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Effects of Microplastics on Aquatic Animals: A Case Study on Daphnia

Karima Wardak[1], Mohammad Dawod Shirzad[2]

[1,2] Kabul University, Department of Environmental Science, Faculty of Environment, Kabul, Afghanistan

E-mail: mshirzad.ku@gmail.com (corresponding author)

ABSTRACT

Plastic is one of the most significant technological products of the twentieth century. Its environmental impact includes the release and accumulation of toxins and contaminants, as well as endocrine disruption in aquatic organisms. Microplastics, in particular, are increasingly present in freshwater ecosystems; however, their specific effects on small aquatic organisms, such as Daphnia, remain poorly understood. In this study, various types of virgin plastics (HDPE, LDPE, LLDPE, PA6, PA6.6, PVC (rigid and flexible), PP, PS, TPU) were obtained from Hi-Tech Polymer Products in Ludhiana. These plastics were ground into small particles and measured using a micrometer, yielding a mean particle size of 0.398 µm. The chemical structures of the microplastics were identified using FTIR spectroscopy. The *Daphnia* culture was maintained in 1000 ml glass beakers. Microplastic particles and small elongated fibres, approximately 300 µm in size, were introduced into the aquatic environment to evaluate ingestion and toxicity. The findings revealed that *Daphnia* ingested long synthetic fibres and that exposure to microplastics negatively affected their normal biological functions. Mortality increased with both exposure time and microplastic concentration. This study contributes to the understanding of microplastic toxicity in freshwater ecosystems and emphasizes the vulnerability of *Daphnia* to plastic pollution.

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INTRODUCTION

Microplastics are increasingly present in freshwater ecosystems, yet their specific effects on small aquatic organisms, such as Daphnia, remain poorly understood. Daphnia, a key zooplankton species in aquatic food chains, may suffer physiological and reproductive harm from ingesting microplastics, posing a threat to ecosystem stability. The formation of plastic through the use of heat is a fundamental step in nearly all plastic manufacturing processes. Plastic is one of the most powerful technological products of the twentieth century (Katzenberger, 2015). It is among the most widely used and adaptable materials globally, with remarkable features that allow it to be shaped and applied in diverse contexts (Dewar-Fowler, 2017). These engineering materials are used in nearly every level of life, and no other material currently offers an equivalent substitute. It is essential to acknowledge the behavior of plastic throughout the various stages of its production and use, including recovery and repurposing (Rusu and Rusu, 2003). The tools and techniques used to develop this understanding are referred to as plastic examination. The analysis of plastic can be widely divided into two main categories. The physical analysis of plastic refers to the evaluation of the material's physical properties and behavior. Thermal behavior, power, and flow properties fall into this category, such as failure and morphological characteristics. The Chemical analysis of plastic pursues to investigate the characteristics of polymer compounds. A combination of these two broad approaches has been successfully used to link the conduct of plastics, their chemical composition, and structural integrity (Smith, 2017).

According to Mantovano et al. (2018), plastics come in various types, including polyethylene and polyamide, among others. Polyethylene is an ethylene polymer ($CH_2 = CH_2$) that is commonly used in daily life, such as in food packaging, shampoo bottles, and bulletproof balloons, among other applications—multiple types of polyethylene with the maximum chemical formula (C_2H_4)nH2. There are various types of polyethylene, including linear low-density polyethylene (LLDPE), high-density polyethylene (HDPE), low-density polyethylene (LDPE), and ultra-high-molecular-weight polyethylene (UHMWPE).

Microplastics are defined as polymer units measuring less than 5 mm (Katzenberger, 2015). A smaller border is not yet clear. These units may be caused by plastic disruption due to collapse and instability, but they will enter the environment as primary microplastics (Shim et al.). Plastic particles have been detected in all the world's oceans and many freshwater systems (Felsing et al., 2018). Pollution: The global assessment, as formed in Marine Nursing Surveys, increases by only one percent (0.1%) of the annual worldwide growth, perhaps due to specimen methods that employ low particle size delivery and accumulation in any matrix or sample framework. Do not make approximations based on solid waste in coastal countries and its relation to 1.7 percent to 4.6 percent of the plastic waste production inside the sea (Rainieri et al., 2018). Plastic is consumed through the food chain, allowing humans to be exposed to it (Bergmann et al., 2015).

An evaluation conducted by the Secretariat of the Convention on Biological Diversity and its technical board in 2012 found that plastics were present in more than 80% of examined aquatic organisms—either externally attached, ingested, or otherwise absorbed. The presence of microplastics was reported explicitly in 11% of these cases (Smith, 2017). The effects of plastics can include the concentration and transfer of toxins and contaminants, tissue damage, and endocrine disruption (Smith, 2017). Prior studies on the effects of microplastics in freshwater environments have primarily focused on vertebrates, which are often highlighted in the media due to their visible reactions to plastic ingestion. However, studies on invertebrates, such as Daphnia, remain sparse and underdeveloped. Given *Daphnia's* ecological importance and high sensitivity to pollutants, it offers an ideal model for understanding the toxicological mechanisms of microplastic exposure. However, this area remains underexplored, particularly in experimental studies that use real polymer particles and quantify mortality under controlled laboratory conditions.

Eco-friendly plastics encompass a diverse range of polymers (Chen et al., 2019). The most common types of polymers in aquatic environments reflect the composition of widely manufactured consumer products: Polyethylene (PE), including LDPE and HDPE; Polypropylene (PP); Polystyrene (PS); Polyamide (nylon); and Cellulose Acetate (CA). These materials frequently degrade into microplastics and are often sourced from bags (LDPE), containers (HDPE), ropes (PP), packaging foams (PS), textiles (PA), and filters (PVC, PET) (Aljaibachi and Callaghan, 2018). Different polymers possess distinct molecular and crystalline structures, which affect their density and environmental behavior (Aljaibachi and Callaghan, 2018). For instance, the relatively lower densities of PE, PP, and PS (≤1.05 kg/l) make them buoyant in water, while denser plastics like PVC and PET (≥1.37 kg/l) tend to sink. Plastic additives such as plasticizers and flame retardants, which contribute to the functionality of polymers, may also influence their environmental toxicity (Mahon et al., 2014). Furthermore, plastics can adsorb and transport other hazardous substances—including hydrophobic pollutants and persistent organic chemicals—thus increasing ecological and health concerns (Oladejo, 2017; Akinro et al., 2012). Whether these microplastic particles act as vectors for harmful chemicals into aquatic organisms, including *Daphnia*, warrants further investigation (Chen et al., 2018).

In response to these gaps, this study investigates explicitly the toxic effects of microplastic particles on *Daphnia*, a freshwater invertebrate species widely used in ecotoxicological testing.

The objectives of this study are as follows:

1. To measure the role of microplastics in the mortality of *Daphnia* under controlled laboratory conditions.
2. To assess whether microplastics can be lethal to freshwater invertebrates and to what extent mortality correlates with exposure concentration and duration.

METHODS AND MATERIALS

Study Area

The study was conducted in Panchkula, Punjab. Punjab is a northern Indian state known for its diverse water resources, making it a relevant setting for freshwater ecotoxicological studies. This paper is research-based; in this research, various laboratory tools, including Fourier Transform Infrared Spectroscopy (FTIR) and a stereo microscope, as well as other essential instruments, were utilized. FTIR was specifically selected for this study due to its high accuracy in identifying the chemical structure of polymer particles based on their functional groups and characteristic absorption spectra. This technique is widely accepted in microplastic analysis for its ability to detect different plastic types non-destructively and with high sensitivity. Books, scientific articles, and credible websites were also used as secondary data sources to support the research.

Sample Collection

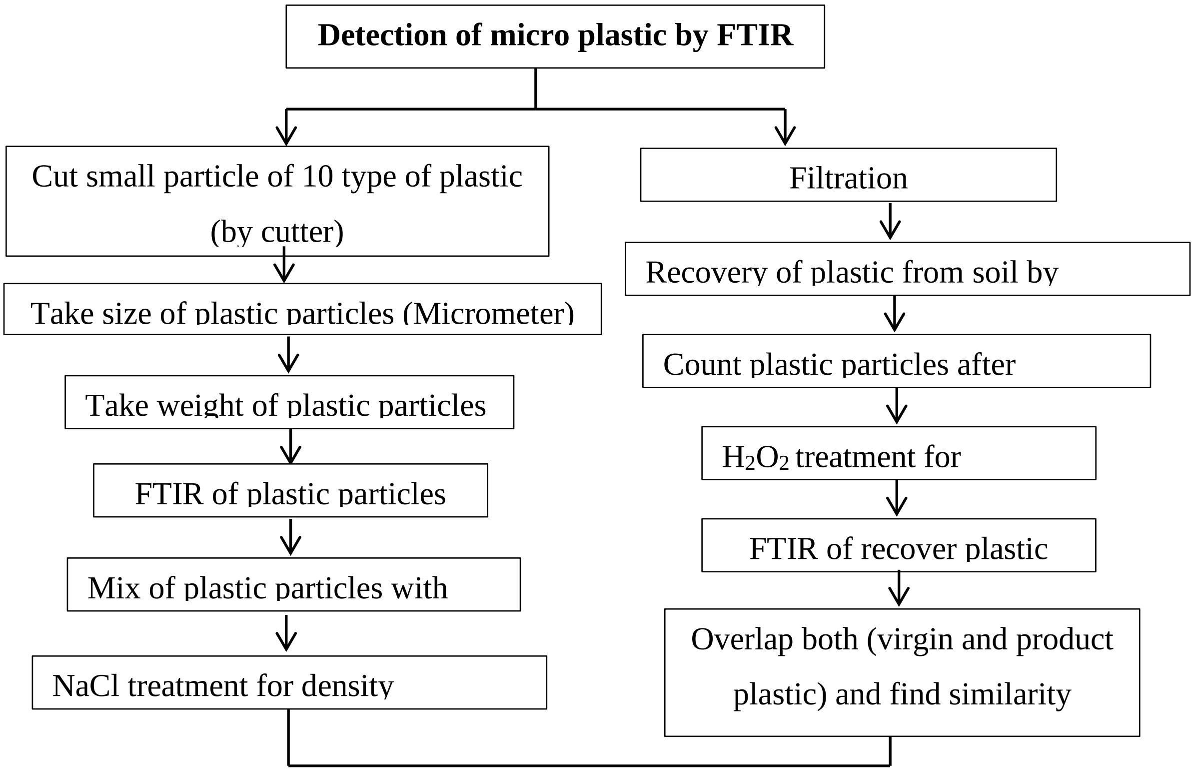

***Figure 1**. Shows detection of Microplastic by FTIR*

Data Collection and Analysis

Culture of Daphnia*.* The most important environmental factor for the success of *Daphnia* is the temperature, which should remain around 20°C (68°F). *Daphnia*, commonly known as water fleas, were selected as the model organism due to their ecological relevance in freshwater ecosystems, short life cycles, sensitivity to pollutants, and wide use as standard bioindicators in ecotoxicology. Their transparent bodies also allow for easy observation of ingested particles under a microscope, making them ideal for studies on ingestion and mortality involving microplastics.

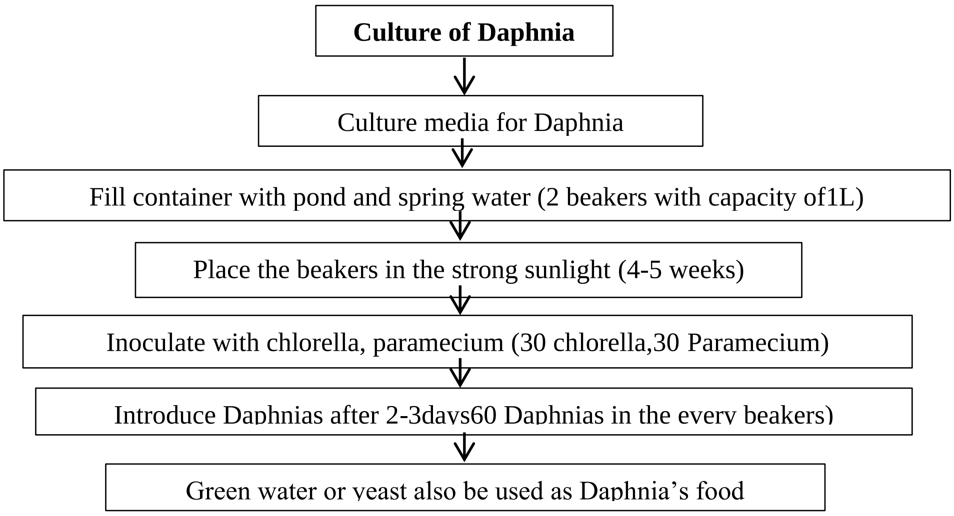

***Figure 2**: Shows the culture of Daphnia*

Ingestion of Microplastics into Daphnia

After 1-hour, 2-hour, and 24-hour exposure to microplastics (latex, 8 µm) in each Petri dish, *Daphnia* specimens were inspected for signs of immobility. The absence of heartbeat confirmed mortality. The death results were recorded as the percentage of dead *Daphnia* for each concentration, as suggested by Shim et al. (2016). The active (alive) and inactive (dead) rates of *Daphnia* were compared with those of control groups. To observe the presence of microplastic particles inside the gastrointestinal tract of *Daphnia*, a stereo microscope was employed.

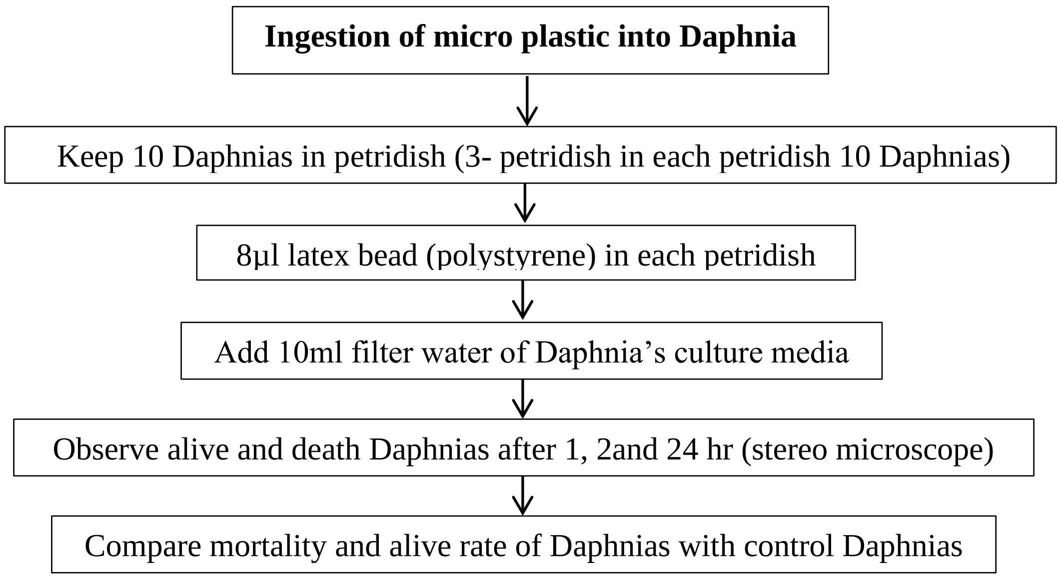

Figure 3*: Shows the steps of ingestion of microplastic into Daphnia*

FINDING AND DISCUSSION

The presence of microplastics in freshwater ecosystems poses a significant ecological threat, particularly to small filter-feeding organisms such as Daphnia. As a keystone species in aquatic food webs, Daphnia play a critical role in nutrient cycling and energy transfer. Studies have demonstrated that Daphnia unintentionally ingest microplastic particles. Sub-lethal effects, including impaired reproduction, growth inhibition, and behavioral changes, have also been observed, indicating a disruption in vital biological processes (Rehse et al., 2016). These particles can physically block the digestive tract and alter energy intake, directly affecting survival rates (Besseling et al., 2014). Additionally, microplastics can act as vectors for other contaminants, such as heavy metals and persistent organic pollutants, thereby enhancing their toxicity (Rainieri et al., 2018). The combination of microplastics and chemical stressors can result in additive or even synergistic effects on aquatic invertebrates (Felsing et al., 2018).

Furthermore, the particle size, shape, and type of polymer influence toxicity outcomes (Chae & An, 2017). Smaller particles, such as nanoplastics, can penetrate tissues more deeply, raising concerns over bioaccumulation and trophic transfer (Mattsson et al., 2017). The physiological stress responses in Daphnia include oxidative damage and immune disruption (Oliveira et al., 2013).

Understanding these effects is crucial for ecological risk assessments. Since Daphnia serve as a primary food source for fish, disruptions in their population can affect entire freshwater food webs (Rist et al., 2017). Their role in water clarity and nutrient cycling also underscores the ecological implications of microplastic pollution (Cole et al., 2015). These effects not only threaten individual health but can cascade through trophic levels, affecting ecosystem structure and function. Additionally, microplastics may act as vectors for other pollutants, compounding their toxicity. Given their ecological importance and sensitivity, Daphnia serve as an effective model for assessing the toxicity of microplastics in freshwater systems. However, further research is needed to evaluate long-term and multi-generational impacts under realistic environmental conditions. For detection of Microplastic by FTIR, first of all took types of virgin plastic (HDPE, LDPE, LLDPE, PA6, PA6.6, PVC(r), PVC (f), PP, PS, TPU) from Hi – TECH POLYMER PRODUCTS company located in Ludhiana, they are common types of plastics. Firstly, cut the type of virgin plastic into small particles and measured the size of the standard virgin plastic particles using a micrometer. Mean of virgin plastic particles was 0.398µm and standard deviation ± 0.0348—more details in the given Table 1.

Table 1: *This table shows the Average size of virgin plastic, 2025*

NO	Type of plastic	Size in mm	Size in µm
1	High-Density Polyethylene (HDPE)	0.35mm	350µm
2	Low Density polyethylene(LDPE)	0.38mm	380µm
3	Linear Low Density polyethylene (LLDPE)	0.44mm	440µm
4	Polyamide Chloride (Rigid) PVC(r)	0.44mm	440µm
5	Polyvinyl Chloride (flexible) PVC (f)	0.37mm	370µm
6	Polyamide (PA 6.6)	0.35mm	350µm
7	Polyamide (PA 6)	0.39mm	390µm
8	Thermo Plastic Polyurethane (TPU)	0.41mm	410µm
9	Polystyrene (PS)	0.43mm	430µm
10	Polypropylene (PP)	0.42mm	420µm

A precision balance was used to measure the weight of microplastics. The mean of virgin plastic particles was 0.77 ± 0.078102 as detailed in Table 2.

Table 2: *Average of virgin plastic particles, 2025*

No	Type of Plastic	Weight
1	Thermo Plastic Polyurethane (TPU)	0.7mg
2	Polystyrene (PS)	0.7mg
3	High-Density Polyethylene (HDPE)	0.9mg
4	Polyamide Chloride (Rigid) PVC(r)	0.8mg
5	Linear Low Density polyethylene (LLDPE)	0.7mg
6	Polypropylene (PP)	0.8mg
7	Polyamide (PA 6)	0.9mg
8	Polyamide (PA 6.6)	0.7mg
9	Polyvinyl Chloride (flexible) PVC (f)	0.8mg
10	Low Density polyethylene(LDPE)	0.7mg

After measuring the size and weight of virgin plastic particles. 50 particles of microplastic mixed with 102g of microplastic-free soil. Provided solution of 130g NaCl + 500 mL distilled water. Aeration by motor for the separation of microplastic particles from soil particles. Add NaCl solution and remove the upper layer of the mixture. After filtration, the remaining material from the filter was kept in a Petri dish, and 30% H2O2 was added for the digestion of biogenic particles. The sample was kept for 15 days. Find the microplastic particles (recovery plastic). A high percentage of recovered particles were PP (42% %, a low percentage of recovered particles were PVC(12%) 12%, more details in Table 3:

***Table 3**: Recover particles of virgin plastic 2025*

No	Type of plastic	Total particles	Recover particles	Percentage
1	HDPE	50	14	28%
2	LDPE	50	15	30%
3	LLDPE	50	11	22%
4	PVC(f)	50	6	12%
5	PVC (r)	50	9	18%
6	PA 6	50	14	28%
7	PA 6.6	50	5	10%
8	PP	50	21	42%
9	PS	50	12	24%
10	TPU	50	17	34%

Different product samples were cut into small particles and underwent the same procedure as for the standard of virgin plastic; more details are provided in Table 4.

***Table 4**: List of Products used in the experiment,2025*

HDPE	**Bottle caps**
LDPE	Carry bags
LLDPE	Packaging material
PS	Disposable items
PP	Tips of the micropipette
TPU	Mobile cover
PVC(r)	Shampoo bottle
PVC(f)	Electric pipe
PA6	Tooth brush
PA6.6	Rope

FTIR: Infrared (IR) spectroscopy is known as vibration spectroscopy (Felsing et al., 2018). For decades, it has been used as a tool to identify and analyze polymeric materials; in fact, the requirement for an artificial polymer analysis method was the basis for the early development of infrared instrumentation during the Second World War (Rusu and Rusu, 2003).

Identification process and the chemical structures of microplastics as obtained from their infrared spectra. Many plastics share standard features, making it convenient to categorize them into groups. A popular method for classifying polymers is by their modes of application. For instance, some polymers such as (HDPE, LLDPE, LDPE, PA6, PA6.6, PVC(r), PVC(f), PP, PS, TPU) are classified as thermoplastics. This enables us to utilize the correlation between the functional groups of polymers and their characteristic infrared frequencies. The purpose of this section is not only to confirm the identification, but also to characterize certain plastic types. Although methods to determine microplastics by IR spectra. Today, most work is performed using Fourier transform infrared (FTIR) spectroscopy.

The IR spectrum of polyethylene (PE) is shown in the Spectra Reference in the Appendix (Rusu and Rusu). If we consider the whole macromolecule as a single linear chain, theoretically, we will encounter a long chain of methylene groups, $(CH_2)n$, with two end groups (usually a methyl group). Because this chemical structure is simple, it consists only of carbon and hydrogen atoms. The primary bending mode of the CH_2 groups is observed in the IR spectrum at 1475 and 1463 cm-1. Most of the branch structure is free(Rusu and Rusu, 2003). HDPE has a high density and is sometimes not transparent, but it resembles the appearance of white milk. The HDPE from 1400 to 1330 includes two peaks. Then, the substance is HDPE, as detailed in Graph 1. In the case of LDPE, the group includes three peaks in the 1330-1400 area. C-H is the stretching area, CH_3 is a functional group; more details are provided in the given figures.

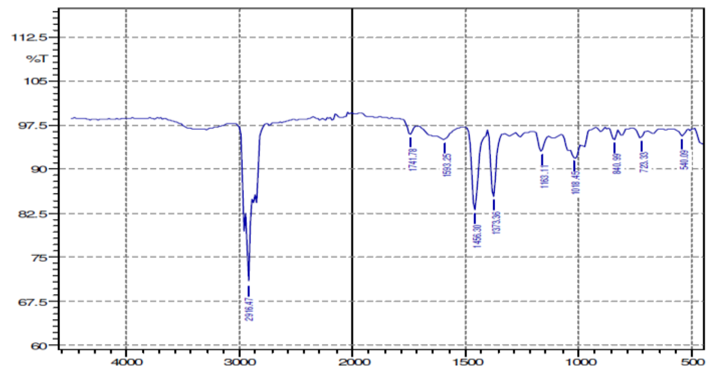

Figure 4. *Characteristic peaks of HDPE 2025*

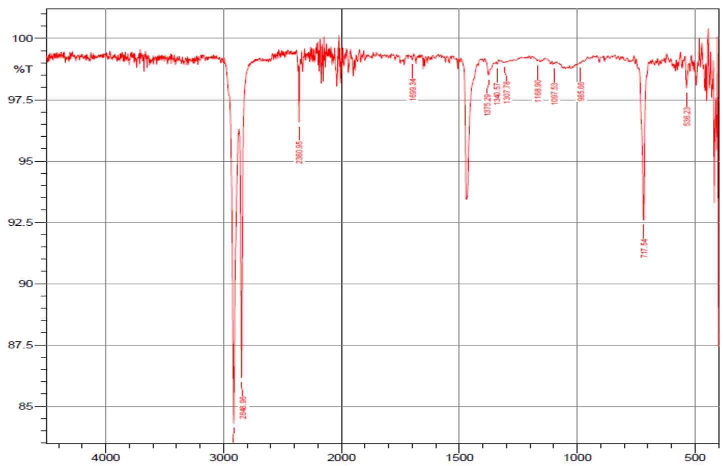

Figure 5. *Characteristic peaks of LDPE*

Culture of Daphnia

For the culture of Daphnia, two glass beakers were used, each with a 1000ml capacity. Firstly, fill the beakers with aquarium water and keep them for one week, allowing the water to sit undisturbed before adding the Daphnia. After one week, inoculate with 30 Chlorella and 30

Paramecium, place the beaker under strong sunlight 3-4hr every day. After two days of inoculation, 60 Daphnia were added to each beaker and kept for one month. Green water and yeast are used as food for Daphnia.

Identification of Daphnia: These small, side-lobed "water fleas" are characterized by a body enclosed in a transparent, shell-like structure. Its smooth and transparent bodies, Daphnia are an ideal organism for initiating biological tests and experiments (FLINN Scientific BioFex). Daphnia moves slowly. They have large second antennae that appear to correct the swimming regeneration and help with the movement of four to six pairs of swimming legs. During the spring and summer, females are very abundant. The eggs generally develop through the gut partition and may be seen in the bare chamber (FLINN Scientific Biofex). Figure 6 shows the culture media, and Figure 7 shows the Daphnia in the culture media.

***Figure 6**: Culture media of Daphnia*

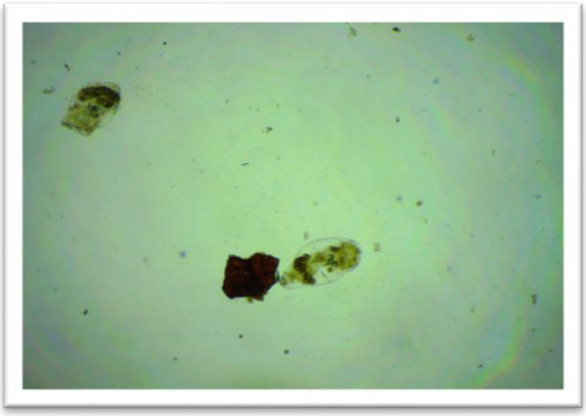

***Figure 7**: Daphnia in the culture media*

Daphnia's lifespan is about eight weeks. Daphnia reaches puberty in 5 to 10 days, at which point it produces offspring for a few months. The most important environmental factor for the success of Daphnia is that it should have a pH of around 7.8. Sunlight is the most important factor for daphnia culture. Daphnia culture is kept under sunlight (3-4 hours per day), allowing for the growth of chlorella.

Ingestion of Microplastics into Daphnia

8µm latex +10ml filters of water from Daphnia's culture media + 10 Daphnias in each petri dish, three petri dishes for the Dose and three petri dishes for control Daphnias. After 1, 2, and 24 hours, observed by a stereo microscope, and compared with the mortality and alive rate of Dose Daphnias and control Daphnias, the mean mortality is 2.83 ± 2.32. More details in Table 5.

***Table 5**: Observation after 1, 2, and 24hours, 2025*

Concentration	Duration	Total	Dead	Alive	Percentage of dead	Mortality
		10	0	10	0%	1±1
8µl	Dose (1hr)	10	1	9	10%	
		10	2	8	20%	
		10	0	10	0%	0±0
Control	(1hr)	10	0	10	0%	

		10	0	10	0%	
		10	3	7	30%	2.3±0.57
8 µl	(2hr)	10	3	7	30%	
		10	4	6	40%	
		10	0	10	0%	0±0
Control	(2hr)	10	0	9	0%	
		10	0	9	0%	
		10	6	4	60%	6.3±0.57
8 µl	(24hr)	10	6	4	60%	
		10	7	3	70%	
		10	0	9	10%	0±0
Control	(24hr)	10	0	9	10%	
		10	0	9	10%	

Observation by stereo microscope reveals a high mortality rate of 6.3% within 24 hours in Dose Daphnia. Zero mortality (0%) was observed during the 1-hour, 2-hour, and 24-hour periods in control Daphnias, indicating that microplastics affected the everyday life of Daphnias—more details in Figure 5.

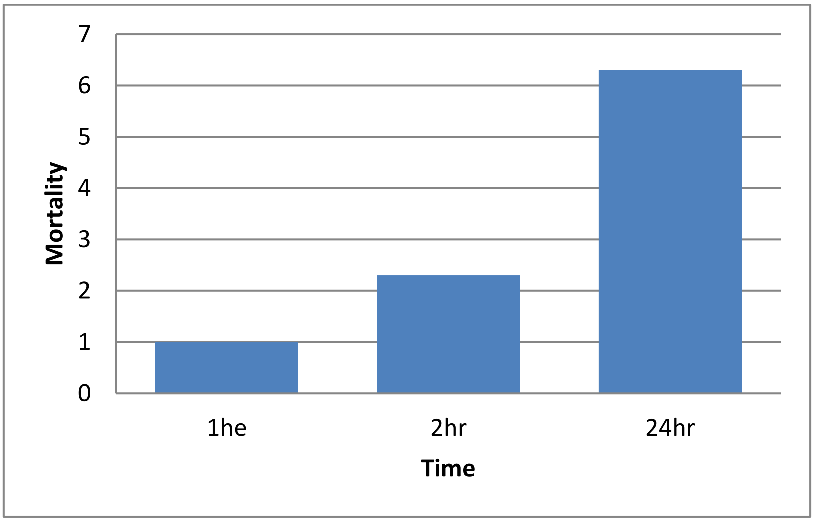

***Figure 8**. The mortality percentage in the case of the Dose and control Daphnias over time*

Ingestion of Different Concentrations of Microplastic into Daphnia

For every concentration, three petri dishes were used, in each of which 10 Daphnias were kept with 5 ml of distilled water. Concentration of latex was 1µl, 2µl, 4µl, 6µl,8µl, but at the same time for all concentrations. After 1 hour, observed by stereo microscope and counted, the mean mortality of the dead and alive Daphnias is 4.33 ± 1.72. More details in Table 6.

***Table 6**: Percentage of dead Daphnias based on different concentrations of latex dose.2025*

Dose	Duration	Total	Alive	Dead	Percentage	Mean of Mortality
		10	8	2	20%	2.3±0.57
1µl	1 hr	10	8	2	20%	
		10	7	3	30%	
		10	7	3	30%	3.3±0.57
2 µl	1hr	10	7	3	30%	
		10	6	4	40%	

		10	5	5	50%	5.3±0.57
4 µl	1hr	10	5	5	50%	
		10	4	6	60%	
		10	4	6	60%	6.3±0.57
6 µl	1hr	10	4	6	60%	
		10	3	7	70%	

Figure 9 shows the dead percentage of Daphnias based on different concentrations in the Dose and control Daphnias. A high concentration of 6 µl of latex shows a high mortality rate of Daphnia.

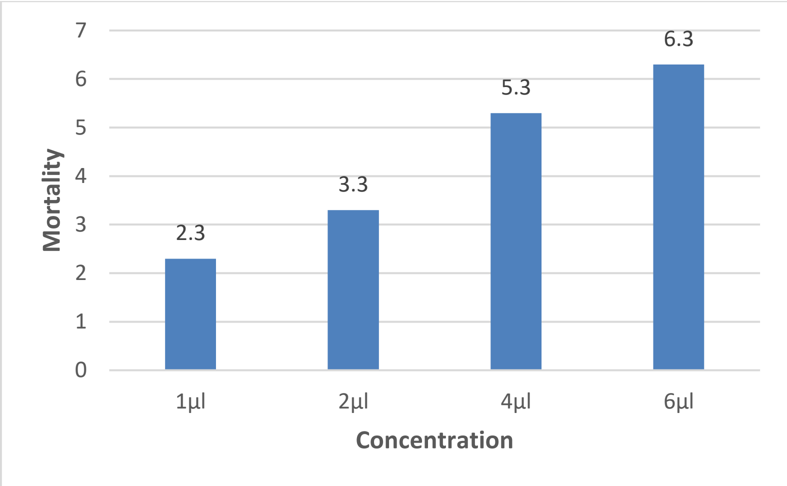

Figure 9. *Mortality percentage in Dose Daphnia. Latex is available in different concentrations*

In the given table, a comparison is made between a high concentration of latex (8 µl) and a low concentration (1 µl) after durations of 1 hr, 2 hr, and 24 hr, as observed by a stereo microscope—more details are provided in Table 7.

Table 7. *High concentration and low concentration of latex in duration of time, 2025*

Concentration	Time	Total	Dead	Alive	Dead percentage	Mean of Mortality
		10	0	10	0%	0.33±0.57
	1hr	10	0	10	0%	
		10	1	9	10%	
		10	1	9	10%	2±1
1 µl	2hr	10	2	8	20%	
		10	3	7	30%	
		10	4	6	40%	4.66±1.15
	24hr	10	4	6	4%	
		10	6	4	60%	
		10	0	10	0%	1±1
	1hr	10	1	9	10%	
		10	2	8	20%	
		10	3	7	30%	3.33±0.57
8 µl	2hr	10	3	7	30%	
		10	4	6	40%	
		10	6	4	60%	6.33±0.57
	24hr	10	6	4	60%	
		10	7	3	70%	

For every concentration used, three Petri dishes were prepared, in each of which 10 Daphnia were kept with 5 mL of distilled water. The concentrations of latex were 1µl, 2µl, 4µl, 6µl, and 8µl, but the same for all concentrations—more details in the given Table 8.

Table 8. Mortality of Daphnias in the 1-hr inoculated by different concentrations of latex (1 µl,2 µl, 4 µl, 6 µl, 8 µl), 2025

Concentration	1 hr (Mean±std)
Control	0±0
1 µl	2.33±0.57
2 µl	3.33± 0.57
4 µl	5.33 ± 0.57
6 µl	6.33 ± 0.57
8 µl	7.33 ± 0.57

Table 9: *Comparison of mortality in high concentration of latex 8 µl and low concentration 1µl in different times (1hr, 2hr, 24hr),2025*

Concentration	1hr	2hr	24hr
Control	0 ± 0	0 ± 0	0 ± 0
1 µl	0.33 ± 0.57	2.0 ± 1.00	4.67 ± 1.15
8 µl	1.00 ± 1.00	3.33 ± 0.57	6.33 ± 0.57

Result of performance as Mean standard deviation (N=3), 1 &8 concentration of latex. Different lowercase alphabets show defense in mortality rates concerning concentration and duration of time.

CONCLUSION

The increasing presence of microplastics in freshwater ecosystems presents a growing environmental concern, particularly for small aquatic organisms like *Daphnia*. As essential components of aquatic food chains, *Daphnia* are highly susceptible to microplastic ingestion, which can impair their feeding, growth, reproduction, and overall survival. These sub-lethal effects may disrupt the balance of ecosystems and threaten biodiversity. Given their ecological significance and sensitivity, *Daphnia* serve as an important bioindicator for assessing microplastic pollution. The current study confirms that even short-term exposure to microplastic particles can result in significant mortality in *Daphnia*, particularly at higher concentrations. Therefore, urgent action is needed to reduce plastic pollution sources and to investigate further the long-term ecological consequences of microplastics in aquatic environments.

While the study demonstrates acute toxic effects of microplastic particles on *Daphnia*, it is limited by its short observation window (up to 24 hours), laboratory-controlled conditions, and the exclusive use of latex microplastic particles. The interactions of mixed plastic types with varying environmental factors, such as light, pH, or dissolved oxygen, and chronic exposures were not addressed. Additionally, the study focused solely on mortality as an endpoint, leaving out potential effects on behavior, reproduction, or genetic expression.

Future research should consider longer-term and multi-generational studies to assess the effects of chronic exposure. Investigations involving multiple plastic types and environmentally aged particles would also provide more realistic insights. Exploring biochemical markers, sub-lethal responses, and trophic transfer through food webs will further strengthen our understanding of microplastic toxicity in freshwater ecosystems.

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CONFLICT OF INTEREST

The authors declare that there are no competing financial or personal interests that could have influenced the work reported in this article.

AUTHOR'S CONTRIBUTIONS

All authors contributed significantly to the development of this article. Karima Wardak conceptualized the study, led the writing, conducted the data analysis, and contributed to drafting the manuscript. M. Dawod Shirzad reviewed the literature and provided critical revisions. All authors read and approved the final version of the manuscript.

DATA AVAILABILITY

The datasets generated and analyzed during the current study on the effects of microplastics on Daphnia are not publicly available, but are available from the corresponding author upon reasonable request. All data generated or analyzed during this study on the effects of microplastics on aquatic animals (Daphnia) are included in this published article.

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