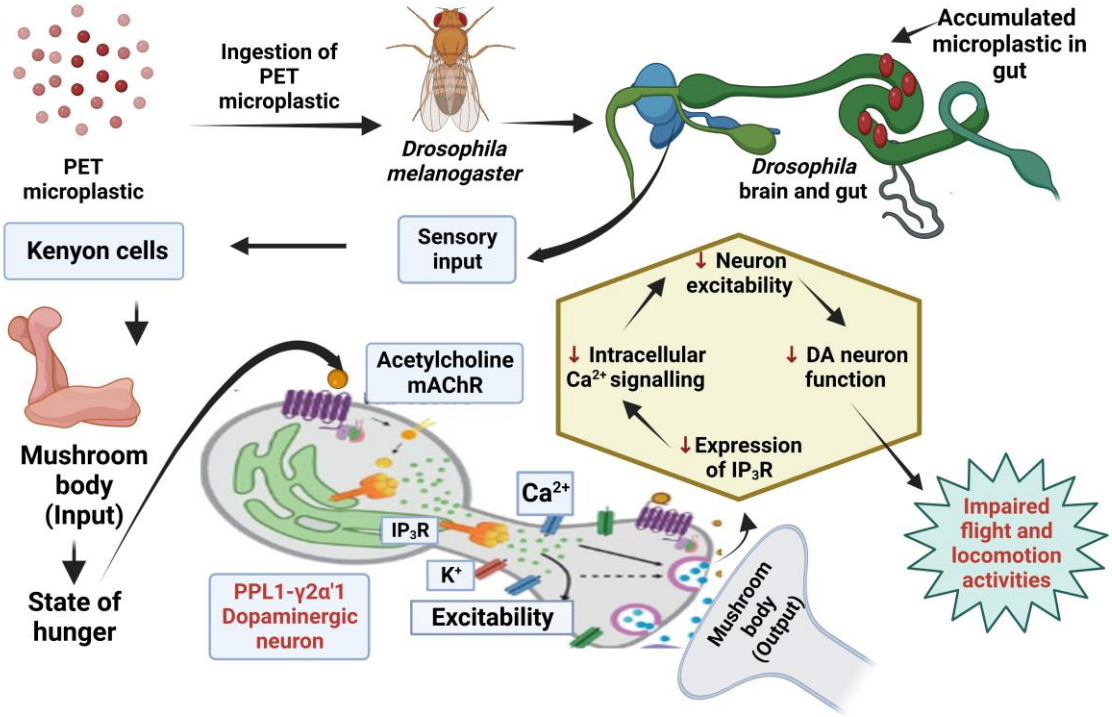

Figure 7.1. Schematic illustration of PET microplastic accumulation in *Drosophila* and its possible effects on the function of dopaminergic neurons, which could be responsible for fly behavior and locomotion. DA—dopaminergic, Ca—calcium ions, K—potassium ions.

Similar to the findings in other organisms, our study revealed a decrease in crawling speed in *Drosophila* larvae exposed to PET microplastics compared with their normal crawling speed after being exposed to high concentrations of PET microplastics. Previous studies (Yin et al., 2019) reported decreased swimming activity in *Sebastes schlegeli*, which supported our findings. Moreover, exposure to microplastics in *C. elegans* has been shown to inhibit acetylcholinesterase function and alter neurotransmitter levels, leading to behavioral abnormalities (Prust and Westerink, 2020). In addition to locomotor impairments, we observed delayed development, which refers to a decrease in the emergence percentage of *Drosophila* in groups after exposure to high doses. This decrease could be attributed to the disruption of vital processes during early development caused by exposure to ROS (Zhu et al., 2022). ROS exposure during organogenesis can lead to delayed or abnormal development and can subsequently delay emergence (Stenvall et al., 2011). However, the impact of ROS on development are complex and context dependent. While excessive ROS can be harmful,

they also play a role in signaling processes that regulate normal development (Yang et al., 2010). The timing of emergence may be determined by the delicate balance between ROS-mediated signaling and oxidative stress (Lozinsky et al., 2013). To support this study, previous studies (Cole et al., 2015; Tongo and Erhunmwunse, 2022) reported that exposure to polystyrene microplastic copepods and brown shrimp delayed development. *Drosophila* is a crucial decomposer species**,** playing a role in organic matter recycling. Microplastic exposure affects larval development and feeding behavior, potentially disrupting nutrient cycling in ecosystems. Similar impacts in soil invertebrates (e.g., earthworms) could affect soil health, microbial diversity, and plant growth. Our study demonstrated that the lifespan of female *Drosophila* decreased after consumption of high concentrations of PET microplastics, but for male flies, there was no effect on the lifespan. Another study (Singh et al., 2010) also revealed changes in the life span of *Drosophila* after the consumption of microplastics. Previous studies have shown that the mortality of the marine species *Clarias gariepinus* (African catfish) increases by 10% after being exposed to a high concentration (2 g/L) (Yu et al., 2018). Our findings provide preliminary information for future research on the impact of PET microplastics on *Drosophila*. The impaired locomotion and negative effects on development and life span following plastic consumption could be related to neurological signaling defects and genetic conditions, such as maternal effects, changes in segmentation, or homeotic genes; therefore, further research regarding molecular and cellular aspects is needed to determine the reasons for these challenges considering the findings of this research. Exposure to microplastics has been linked to oxidative stress and neurodegeneration in *Drosophila*, leading to impaired locomotor activity and learning defects. Given the conservation of neurobiological pathways between *Drosophila* and humans, this raises concerns about potential neurological disorders such as Alzheimer's and Parkinson's disease due to chronic microplastic exposure. According to an available study (Song et al., 2019), snails subjected to extended exposure to PET microfibers exhibited a reduction in food consumption and excretion, resulting in the impairment of villi in the stomach and intestine. Moreover, it has been shown that this particular type of intestinal injury has the potential to diminish nutritional absorption in *Daphnia* (Kim et al., 2021). In *Drosophila*, gastrointestinal injury was also observed after polystyrene microplastic exposure (Zhang et al., 2020). The studies have demonstrated that microplastics

accumulate in the gut, hemolymph, and fat body, leading to systemic inflammation and tissue damage. In humans, prolonged ingestion of microplastics through food and water may cause intestinal inflammation, immune dysregulation, and metabolic disorders. It also serves as prey for birds, amphibians, and other insects. If microplastics bioaccumulate in their tissues, they could transfer pollutants up the food chain, leading to toxicity in higher organisms, including humans. Oxidative stress is one of the outcomes linked to exposure to microplastics (Ferrante et al., 2022). These findings are in agreement with previous research (Yu et al., 2018) showing that after the accumulation of polystyrene microplastics, oxidative stress increases within the liver of *Eriocheir sinensis*; moreover, antioxidant enzyme activities in *Brachionus calyciflorus* change after being exposed to polystyrene microplastics, and increased SOD and CAT activities are observed. The reproductive health of flies has been found to be impaired, which is a novel topic for future research (Liang et al., 2021). In female flies, cellular toxicity in the mature follicles of the ovaries may be responsible for changes in or effects on reproductive health because several variables may be connected to this toxicity, such as female fertility, fecundity, the egg distribution chamber, embryo development, yolk protein-encoding gene expression, meiotic crossing over, and maternal protein localization (Vimal et al., 2019). In male flies, toxicity occurring in the accessory gland and testis could also lead to changes in sperm morphology, sperm formation, sperm count, courtship behavior, and sperm motility (Tiwari et al., 2011). For the first time, a recent study revealed the presence of PVC, PS, and PE microplastics in testis and semen samples of human beings (Zhao et al., 2013). The accumulation of polystyrene microplastics in *Danio rerio* (zebrafish) decreased the fertility rate and severely affected gonad morphology (Gupta et al., 2023). Oral ingestion of microplastics in mice leads to infertility, a reduction in fertilization, and embryo development; furthermore, during the gestation and lactation periods, exposure to microplastics results in transgenerational microplastic reproductive toxicity (Zhang et al., 2023). The hazardous effects that were determined by our research on reproductive health could cause alterations in the expression of genes that are responsible for reproduction, so further research at the molecular level using transcriptomic analysis or any scientific method is needed to determine the variation in genes. When organisms consume microplastics, they can accumulate and be passed on to their offspring, leading to transgenerational or

multigeneration toxicity (Li et al., 2022), as shown in the schematic representation in Figure 7.2

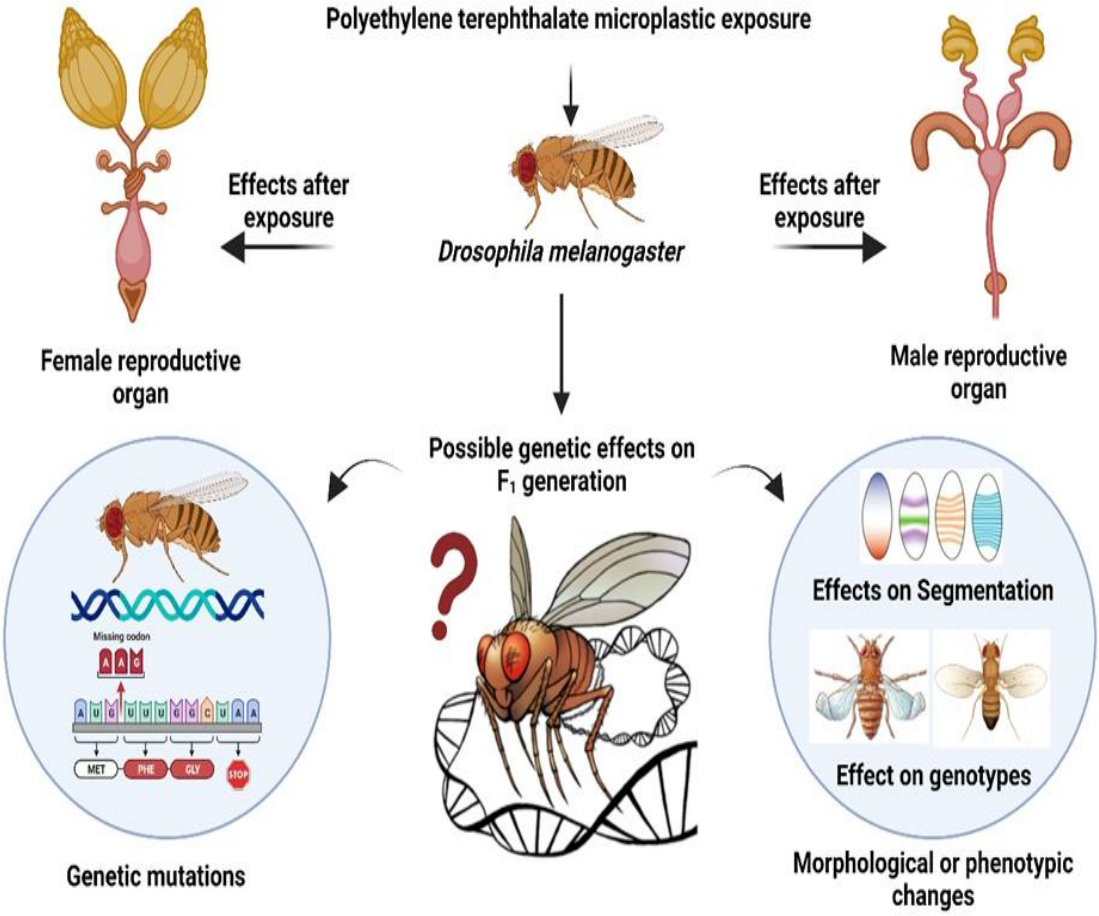

Figure 7.2. Schematic representation of possible transgenerational effects on *Drosophila melanogaster* after exposure to PET microplastics.

Life cycle assessments indicate that reproductive health alterations and toxicity may manifest during various stages, such as gamete production, embryogenesis, hatching, secondary maturation, or the transformative processes involved in these crucial biological activities (Chen et al., 2023). A study revealed that the presence of polyethylene microplastics in the soil at a concentration of 0.5% resulted in a 70% decrease in earthworm reproduction for both the parent (F0) and first filial (F1) generations compared with soil without microplastic contamination. Additionally, notable DNA damage was identified in the F0 generations after a 28-day period (Sobhani

et al., 2022). *Drosophila* studies have shown that microplastics can induce reproductive toxicity, leading to decreased fecundity, altered sperm morphology, and reduced embryonic development. These effects are comparable to findings in mammals and suggest potential risks of microplastic exposure on human fertility and fetal development. *Drosophila* indicate that microplastics, especially those containing endocrine-disrupting chemicals (EDCs) like phthalates and bisphenols, can alter hormone signaling. In humans, similar endocrine disruptions may contribute to metabolic disorders, obesity, thyroid dysfunction, and reproductive anomalies. At the molecular level, the expression of the *hsp83* and *hsp70* genes was upregulated. Similarly, the upregulation of *hsp 70* expression was detected in the giant river prawn *Macrobrachium rosenbergii* subjected to microplastics composed of polystyrene and polyethylene (Jaikumar et al., 2021). The heat shock response facilitated by heat shock transcription factor 1 (HSF1) is impeded by a mixture of di-(2-ethylhexyl) phthalic acid (DEHP) and polypropylene microplastics (Yang et al., 2023); this combination induces neurotoxicity in immature mice via neuronal apoptosis and neuroinflammation. While many studies have examined the effects of different microplastics and stress genes, the precise HSP that serves as an early indicator of cellular redox changes and can be used to detect microplastic toxicity remains uncertain. However, our research has now produced conclusive results at the gene level regarding this aspect. Elevated levels of HSPs are a defensive reaction to stress (Ciocca et al., 2005), and moreover, *hsp26*, *hsp90,* and *hsp70* are stress-inducible HSPs that have been the subject of extensive research (Tausif et al., 2024). Previous studies have demonstrated that malignancy is characterized by a substantial increase in the expression and activity of these chaperones, which are also susceptible to various stimuli that induce cell death (Somu et al., 2024). Under normal conditions, HSF-1, a transcription factor, is retained in the cytoplasm by HSPs such as *hsp 90* and *hsp 70*, which bind to HSF1, preventing its transcriptional activity (Verma et al., 2021). However, according to our study, we believe that the mechanism for stress gene regulation in response to stress induced by PET microplastics is as follows: HSPs detach from this complex, activating HSF1. Once activated, HSF1 moves into the nucleus and binds to specific sequences called heat shock elements (HSEs) located upstream of heat shock gene promoters, thus initiating the transcription of HSP genes, as illustrated in Figure 7.3. Our study sheds light on the toxicity of

microplastics through the involvement of different HSP genes in defense mechanisms against PET microplastics. Ultimately, our findings suggest that the overexpression of specific stress genes could indicate impending health threats from microplastics, serving as early indicators of toxicity (Somu et al., 2024). Numerous studies have highlighted metabolic disturbances, neurotoxicity, and increased cancer risk in humans following exposure to microplastics (Kuppuswami and Senthikumar, 2023). Whether the same stress gene functions as a stress biomarker for all forms of microplastics remains unknown; therefore, additional comparative research between various microplastics and stress genes is necessary to answer this question. Therefore, the present research should further investigate genotoxicity because when assessing the potential adverse consequences of environmental contaminants, genotoxicity has emerged as an essential biomarker. DNA damage is widely acknowledged for its profound influence on various health conditions, including gene and chromosome mutations, cancer development, and the aging process (Mohamed et al., 2017). Genotoxicity is commonly considered a reliable biomarker for carcinogenesis, as it is crucial in both the initiation and progression of cancer (Tagorti and Kaya, 2022). Despite the importance of biomarkers, few studies have assessed the possible genotoxic impacts of PET MP. The results of our study provide initial data that might serve as a foundation for further investigations into the effects of PET MP on *Drosophila*. Additional investigations are needed to explore the transgenerational impacts and ascertain the extent to which an organism's body is resistant to microplastic toxicity. If such resistance exists, it is crucial to understand the specific level at which it manifests and to elucidate the physiological or genetic changes that occur within the organism's body to facilitate this.

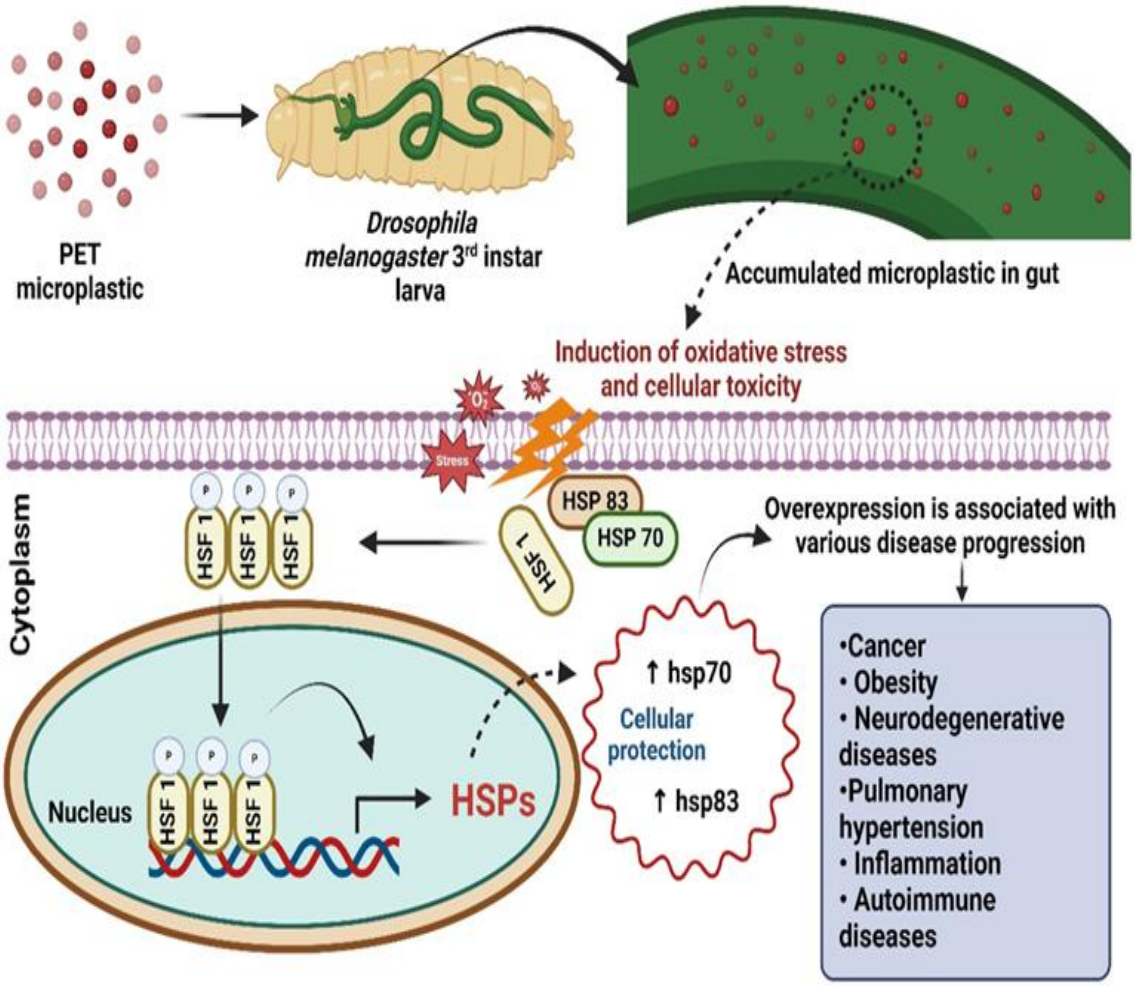

Figure 7.3. Schematic representation of the accumulation of PET microplastics and the role of heat shock proteins in promoting cellular protection for cell survival.

Overall, our research revealed that microplastics even affect nontarget organisms in many ways. The increased expression of *hsp 83* and *hsp 70* is associated with various diseases. At the genetic level, the increased expression of *hsp 83* in *Drosophila melanogaster* has important ramifications for both fundamental and applied biological research (Rokusek, 2023). The *Drosophila* counterpart of *hsp 90*, a well-researched chaperone protein involved in the stress response, stabilization, and folding of proteins, is called *hsp 83* (Liang et al., 2023). There are several major explanations for the importance of increasing *hsp 83* expressions in *Drosophila*. *hsp 83* has a protective role in cells against a range of stressors, such as oxidative stress, thermal shock, and environmental pollutants. Researchers can investigate how improved stress resistance impacts overall organismal health and survival by upregulating *hsp 83* expressions

(Rowinska et al., 2024). *Drosophila* is a well-researched model organism that is perfect for examining the molecular processes involved in the stress response. These investigations have provided insights that can be applied to comprehend comparable mechanisms in higher creatures, such as humans (Silvestro et al., 2023). A common feature of neurodegenerative illnesses such as Alzheimer's disease and Parkinson's disease is the aggregation of misfolded proteins, which can be prevented by upregulating *hsp 83* expressions. Potential treatment therapies can be screened, and these disorders can be studied via *Drosophila* models with high *hsp 83* levels (Abou-Shaara, 2024). The stabilization of several oncogenic proteins is facilitated by *hsp 83*. Research on the consequences of elevated *hsp 83* expressions in *Drosophila* can aid in the development of *hsp* -targeted treatments and shed light on the biology of cancer. *hsp 83* functions in several developmental stages. Researchers can study the effects of chaperone proteins on development and differentiation by varying their expression (Marta et al., 2024). Increased *hsp 83* levels have the potential to reduce aging-related effects by preserving protein homeostasis and minimizing cellular damage. Research on *Drosophila* can provide insight into how *hsp 83* functions throughout aging and possible ways to support healthy aging. Because *Drosophila* is genetically accessible, scientists may examine how *hsp83* interacts with other genes (Villada and Bedoya, 2024). This may reveal networks and processes related to protein homeostasis and the stress response. Elucidating the epigenetic regulation of *hsp 83* expression might help researchers better understand the more general regulatory processes governing gene expression under stressful circumstances (Rai, 2023). The activities of *hsp 83* in *Drosophila* and other animals may be compared to help researchers understand how stress response systems have evolved to be both diverse and evolutionarily conserved. Research on how *Drosophila*'s capacity to adapt to environmental changes is impacted by elevated *hsp 83* expressions might shed light on how organisms develop stress tolerance (Singh et al., 2024).

7.1 Significance of upregulated *hsp* levels in various diseases

7.1.1 Cancer

hsp 70 and Cancer:

Growth and Survival of Tumors: High expression of *hsp 70* is frequently observed in a number of malignancies, such as lung, prostate, and breast cancers. By stabilizing

oncoproteins and preventing apoptosis or programmed cell death, *hsp 70* aids in the survival of cancer cells (Alimardan et al., 2023).

Chemoresistance: Increased expression of *hsp 70* is linked to chemoresistance, which makes cancer cells more challenging to eradicate (Wong et al., 2023).

Prognostic marker: Aggressive tumor behavior and a poor prognosis are frequently correlated with high *hsp 70* expressions (Guliy et al., 2023).

hsp 90 in Relation to Cancer

Oncoprotein Stabilization: A variety of oncoproteins that promote the growth of cancer, such as HER2, BCR-ABL, and mutant p53, are stabilized and activated by *hsp 90*. The proliferation and survival of cancer cells are promoted by increased *hsp 90* expressions (Youssef et al., 2023).

Cancer Cell Proliferation: *hsp 90* promotes the proliferation of cancer cells by facilitating the proper folding and function of proteins involved in the cell cycle**.**

Targeting for Therapy: Because *hsp 90* inhibitors can degrade client oncoproteins and stop tumor growth, they are being developed as cancer therapies (e.g., derivatives of geldanamycin) (Basset et al., 2023).

7.1.2 Neurodegenerative conditions

Neurodegenerative Diseases and HSP70:

Alzheimer's disease: By stopping the aggregation of tau and amyloid-beta proteins, increased *hsp 70* expressions can be neuroprotective. On the other hand, aberrant *hsp 70* can also obstruct regular protein breakdown processes (Venediktov et al., 2023).

Parkinson's Disease: A crucial protein, misfolded alpha-synuclein, is facilitated by *hsp* 70. Although it has therapeutic potential, increasing *hsp 70* expression needs to be carefully controlled (Ghosh et al., 2023).

Huntington's disease: *hsp* 70 has the ability to reduce the toxicity of the aggregation of mutant huntingtin proteins, which may halt the course of Huntington's disease (Evgenev et al., 2023).

Neurodegenerative Diseases and HSP90:

Amyloid-beta and tau aggregation: Tau and amyloid precursor protein (APP) folding and stabilization are facilitated by *hsp 90*. In Alzheimer's disease, the buildup of misfolded proteins may be worsened by elevated *hsp* 90 expressions.

Synaptic function: *hsp* 90 is essential for preserving the plasticity and function of synapses. *hsp 90* dysregulation can deteriorate synaptic function and accelerate the course of neurodegenerative diseases.

7.1.3 Other medical conditions

Cardiovascular Diseases:

Atherosclerosis: By regulating inflammatory responses and cell survival, increased expression of *hsp 70* and *hsp 90* in smooth muscle and endothelial cells can aid in the development of atherosclerosis (Chiosis et al., 2023).

Ischemic Injury: By preventing apoptosis and promoting cell survival, increased *hsp 70* expressions can shield cardiac cells from ischemic injury (such as that which occurs after a heart attack) (Bahr et al., 2024).

inflammatory illnesses

Rheumatoid Arthritis: Elevated *hsp 70* expressions in rheumatoid arthritis patients' synovial tissues can influence inflammation and immunological responses, which in turn can influence the course of the illness (Kielbowski et al., 2024).

Inflammatory Bowel Disease: Prolonged overexpression of *hsp 70* may impact immunological responses and gut homeostasis, although elevated levels in the gut epithelium can shield against damage caused by stress (Su et al., 2024).

Infectious diseases:

Viral infections: Certain viruses require the host's *hsp 70* and *hsp 90* to aid in their reproduction. Thus, elevated HSP expression may promote the viral life cycle and worsen infections (Wongchitrat et al., 2024).

Bacterial infections: Some bacteria have the ability to cause host cells to produce HSP, which helps the germs survive and evade immunological reactions (Ugoala, 2023).

Therapeutic consequences

HSP Inhibitors: Research on the use of particular inhibitors to target *hsp 70* and *hsp 90* is ongoing in the context of neurological and cancer disorders. Clinical studies have demonstrated the potential of *hsp 90* inhibitors as cancer treatments (Zhao et al., 2023).

HSP-Inducing Agents: On the other hand, stimulating *hsp 70* expression may provide neuroprotection and impede the progression of disorders such as neurodegenerative disorders (Belenichev et al., 2023). Increased expression of *hsp 70 and hsp 83* has been linked to a number of illnesses, including neurological conditions and cancer (Kauts et

al., 2024). Comprehending the functions of these heat shock proteins in the etiology of diseases is essential for formulating focused treatment approaches (Jeyachandran et al., 2023). The activation of HSPs in response to genotoxic stress is one of the conserved cellular stress response mechanisms shared by humans and *Drosophila*. Owing to this conservation, the results obtained from *Drosophila* can be applied to humans (Fonseca-Carvalho et al., 2024). In genetic and toxicological research, *Drosophila* is a well-respected model organism. The knowledge gathered from research on *Drosophila* HSP responses to microplastic-induced genotoxicity can help us comprehend comparable human mechanisms (Sanders et al., 2023). Research on *Drosophila* has demonstrated that exposure to microplastics can result in chromosomal abnormalities and an increase in DNA strand breaks, two indicators of DNA damage (Aloisi et al., 2024). *Drosophila* exhibit increased production of HSPs, including *hsp 70 and hsp 90*, in response to this genotoxic stress. These HSPs aid in damage management and protection of cellular integrity. Microplastics can enter the human body through the mouth (with food and water) or the respiratory system (with airborne particles). The genotoxic effects observed in *Drosophila* point to comparable concerns for human DNA damage (Dahleh and Prigol, 2023). The observation that HSPs are upregulated in *Drosophila* in response to genotoxicity generated by microplastics suggests that a comparable defense mechanism may be triggered in humans (Mukherjee et al., 2024). This reaction is essential for preventing DNA damage and preserving the integrity of cells. HSPs may be used as biomarkers to identify and track human exposure to microplastics and their genotoxic consequences (Kauts et al., 2024). Comprehending the ability of HSPs to react to genotoxicity caused by microplastics might help in the development of therapies that improve cellular resistance, possibly by modifying HSPs (Panizzolo et al., 2023).

7.2 Potential mitigation strategies and applications

Governments and regulatory bodies should enforce restrictions on the production and sale of single-use plastics, including plastic straws, cutlery, and packaging. Encouraging alternatives such as biodegradable materials and compostable plastics can significantly reduce plastic waste. Manufacturers should focus on developing sustainable packaging and reducing unnecessary plastic components in consumer products. The use of natural

fiber-based textiles can also help reduce microplastic shedding from synthetic fabrics (Mclaughlin et al., 2022). Encourage responsible consumer behavior public awareness campaigns should educate individuals on reducing plastic use, opting for reusable products, and making eco-conscious purchasing decisions. Efficient waste segregation and recycling infrastructure can prevent plastic materials from breaking down into microplastics. Establishing incentive-based recycling programs can encourage higher participation rates in plastic waste management (Kutralam-Muniasamy et al., 2024). Microplastics from personal care products, laundry wastewater, and industrial discharge can be effectively removed by advanced filtration systems such as membrane bioreactors, nano-filters, and activated carbon treatment. Implementing these technologies in wastewater treatment plants can prevent microplastics from entering aquatic ecosystems. Installing microfiber filters in washing machines and promoting the use of washing bags can minimize the release of microplastics from synthetic fabrics into water systems. Certain microorganisms and enzymes have shown potential in breaking down plastic polymers. Biotechnological advancements in microbial plastic degradation could offer a sustainable solution for microplastic accumulation (Auta et al., 2017). Developing biodegradable and compostable polymers derived from natural sources, such as PHA (Polyhydroxyalkanoates) and PLA (Polylactic Acid)**,** can provide a sustainable alternative to conventional plastics. Nanotechnology-Based Filtration Systems**,** incorporating nanoparticle-based adsorbents and filtration membranes in industrial effluent treatment can effectively trap microplastics before they reach the environment. Governments should establish stringent policies on plastic production, disposal, and microplastic emissions. Implementing extended producer responsibility (EPR) programs can hold manufacturers accountable for the environmental impact of their products. Establishing global monitoring frameworks for microplastic contamination in air, water, and soil can help track pollution levels and ensure compliance with environmental safety standards (Limb, 2021**).** Developing Sustainable Alternatives**,** research institutions and industries should invest in eco-friendly material innovations that replace conventional plastic products. More research is needed to understand the long-term effects of microplastics on human health, wildlife, and ecosystem stability**,** leading to data-driven mitigation strategies**.**

CHAPTER 8

CONCLUSION

In general, our research indicated that the toxicity of PET MPs is dose dependent. Specifically, we observed that an increase in plastic concentration corresponds to a heightened toxicological impact. At elevated concentrations, PET MPs induced significant cytotoxicity, oxidative stress, and reproductive damage in the model organism *Drosophila melanogaster*. The behavior, locomotion, development, and life span (female) of flies are adversely affected. Furthermore, our study also determined the expression of stress gene biomarkers responsible for microplastic-induced toxicity. With respect to the population, our research results suggest that a certain plastic concentration is permissible for use. Nevertheless, owing to the pervasive nature of plastic in our everyday existence, it is imperative that we not completely dismiss its significance. Nevertheless, when the concentration is above a certain level, it becomes a matter of significant apprehension. One important response of organisms to the toxicity of microplastics is increased expression of HSPs. This defense system helps reduce the harmful effects of inflammation, oxidative stress, and damage caused by microplastics. Comprehending this correlation is vital in evaluating the influence of microplastics on the well-being of organisms and formulating approaches to address environmental contamination and its biological effects. The analysis of the underlying toxicity suggested that plastic has a persistent and incremental impact on daily life. Furthermore, investigating how microplastics impact the physiology of insects is crucial. This is because insects are considered potential natural plastic degraders. Recent research using advanced genetic techniques has suggested that *Drosophila melanogaster* is served as a model organism for the development of highly degrading insects. The findings of this study provide valuable insights into the significant consequences of microplastic contamination and emphasize the urgent need for the implementation of efficient mitigation measures. Hence, more investigations are necessary to assess the impact of microplastics at the molecular level. Studying the genotoxicity of microplastics in *Drosophila* offers important information about possible threats to human health, especially when considering heat shock protein reactions. These findings are relevant because stress response mechanisms in humans and *Drosophila* are conserved. Researchers can attain a better understanding of the health consequences of micro and nano plastic exposure for humans by examining the processes underlying HSP overexpression and its protective effects in *Drosophila*. This will facilitate the creation

of more effective risk assessments, biomarkers, and treatment approaches. While the research establishes a baseline for understanding the physiological and ecological risks associated with microplastics, effective mitigation requires multifaceted environmental, policy, and industrial interventions**.** Countries that have enforced bans on plastic bags and microbeads have reported a significant reduction in microplastic pollution. Expanding such regulations to include synthetic fibers from textiles and industrial plastic discharge can further mitigate contamination. Governments and health organizations should establish exposure thresholds for microplastics in drinking water and food. Further studies should investigate biomarkers of microplastic exposure and their correlation with chronic diseases. Educating the public on plastic alternatives, dietary choices, and proper waste disposal can help mitigate exposure risks. Textile and packaging industries should invest in low-shed materials and microplastic capture technologies to minimize environmental discharge. Engineering high-efficiency microplastic filters for water treatment facilities and household appliances (e.g., washing machines) can significantly reduce microplastic emissions at the source. By integrating scientific findings with policy frameworks, industrial innovation, and public health initiatives, we can work toward a sustainable and microplastic-free future**.**

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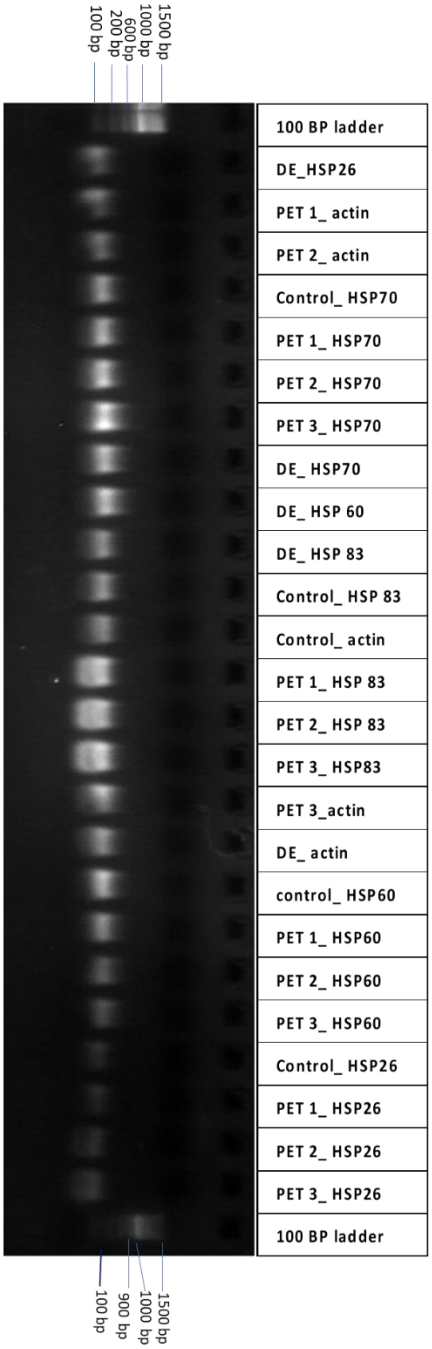
APPENDICES

RESEARCH PUBLICATIONS AND CONFERENCES

Supplementary Data



Full gel image RT-PCR

PUBLICATIONS

Title: - The evidence of in-vivo and in-vitro studies on microplastic and nano plastic toxicity in mammals: A possible threat for an upcoming generation?

Reference: - Kauts, S., Shabir, S., Yousuf, S., Mishra, Y., Bhardwaj, R., Milibari, A. A., ... & Singh, M. P. (2023). The evidence of in-vivo and in-vitro studies on microplastic and nano plastic toxicity in mammals: A possible threat for an upcoming generation? *Physics and Chemistry of the Earth, Parts A/B/C*, 103511.

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The evidence of *in-vivo* and *in-vitro* studies on microplastic and nano plastic toxicity in mammals: A possible threat for an upcoming generation?

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ABSTRACT

Plastic pollution is a pressing global environmental concern, exerting significant impacts on both aquatic and terrestrial ecosystems in recent decades. The detrimental effects of plastic on various species within these habitats have raised serious alarm. Regrettably, human exposure to microplastics (MPs) and nanoplastics (NPs) through contaminated food has become unavoidable, but the comprehensive extent of their consequences remains largely unexplored. This review aims to advance existing research and conduct a comprehensive evaluation of current studies pertaining to the potential toxicity and negative impacts of micro- and nano-plastics on soil, water, and land, with a specific focus on their implications for human health. Through meticulous examination of the existing literature, we aim to provide a succinct overview of the potential risks associated with micro and nano plastics, thereby guiding and emphasizing the direction for future research in this domain and addressing critical knowledge gaps. The study underscores that extant research has established a clear association between micro and nano plastics and their adverse effects on the digestive system, cell lines, and reproductive health. Nevertheless, deeper investigations are imperative to achieve a better understanding of the cellular, molecular, neurological, and systemic toxicity caused by these minute plastic particles. Information presented in the review provides a scientific understanding of the hazards posed by micro and nano plastics to both the environment and human health is essential for formulating effective mitigation strategies and regulatory measures.

1. Introduction

Plastic pollution in the environment is receiving a lot of attention currently and inappropriate unloading of discarded or abandoned plastic waste pollutes the environment (Pinto et al., 2019). Since the 1940s, when the widespread use of plastics in fields as diverse as industry, agriculture, medicine, and consumer goods emerged, the plastics sector has grown exponentially (Peng et al., 2022). It's not unexpected that people are being exposed to microplastics (anything less than 5 mm in size) since they are pervasive environmental pollutants found in water and food (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016). Secondary microplastics, such as those released from strewn plastic trash, are the consequence of the breakdown of primary microplastics, which are manufactured purposely for technical uses such as abrasive in personal care items (Andrady, 2017). The removal of household wastewater discharge, sewage ooze garbage, and plastic waste from agrarian soils is a major challenge for natural decontamination (Chae and An, 2018). Plastic is widely utilized due to its exceptional utility in facilitating the convenient transportation of daily essential items for individuals. Its practicality stems from its versatility, durability, and lightweight properties, which make it an ideal material for various applications in modern life. (Mafuta et al., 2021). Once in the

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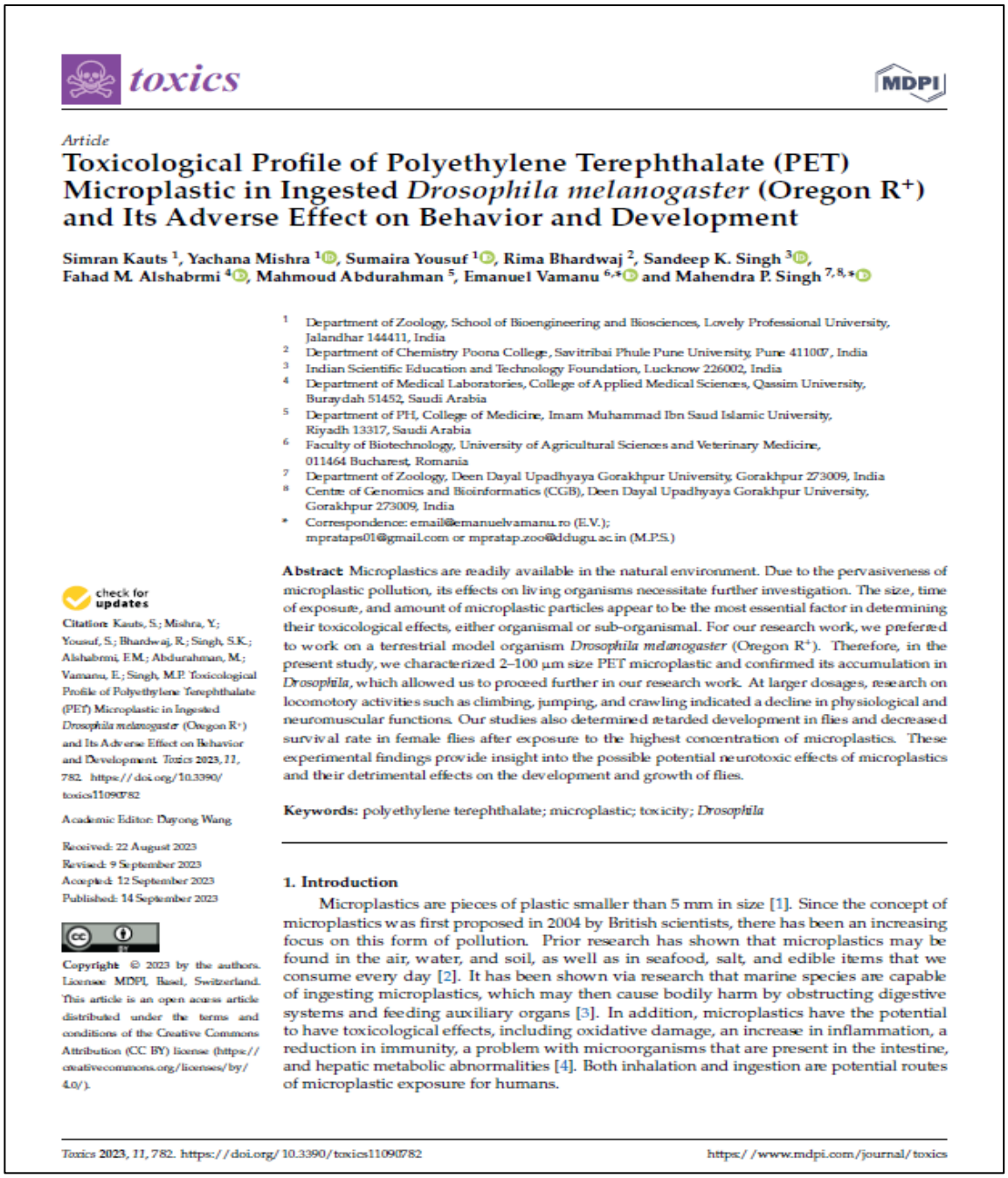
Available online 14 November 2023

Title: - Toxicological Profile of Polyethylene Terephthalate (PET) Microplastic in Ingested *Drosophila melanogaster* (Oregon R+) and Its Adverse Effect on Behavior and Development

Reference: - Kauts, S., Mishra, Y., Yousuf, S., Bhardwaj, R., Singh, S. K., Alshabrmi, F. M., ... & Singh, M. P. (2023). Toxicological Profile of Polyethylene Terephthalate (PET) Microplastic in Ingested *Drosophila melanogaster* (Oregon R+) and Its Adverse Effect on Behavior and Development. *Toxics*, *11*(9), 782.



toxics

MDPI

Article

Toxicological Profile of Polyethylene Terephthalate (PET) Microplastic in Ingested *Drosophila melanogaster* (Oregon R+) and Its Adverse Effect on Behavior and Development

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Abstract: Microplastics are readily available in the natural environment. Due to the pervasiveness of microplastic pollution, its effects on living organisms necessitate further investigation. The size, time of exposure, and amount of microplastic particles appear to be the most essential factor in determining their toxicological effects, either organismal or sub-organismal. For our research work, we preferred to work on a terrestrial model organism *Drosophila melanogaster* (Oregon R^+). Therefore, in the present study, we characterized 2–100 µm size PET microplastic and confirmed its accumulation in *Drosophila*, which allowed us to proceed further in our research work. At larger dosages, research on locomotory activities such as climbing, jumping, and crawling indicated a decline in physiological and neuromuscular functions. Our studies also determined retarded development in flies and decreased survival rate in female flies after exposure to the highest concentration of microplastics. These experimental findings provide insight into the possible potential neurotoxic effects of microplastics and their detrimental effects on the development and growth of flies.

Keywords: polyethylene terephthalate; microplastic; toxicity; *Drosophila*

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Citation: Kauts, S.; Mishra, Y.; Yousuf, S.; Bhardwaj, R.; Singh, S.K.; Alshabrmi, F.M.; Abdurahman, M.; Vamanu, E.; Singh, M.P. Toxicological Profile of Polyethylene Terephthalate (PET) Microplastic in Ingested *Drosophila melanogaster* (Oregon R+) and Its Adverse Effect on Behavior and Development. *Toxics* **2023**, *11*, 782. https://doi.org/10.3390/toxics11090782

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1. Introduction

Microplastics are pieces of plastic smaller than 5 mm in size [1]. Since the concept of microplastics was first proposed in 2004 by British scientists, there has been an increasing focus on this form of pollution. Prior research has shown that microplastics may be found in the air, water, and soil, as well as in seafood, salt, and edible items that we consume every day [2]. It has been shown via research that marine species are capable of ingesting microplastics, which may then cause bodily harm by obstructing digestive systems and feeding auxiliary organs [3]. In addition, microplastics have the potential to have toxicological effects, including oxidative damage, an increase in inflammation, a reduction in immunity, a problem with microorganisms that are present in the intestine, and hepatic metabolic abnormalities [4]. Both inhalation and ingestion are potential routes of microplastic exposure for humans.

Title: - Impact of Polyethylene Terephthalate Microplastics on *Drosophila melanogaster* Biological Profiles and Heat Shock Protein Levels.

Reference: - Kauts, S., Mishra, Y., & Singh, M. P. (2024). Impact of Polyethylene terephthalate Microplastics on *Drosophila melanogaster* Biological Profiles and Heat Shock Protein Levels. Biology, 13(5), 293.

biology

MDPI

Article

Impact of Polyethylene Terephthalate Microplastics on *Drosophila melanogaster* Biological Profiles and Heat Shock Protein Levels

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Simple Summary: The threat posed by microplastic toxicity to organisms is growing substantially, and the ramifications of daily microplastic usage cannot be disregarded. The extent of toxicological research pertaining to microplastics has increased due to the grave and alarming nature of microplastic pollution. We have, therefore, conducted research in an effort to determine the toxicological impact of microplastics on the cellular and genetic levels. The toxicity of accumulated polyethylene terephthalate microplastics on *Drosophila melanogaster* has been determined by our research. At higher concentrations of microplastics, cellular and reproductive toxicities have been observed, which correspond to elevated oxidative stress, identified through the analyses of various oxidative stress markers' activities. Furthermore, the levels of heat shock proteins have been identified, contributing to the understanding of the primary defense mechanism against the toxicity of microplastics. The study has provided significant and concerning insights into the escalating health risks posed by microplastics. It appears that microplastics are inducing genetic alterations; therefore, further investigation should be undertaken at the genetic level to clarify the potential transgenerational consequences that pose a significant risk to future generations.

Abstract: Microplastics and nanoplastics are abundant in the environment. Further research is necessary to examine the consequences of microplastic contamination on living species, given its widespread presence. In our research, we determined the toxic effects of PET microplastics on *Drosophila melanogaster* at the cellular and genetic levels. Our study revealed severe cytotoxicity in the midgut of larvae and the induction of oxidative stress after 24 and 48 h of treatment, as indicated by the total protein, *Cu-Zn SOD*, *CAT*, and MDA contents. For the first time, cell damage in the reproductive parts of the ovaries of female flies, as well as in the accessory glands and testes of male flies, has been observed. Furthermore, a decline in reproductive health was noted, resulting in decreased fertility among the flies. By analyzing stress-related genes such as *hsp83*, *hsp70*, *hsp60*, and *hsp26*, we detected elevated expression of *hsp83* and *hsp70*. Our study identified *hsp83* as a specific biomarker for detecting early redox changes in cells caused by PET microplastics in all the treated groups, helping to elucidate the primary defense mechanism against PET microplastic toxicity. This study offers foundational insights into the emerging environmental threats posed by microplastics, revealing discernible alterations at the genetic level.

Keywords: *Drosophila*; toxicity; heat shock proteins; oxidative stress; cell damage; reproductive capacity; microplastic; environmentally relevant

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Citation: Kauts, S.; Mishra, Y.; Singh, M.P. Impact of Polyethylene Terephthalate Microplastics on *Drosophila melanogaster* Biological Profiles and Heat Shock Protein Levels. *Biology* **2024**, *13*, 293. https://doi.org/10.3390/biology13050293

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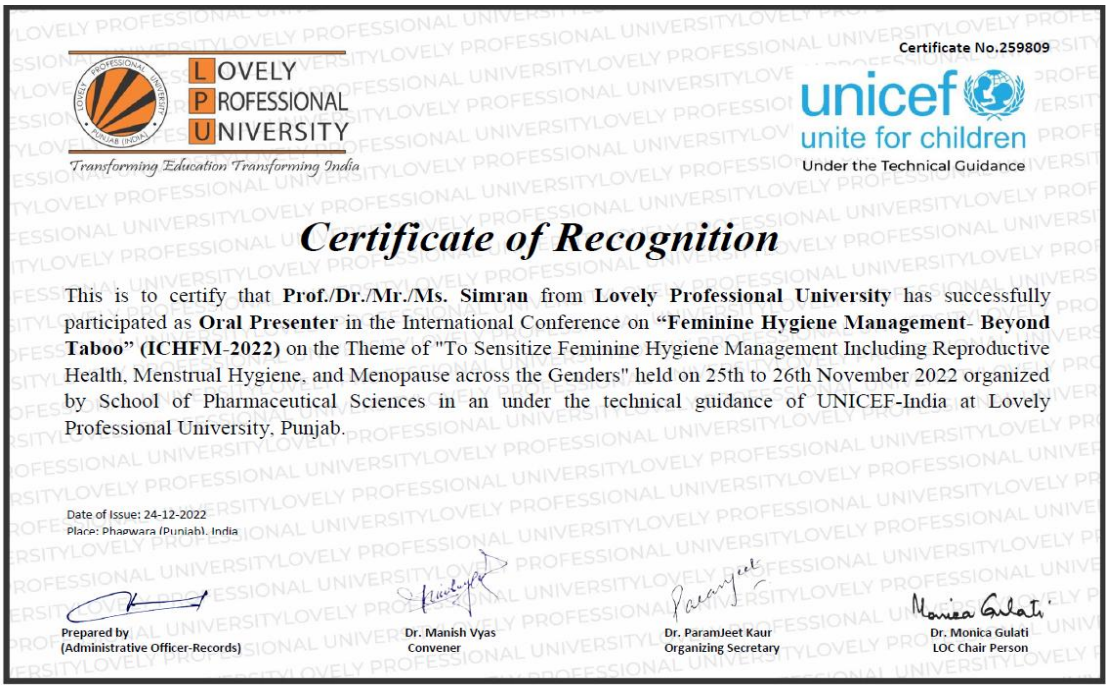
CONFERENCES

1. Oral Presenter at the International Conference on “Feminine Hygiene Management- Beyond Taboo” (ICHFM-2022) on the Theme of "To Sensitize Feminine Hygiene Management Including Reproductive Health, Menstrual Hygiene, and Menopause across the Genders" held on 25th to 26th November 2022 organized by School of Pharmaceutical Sciences in an under the technical guidance of UNICEF-India at Lovely Professional University, Punjab.

2. Oral presenter at International Conference on Microbial Bioprospecting Towards Sustainable Developments Goals (ICMBSDG 2023)24-25 Nov, 2023. Organized by Association of Microbiologist of India-LPU Unit, Department of Microbiology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India. In association with Society of Chemical and Synthetic Biology, India

3. Oral presenter in 5th International Conference on Recent Advances in Fundamental and Applied Sciences (RAFAS 2024) organized by School of Chemical Engineering & Physical Sciences on 19-20 April 2024 at Lovely Professional University, Punjab.



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Certificate of Presentation

This is to certify that **Dr./Mr./Ms. Simran** of **Lovely Professional University, Phagwara** has given **Oral** presentation on **Accumulation of PET microplastic and its effects on Drosophila melanogaster** in the **5th International Conference on Recent Advances in Fundamental and Applied Sciences (RAFAS-2024)** held from 19th to 20th April 2024, organized by School of Chemical Engineering and Physical Sciences, Lovely Faculty of Technology and Sciences, at Lovely Professional University, Punjab.

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(RAFAS-2024)

Head of Faculty
Lovely Professional University