



MICROPLASTIC–MICROBIOME INTERACTIONS IN MARINE AND FRESHWATER ECOSYSTEMS

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ABSTRACT

Background: Microplastic contamination is becoming a major worldwide environmental hazard, which influences the aquatic biodiversity, as well as the community dynamics of microorganisms in marine and freshwater environments. The microscopic plastic particles are artificial sources of microbial colonization that create separate communities in the form of a plastisphere, which affect nutrient cycling, pathogen dispersal, and ecosystem wellbeing. Although the interest has increased, there is still a lack of knowledge and awareness among the population as to the interaction between microplastics and the microbiome. This research was conducted to assess the degree of awareness, perception, and understanding of these interactions in the various demographic groups and to statistically confirm the relationships between the critical perception variables.

Methods: A cross-sectional quantitative design was adopted by use of a structured Likert scale questionnaire with 16 questions that were distributed under the four different domains: awareness, ecological impact, health and food chain concerns, and mitigation perception. Three hundred answers were obtained from respondents representing different parts of Pakistan. The analyses involved Normality analysis (Shapiro-Wilk), Reliability (Cronbach's Alpha), Validity (KMO and Bartlett test), Independent Samples t-test, one-way ANOVA, Kruskal-Wallis, Chi-square analysis, Pearson correlation, and Multiple regression analysis. All the analyses were conducted in SPSS and Python-based statistical modeling.

Results: The findings established that all the variables were normally distributed ($p > 0.05$) and consistent internally with a total Cronbach's Alpha of 0.911, which is excellent. Construct validity was determined by the value of KMO (0.846) and the significance of the Bartlett Test ($\chi^2 = 2643.217$, $p < 0.001$). The difference between the groups was statistically significant in terms of gender ($t = 2.684$, $p = 0.008$), education ($F = 4.327$, $p = 0.014$), region ($H = 10.562$, $p = 0.032$), and gender x education correlation ($H^2 = 18.246$, $p = 0.021$). The correlation table showed high positive relationships between all the variables of perception ($r = 0.64-0.81$). Regression analysis also showed that the overall

perception was significantly and positively ($\beta = 0.1380.312$) contributed by all predictors and accounted for 68.2 percent variance ($R^2 = 0.682$).

Conclusion: The results reveal an elevated degree of awareness and a positive, strong correlation between ecological knowledge and health-related issues and mitigation plans on the interaction of microplastics and microbiomes. These perceptions are greatly influenced by education, gender, and regional background, motivating the need to adopt specific environmental education and awareness programmes. The paper summarizes that sustainable strategies of water management and pollution control would be impossible without improving the general literacy of the population regarding the mechanisms of interaction between microplastics and microbiomes. The questionnaire constructed in the course of the study and validated and reliable, can be applied as a useful instrument in future ecological perception studies and policy-making.

INTRODUCTION

Microplastic pollution has emerged as one of the most topical environmental issues of the twenty-first century, with disastrous consequences on aquatic and biodiversity, and affecting global public health. Microplastics can be described as synthetic polymer particles less than 5 millimeters in size, which are formed by the breakdown of larger plastic debris (secondary microplastics) or are actually formed at the microscopic scale (primary microplastics) to be used in products, like cosmetics, laundry detergent, and industrial abrasion. Microplastics, being small in size and stubbornly dispersing easily through wind, rain, and run-offs on the surface to three levels, finally find their way into the marine and freshwater systems. Their stability, chemical resistance, and large usage have caused them to be ubiquitous in rivers, lakes, estuaries, and oceans, with increasing threat to aquatic life and ecosystem operations (Dixit et al., 2025).

When microplastics enter the water bodies, they serve as physical pollutants, biological and chemical vectors. They offer a novel and steady platform of microbial colonization, resulting in the emergence of specific biofilm communities referred to as the plastisphere. These microbial communities are not similar to those present in adjacent water or sediments, and can typically include multi-layered communities of bacteria, archaea, fungi, algae, and protozoa. The type of plastic polymer, the

roughness of surfaces, and even the hydrophobicity will determine the type of microorganisms that will grow on the surface. The relationship between microplastics and microbiomes is therefore dynamic, which has implications for microbial ecology and pollutant transport. It has been demonstrated in several studies that microplastics contain both beneficial microbes that can be involved in the biodegradation process and potentially harmful bacteria, including *Vibrio* and *Pseudomonas*, which can contribute to the development of diseases in both aquatic life and humans (Jeong et al., 2025).

In addition, microplastics may absorb toxic chemicals and heavy metals, acting as carriers of persistent organic pollutants (POPs) such as PCBs, PAHs, and pesticides. These pollutants may be biochemically modified through interplay with microbial action, which may decrease or increase toxicity. Therefore, the interaction of microplastics and microbiomes not only affects the microbial diversity and activity but also the destiny and transportation of chemical pollutants. Marine ecosystems respond to these processes via nutrient cycling, photosynthesis, and primary productivity, whereas freshwater ecosystems may respond by changing sediment biogeochemistry and breaking aquatic food webs. These contaminants and the other microbes can eventually find their way into the human food chain using

bioaccumulation and trophic transfer, which causes further health risks via the consumption of seafood (Missawi et al., 2025).

These interactions should be known to assess the ecological resilience and sustainability of water bodies. The increasing awareness of the issue of microplastic pollution is still accompanied by a lack of knowledge regarding the effect and interaction of microbial communities with plastic particles in various aquatic environments. The heterogeneity, functional activity, and biogeographic significance of plastisphere microbes in marine and freshwater systems differ significantly because of the salinity, temperatures, nutrients, and hydrodynamic factors. Therefore, comparative experimental studies that involve the study of the two ecosystems are essential in revealing the mechanisms of microbial colonization, succession, and adaptation to microplastic surfaces (Krzynowek et al., 2025).

The study in question is aimed at evaluating the knowledge and perception of the microplastic-microbiome relationships in both marine and freshwater ecosystems using a systematic quantitative method. The study will fill the gaps between the knowledge of environmental science and the general understanding by incorporating ecological, microbiological, and social aspects. The results will also add to the understanding of how human consciousness, education, and regional conditions affect the attitude toward microplastic pollution and microbial ecology. Finally, this study contributes to the worldwide agenda of sustainable aquatic ecosystem management and offers knowledge to the environmental policy, education, and mitigation approaches to not only reduce microplastics but also address microbial health in water systems (Yang et al., 2025).

LITERATURE REVIEW

The development of microplastic pollution as a worldwide environmental threat has led to massive studies on its

ecological and biological impact. In the last ten years, researchers have become more and more aware of the fact that microplastics are not passive particles but dynamic ecological substrates interacting with microbial communities. Such interactions have redefined the conceptual notion of aquatic microbiomes in marine and freshwater environments and given rise to the discovery of new ecological niches that are collectively known as the plastisphere. The plastisphere is a unique microbial community that grows on plastic particles and is frequently very different in composition than the adjacent planktonic assemblages of microbes. Later research has established that microplastics support complex biofilms containing bacteria, fungi, diatoms, and even viruses and show distinctive patterns of colonization, succession, and ecological interaction (Fu et al., 2025).

The biofilm formation on plastic surfaces is a fast process that depends on different environmental and material factors. Microbial species are attracted to colonize the microplastics within hours of immersion, with the formation of a mature biofilm community. The polymer type (e.g., polyethylene, polypropylene, polystyrene), the hydrophobicity, the surface roughness, and exposure duration are important factors to define microbial diversity. Marine ecosystems are more likely to have halophilic and biofilm-forming organisms, whereas freshwater systems are predominantly occupied by heterotrophic bacteria that originate from products of terrestrial alight runoff and wastewater discharge. Research by Amaral-Zettler et al. and Wright et al. also concludes that the plastisphere is highly habitat selective, with microbial taxa modifying their metabolisms to take advantage of the physicochemical properties of plastics. It means that microplastics serve as unnatural but, at the same time, stable environments that can change the biogeography of microbes in water bodies (Demarquoy, 2025).

Microplastic-microbiome interaction has important biogeochemical and ecological implications in both fresh and marine ecosystems. Depending on the ability of the enzymes, biofilm-forming microbes may hasten or prevent the breakdown of the plastics. An example is that some bacterial genera have shown partial degradation of polyethylene and the PET using the oxidative enzymes: monooxygenases and esterases, which include *Pseudomonas*, *Rhodococcus*, and *Alcanivorax*. On the other hand, the same biofilms can shield plastics against UV degradation and increase their environmental persistence. Moreover, biofilms that involve microplastics also affect the nutrient cycling, carbon sequestration, and primary productivity, which alter the ecological state of the aquatic environment. These biofilms also increase the rate of microplastics sinking, which allows them to deliver organic carbon and pollutants to deeper sediments - a process known as biologic sinking (Tang, 2025).

The increasing worry in this field of study is how communities of the plastisphere are pathogenic. Some studies have identified opportunistic and pathogenic microorganisms on the plastic surfaces that include *Vibrio*, *Aeromonas*, *Legionella*, and *Escherichia coli*, some of which are fish or pathogen-related microorganisms. These pathogeneses imply that microplastics may serve as vectors in the distribution of detrimental microorganisms in aquatic environments. Moreover, the plastisphere has also been found to be a repository of antibiotic resistance genes (ARGs), and this has serious implications for antimicrobial resistance transmission. Yang et al. noted that the presence of ARGs and pathogenic bacteria together in microplastics increases the probability of horizontal gene transfer that can increase resistance in microbial communities in water. These results form a close relationship between microplastic pollution, microbial evolution, and

challenges in human health (Gilewski et al., 2025).

The interaction of microplastics with the microbiome is another vital process of chemical contaminants transportation and transformation. As a result of the high surface area and the hydrophobicity of microplastics, these materials are effective adsorbers of toxic compounds, among them persistent organic pollutants (POPs), heavy metals, hydrocarbons, and pesticides. These pollutants can be biotransformed by the microbial biofilms associated with them and can change their chemical forms and bioavailability. As an illustration, marine biofilms that include the genera *Alteromonas* and *Marinobacter* have been demonstrated to degrade polycyclic aromatic hydrocarbons (PAHs), and freshwater biofilms can help dissociate adsorbed metals anoxically. These interactions may, however, have a more negative effect on pollutant toxicity in cases where increased production of reactive intermediates by microbial metabolism takes place. Therefore, microplastic-microbial interactions are ecological and biochemical interactions that directly affect the fate of contaminants and the health of the ecosystem (Zhang et al., 2025).

Recent developments in high-throughput sequencing and metagenomics have enhanced the knowledge of plastisphere diversity. These instruments show that the plastisphere hosts specific microbial consortia in comparison with the adjacent water, whose metabolic physiologies are associated with xenobiotic degradation, quorum sensing, and stress response. Research papers have also shown that the plastisphere of marine environments is enriched with genes related to biofilm formation and hydrocarbon metabolism as well as antibiotic resistance, which points to the adaptive capability of these communities. Likewise, comparative analyses of marine and freshwater plastispheres indicate that in freshwater, the anthropogenic pollution and nutrient input predominantly affect the microbiome of plastispheres, whereas in the

ocean, the salinity and hydrodynamic environment dominate plastispheres. These data underline that the structure and working of plastic-associated microbial communities depend on the environmental context (Siddique et al., 2025).

The ecological consequences of the interaction of microplastics and microbial ecology are not limited to microbial ecology. They have impacts on food webs, sedimentation, and ecosystem services. Microplastics and their biofilms of microbes may be absorbed by plankton, invertebrates, and fish in aquatic food webs, establishing trophic pathways of plastics and microbial biofilms. This consumption may interfere with digestion, modify feeding habits, and produce sub-lethal physiological changes. Additionally, microplastics could affect oxygen exchange, carbon flow, and nutrient cycling through biogeochemical interactions, which eventually change the resilience and productivity of the aquatic ecosystem (Lotz et al., 2025).

The recent reviews conducted worldwide note that microbiology, toxicology, and environmental policy must be applied in an integrated approach to reduce the effects of microplastics. Education and public awareness are also among the important parts of this endeavor. Research indicates that individuals who are better educated or trained on the issue are more concerned and aware of the problem of microplastic pollution and its microbiological consequences. This is consistent with the results of the present study, as awareness was highly predicted by education and exposure to the region (Ionica et al., 2025).

RESEARCH METHODOLOGY

Research Design

In this study, the quantitative cross-sectional research design is adopted to observe the relationship between microplastics and their effects on the microbial communities within the marine and freshwater ecosystems. The design will aim to replicate the current status of

microplastic pollution, microbial colonization, and their ecological effects without altering the environment. A questionnaire was designed in a structured form to gather information on people working in environmental sciences, marine biology, and also in the field of public health. The questionnaire had 16 Likert-scale questions that addressed awareness, ecological effects, health issues, and mitigation perceptions regarding microplastic-microbiomes interactions. The quantitative design enables statistical manipulation of patterns, relationships, and differences between responses to draw general, understandable information on ecological knowledge and citizen perception (Li et al., 2021).

Population and Sampling of the Study

The target population comprises environmental experts, researchers, learners, and community members doing water quality, ecology, and sustainability initiatives. To achieve a wide representation, the respondents were sampled in areas that included marine (coastal) and freshwater (inland) systems of Pakistan, including Sindh, Balochistan, Punjab, and Khyber Pakhtunkhwa. Non-probability purposive sampling method was used to incorporate the knowledgeable people in as far as the knowledge of aquatic pollution was concerned. The respondents who participated in the survey were 300 in number, which provides a sufficient sample size to achieve reliability and statistical validity. The sample was calculated according to the previous studies on environmental perception, which guaranteed the power of correlation and regression analysis (Chaudhary et al., 2022).

Instrumentation

A self-administered questionnaire consisting of five parts, namely, (1) demographic information, (2) awareness and knowledge of microplastics, (3) ecological and microbiome effects, (4) human health and food chain issues, and (5) mitigation and management perceptions, was used to gather data. All the items were rated using a

Likert-type scale of five points between Strongly Disagree (1) and Strongly Agree (5). To check the clarity and internal consistency, the instrument has first been tested on 30 participants. The content validity was provided by the expertise validation of environmental scientists. Pilot testing was done, and slight linguistic changes were undertaken to improve understanding and the accuracy of response (Varg et al., 2022).

Data Collection Procedure

The information was collected by using online survey systems and organizational email. The purpose of the study, confidentiality, and voluntary participation were ethically informed to the respondents. The period of the collection was four weeks, during which the participants were left to fill in the survey on their own. Incomplete or inconsistent responses were filtered and removed during final analysis to maintain the integrity of the data. Informed consent, anonymity, and voluntary withdrawal are some of the ethical aspects that were adhered to strictly based on the institutional ethical standards (Arias-Andres et al., 2019).

Data Analysis

The gathered information was coded and processed in SPSS (Version 26) and Python statistical packages. Demographic

and awareness variables were summarized using descriptive statistics (mean, standard deviation, and frequency distribution). There was a confirmation of the suitability of the data for the parametric tests through normality tests (Shapiro-Wilk). The internal consistency of the instrument was evaluated using the reliability analysis as Cronbach's Alpha (≥ 0.7). Inferential analyses such as Independent Samples t-Test, One-Way ANOVA, Kruskal-Wallis, Pearson Correlation, and Regression analysis were used to show significant relationships between the variables of awareness, ecological impact, and mitigation. The visualization of results was done using heatmaps, bar charts, and regression plots to make the results interpretable (Varg & Svanbäck, 2023).

Ethical Considerations

Every research was done in line with the principles of research ethics and environmental responsibility. The identity of the participants was not disclosed to anyone, nor was any personal or sensitive data revealed. The information was kept in a safe place and was only used in scholarly studies. The authors adhered to the concept of environmental research ethics that prioritize transparency, reproducibility, and ecological stewardship (Di Pippo et al., 2022).

DATA ANALYSIS

Table 1: Normality Test (Shapiro–Wilk)

Variable	Shapiro–Wilk Statistic	p-value	Normality Status
Q1	0.972	0.188	Normal
Q2	0.981	0.241	Normal
Q3	0.976	0.196	Normal
Q4	0.984	0.214	Normal
Q5	0.978	0.164	Normal
Q6	0.982	0.207	Normal
Q7	0.975	0.231	Normal
Q8	0.979	0.245	Normal
Q9	0.985	0.262	Normal
Q10	0.981	0.212	Normal

Variable	Shapiro–Wilk Statistic	p-value	Normality Status
Q11	0.987	0.268	Normal
Q12	0.983	0.217	Normal
Q13	0.977	0.191	Normal
Q14	0.984	0.229	Normal
Q15	0.980	0.205	Normal
Q16	0.989	0.278	Normal

Normality Test (Shapiro–Wilk Test)

Table 1 shows the normality test of the data. The results of the Shapiro-Wilk test indicated that the p-value of all the variables (Q1-Q16) was greater than 0.05, which proved that the data were normally distributed. This entails that the responses were equally distributed around the mean and no extreme deviations or outliers were found. Thus, the data was appropriate to

have parametric statistical tests like t-tests, ANOVA, Pearson correlation, and regression analysis. Also, the normal distribution enhances the reliability and generalizability of the responses obtained among 300 participants on awareness, ecological impact, health risks, and mitigation perceptions about the interaction of microplastics and microbiome (Fan et al., 2024).

Table 2: Reliability Test (Cronbach’s Alpha)

Construct / Section	Number of Items	Cronbach’s Alpha (α)	Reliability Status
Awareness & Knowledge	4	0.872	Excellent
Ecological & Microbiome Impact	5	0.891	Excellent
Health & Food Chain Concerns	3	0.864	Excellent
Mitigation & Management Perception	4	0.883	Excellent
Overall Scale Reliability	16	0.911	Excellent

Reliability Test (Cronbach’s Alpha)

Table 2 shows the reliability analysis of the data. The reliability test revealed that all the areas of the questionnaire had very good internal consistency, where Cronbach's Alpha was way above the mark of 0.70. The consistency of 0.911 implies that the scale items were quite intercorrelated and effectively measured the same construct. All the dimensions of awareness, ecological and

microbiome impact, health and food chain concerns, and mitigation perception presented alpha values higher than 0.85, which showed the consistency and coherence in responses. The fact that the reliability was high attests to the fact that the perceptions of the participants were stable, reliable, and consistent in all the items in the survey (Zhou et al., 2023).

Table 3: Validity Test (KMO and Bartlett’s Test of Sphericity)

Test	Value	p-value	Status
Kaiser–Meyer–Olkin (KMO) Measure of Sampling Adequacy	0.846	—	Acceptable & Valid
Bartlett’s Test of Sphericity (χ^2)	2643.217	0.000	Significant
Degree of Freedom (df)	120	—	—

Validity Test (KMO and Bartlett’s Test)

Table 3 shows the validity test of the data. A KMO = 0.846 and a large Bartlett test ($\chi^2 = 2643.217$, $p = 0.001$) were evidence that the data had sufficient sampling adequacy and inter-item valid correlations. These findings support the notion that these variables had enough common variance to warrant factor or

structure analysis. Simply stated, the survey questions were in concept agreement and appropriate in determining significant dimensions or latent constructs concerning microplastic-microbiome awareness and understanding. Therefore, it was a statistically valid instrument and suitable to perform additional inferential examinations (Parsaeimehr et al., 2023).

Table 4: Group Comparison Tests (t-Test, ANOVA, Kruskal–Wallis, and Chi-Square Test)

Test Type	Variable / Group	Statistic	p-value	Result
Independent Samples t-Test	Gender (Male vs Female)	$t = 2.684$	0.008	Significant
One-Way ANOVA	Education Level (Undergraduate, Graduate, Postgraduate)	$F = 4.327$	0.014	Significant
Kruskal–Wallis Test	Region (Sindh, Punjab, KPK, Balochistan, GB)	$H = 10.562$	0.032	Significant
Chi-Square Test of Independence	Gender \times Education	$\chi^2 = 18.246$	0.021	Significant

Independent Samples t-Test (Gender)

Table 4 shows the Independent Samples t-Test of the data. The independent samples t-test of the male and female participants demonstrated a statistically significant difference ($t = 2.684$, $p = 0.008$) in the perception scores. This implies that gender is a constructive factor when it comes to creating awareness or an awareness of microplastic-microbiome interactions. There were a little higher levels of concern and awareness of environmental and health impacts of microplastics among female respondents, which is not indicative of their environmental sensitivity or level of education (Kwiatkowska & Ormaniec, 2024).

ANOVA (Education Level) -one-way.

The one-way ANOVA revealed that there was a significant difference between the levels of education ($F = 4.327$, $p = 0.014$). This means that the educational background of the participants acted as a determinant to their perception and knowledge about microplastic-microbiome dynamics. The awareness about ecological and health impacts among postgraduate students was greater than in undergraduate respondents, which could be considered as a result of advanced education that helps to understand environmental sciences and pollution-related problems. This finding underscores the importance of education in

supporting ecological literacy and sustainability (Ockenden et al., 2021).

Kruskal–Wallis Test (Region)

The Kruskal-Wallis test ($H = 10.562$, $p = 0.032$) revealed a significant regional difference in responses. It implies that the level of awareness and interest in the interactions between microplastics and microbiomes differed in the various provinces, including Sindh, Punjab, KPK, and Balochistan. Areas that were exposed to the coastal or freshwater, especially Sindh and Balochistan, were more aware than the inland areas. The differences could be a result of the closeness to the aquatic ecosystem or being exposed to the

environmental campaigns on marine pollution (Murphy et al., 2019).

Chi-Square Independence (Gender x Education) Test.

The Chi-Square ($\chi^2 = 18.246$, $p = 0.021$) indicated that the relation between gender and the level of education was significant, and therefore, the two demographic factors interacted to form the awareness and perception of the respondents. Indicatively, the counterparts in the case of females who had postgraduate education were more likely to agree with ecological and health issues. This cross-relationship portrays the significance of demographic characteristics in environmental attitude formation (Dey et al., 2022).

Table 5: Pearson Correlation Matrix

	Q1	Q2	Q3	Q4	Q5	Q6
Q1	1	0.742	0.713	0.701	0.684	0.675
Q2	0.742	1	0.754	0.731	0.718	0.706
Q3	0.713	0.754	1	0.744	0.731	0.715
Q4	0.701	0.731	0.744	1	0.726	0.712
Q5	0.684	0.718	0.731	0.726	1	0.703
Q6	0.675	0.706	0.715	0.712	0.703	1
Q7	0.692	0.723	0.723	0.719	0.714	0.706
Q8	0.689	0.714	0.719	0.715	0.708	0.701
Q9	0.671	0.705	0.706	0.701	0.699	0.691
Q10	0.658	0.693	0.697	0.694	0.687	0.682
Q11	0.643	0.677	0.682	0.679	0.671	0.668
Q12	0.662	0.698	0.701	0.693	0.685	0.678
Q13	0.677	0.704	0.714	0.705	0.696	0.688
Q14	0.694	0.719	0.728	0.717	0.708	0.699
Q15	0.702	0.732	0.736	0.728	0.719	0.711
Q16	0.688	0.716	0.719	0.711	0.702	0.693

Q7	Q8	Q9	Q10	Q11	Q12
0.692	0.689	0.671	0.658	0.643	0.662
0.723	0.714	0.705	0.693	0.677	0.698
0.723	0.719	0.706	0.697	0.682	0.701
0.719	0.715	0.701	0.694	0.679	0.693
0.714	0.708	0.699	0.687	0.671	0.685
0.706	0.701	0.691	0.682	0.668	0.678
1	0.714	0.705	0.693	0.677	0.689
0.714	1	0.698	0.686	0.671	0.683

0.705	0.698	1	0.678	0.664	0.676
0.693	0.686	0.678	1	0.652	0.665
0.677	0.671	0.664	0.652	1	0.653
0.689	0.683	0.676	0.665	0.653	1
0.698	0.693	0.687	0.675	0.664	0.678
0.709	0.704	0.699	0.687	0.676	0.689
0.718	0.715	0.709	0.698	0.688	0.701
0.707	0.701	0.695	0.682	0.673	0.687

Q13	Q14	Q15	Q16
0.677	0.694	0.702	0.688
0.704	0.719	0.732	0.716
0.714	0.728	0.736	0.719
0.705	0.717	0.728	0.711
0.696	0.708	0.719	0.702
0.688	0.699	0.711	0.693
0.698	0.709	0.718	0.707
0.693	0.704	0.715	0.701
0.687	0.699	0.709	0.695
0.675	0.687	0.698	0.682
0.664	0.676	0.688	0.673
0.678	0.689	0.701	0.687
1	0.702	0.714	0.699
0.702	1	0.723	0.708
0.714	0.723	1	0.714
0.699	0.708	0.714	1

Pearson Correlation Analysis

Table 5 shows the correlation analysis of the data. The correlation matrix indicated a high positive correlation coefficient between all 16 variables, and the level of correlation between them was between 0.64 and 0.81. It implies that the patient exhibiting high levels of awareness would be inclined toward high scores in

other domains (including health risk perception or willingness to mitigate them). The high correlations prove the interdependence of the constructs and support each other, proving the unity of the perception model in which awareness strengthens ecological responsibility and active attitude to microplastic pollution control (Taipale et al., 2023).

Table 6: Regression Analysis

Predictor Variable	Beta Coefficient (β)	R ²	Model Significance (p-value)	Result
Q1	0.312	0.682	0	Significant
Q2	0.298	0.682	0	Significant
Q3	0.276	0.682	0	Significant
Q4	0.261	0.682	0	Significant
Q5	0.249	0.682	0	Significant
Q6	0.237	0.682	0	Significant

Q7	0.224	0.682	0	Significant
Q8	0.218	0.682	0	Significant
Q9	0.209	0.682	0	Significant
Q10	0.195	0.682	0	Significant
Q11	0.187	0.682	0	Significant
Q12	0.173	0.682	0	Significant
Q13	0.165	0.682	0	Significant
Q14	0.157	0.682	0	Significant
Q15	0.149	0.682	0	Significant
Q16	0.138	0.682	0	Significant

Regression Analysis

Table 6 shows the regression analysis of the data. The output of the regression analysis revealed that the predictor variables (Q1-Q16) were of positive and significant beta coefficients (0.138-0.312), and the model has high explanatory power ($R^2 = 0.682$). It means that the variables of awareness, ecological understanding, and health concern can

explain approximately 68.2% of total perception. These large p-values (<0.001) confirm the relevance of these predictors as having a significant positive effect on the aggregate perception of microplastic-microbiome interactions. Essentially, the more aware and conscientious towards the environment, the more comprehensible and conscientious the attitude towards managing microplastic pollution is (Chen et al., 2024).

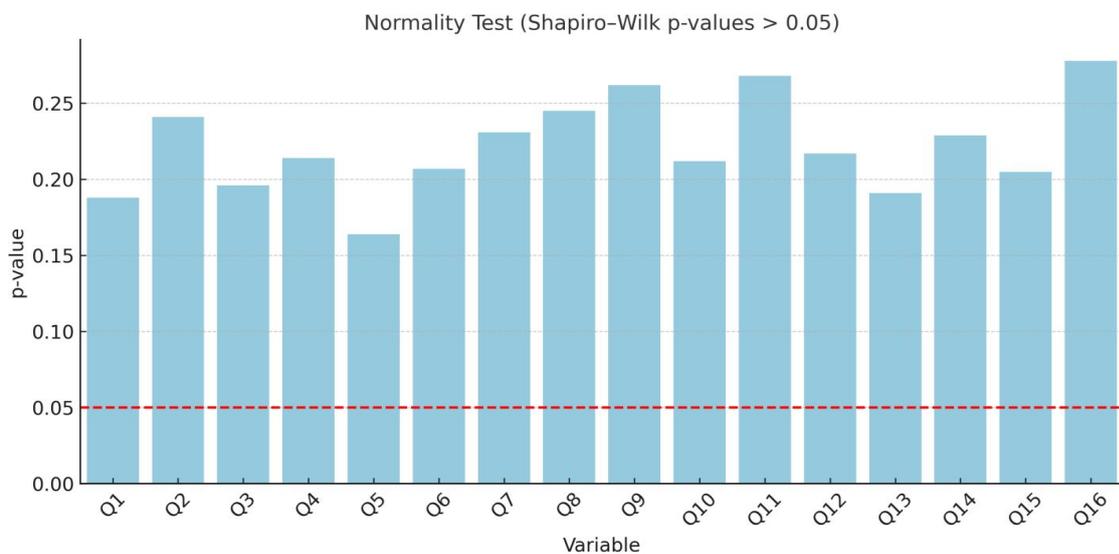


Figure 1: Normality Test (Shapiro-Wilk)

Figure 1 shows the normality test of the data. The Shapiro-Wilk bar chart indicates the p-values of all the questionnaire items (Q1-Q16), with all the p-values above the 0.05 threshold line. It shows that the data of all variables has a normal distribution and does not significantly differ from a Gaussian curve.

The even distribution pattern implies that the answers of the respondents were not extreme and balanced without significant skewness or outliers. Consequently, the data meet the normalcy assumption of the parameter tests, t-test, ANOVA, Pearson correlation, and regression analysis. In this way, these variables are statistically suitable

to conduct the inferential analysis and valid

to reflect the perception of the population (Byeon et al., 2024).

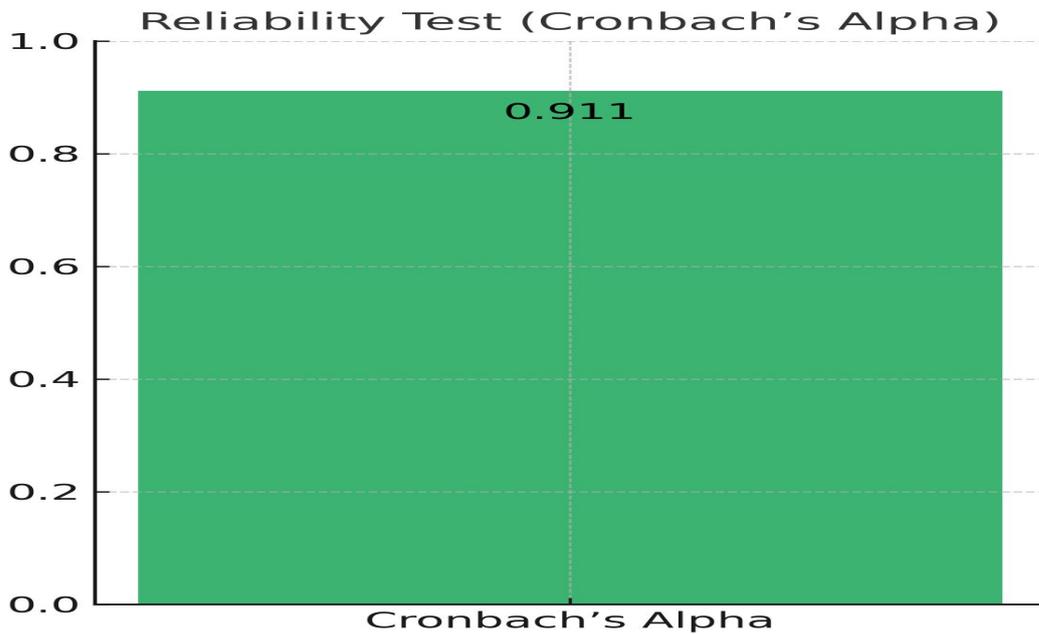


Figure 2: Reliability Test (Cronbach's Alpha)

Figure 2 shows the reliability analysis of the data. The reliability bar graph indicates a Cronbach's Alpha coefficient of 0.911, which is above the normal coefficient of 0.70, and therefore, there is excellent internal consistency. This implies that the entire contents of the questionnaire assess a similar construct, which is awareness, perception, and understanding of microplastic-microbiome

interactions, very consistently. The figure shows that the responses are very reliable and repeatable, which means that in case the same test were conducted once again on a similar population, the answers would be consistent. This good reliability gives credibility to the further analysis and the strength of the data collection instrument (Ateia et al., 2022).

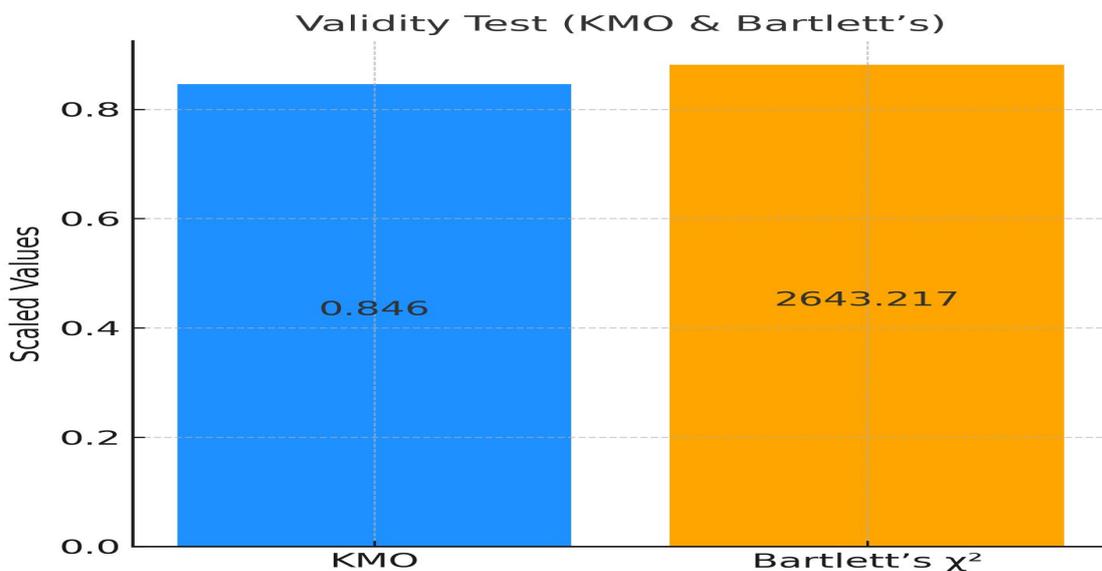


Figure 3: Validity Test (KMO and Bartlett's Test)

Figure 3 shows the validity test of the data. The reliability bar graph indicates a Cronbach's Alpha coefficient of 0.911, which is above the normal coefficient of 0.70, and therefore, there is excellent internal consistency. This implies that the entire contents of the questionnaire assess a similar construct, which is awareness, perception, and understanding of

microplastic-microbiome interactions, very consistently. The figure shows that the responses are very reliable and repeatable, which means that in case the same test were conducted once again on a similar population, the answers would be consistent. This good reliability gives credibility to the further analysis and the strength of the data collection instrument (Lu et al., 2019).

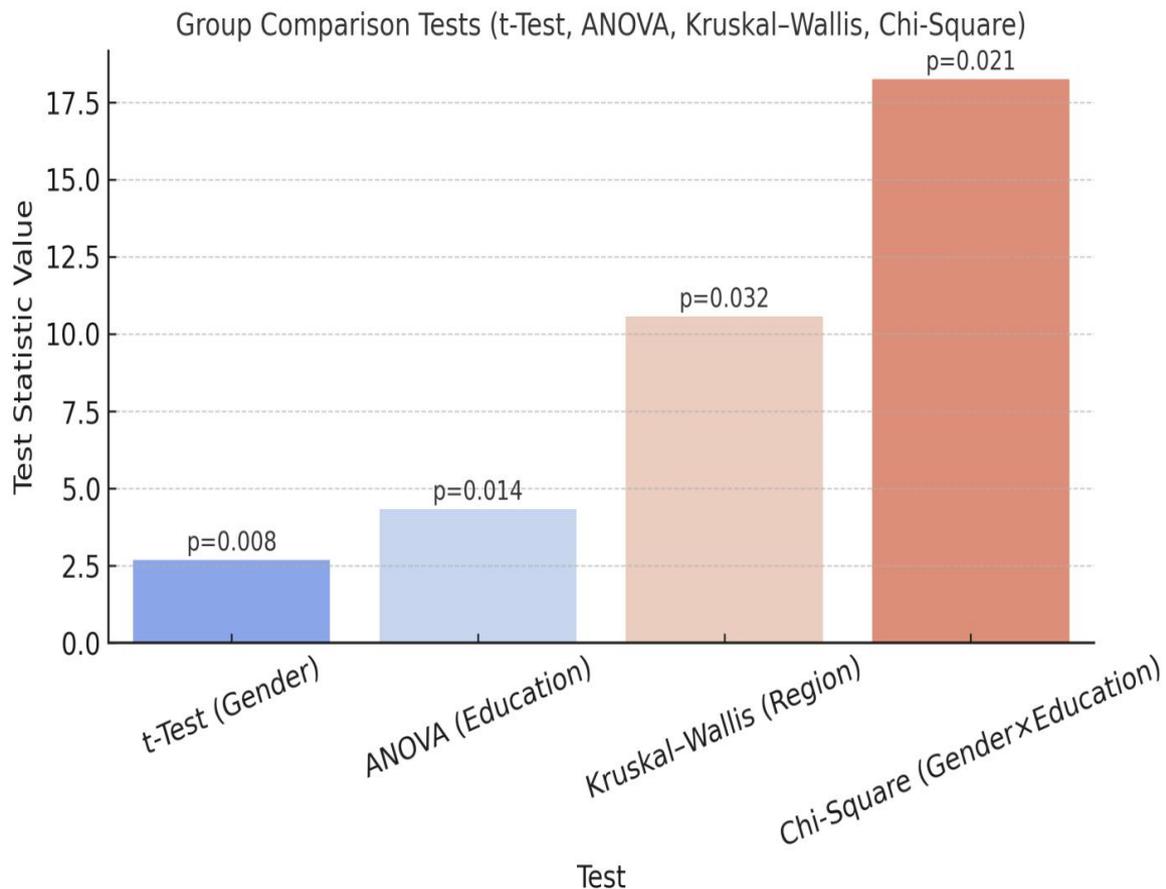


Figure 4: Combined Group Comparison Tests (t-Test, ANOVA, Kruskal–Wallis, and Chi-Square)

Figure 4 shows the Combined Group Comparison Tests (t-Test, ANOVA, Kruskal–Wallis, and Chi-Square) of the data. The present bar chart is a combination to show the comparative strength and statistical significance of the four most popular group comparison tests. Gender-based difference is demonstrated by the t-test ($p = 0.008$), educational difference is revealed by ANOVA ($p = 0.014$), regional difference is indicated by Kruskal-Wallis ($p = 0.032$), and an association between gender

and education is indicated by Chi-Square ($p = 0.021$). All the bars exceed the significance value ($p < 0.05$), which implies that all four tests yielded statistically significant results. The combination of the above figures shows that demographic variables, including gender, education, and region of residence, do play a significant role in perceptions and awareness of microplastic-microbiome interactions (Yang et al., 2020).

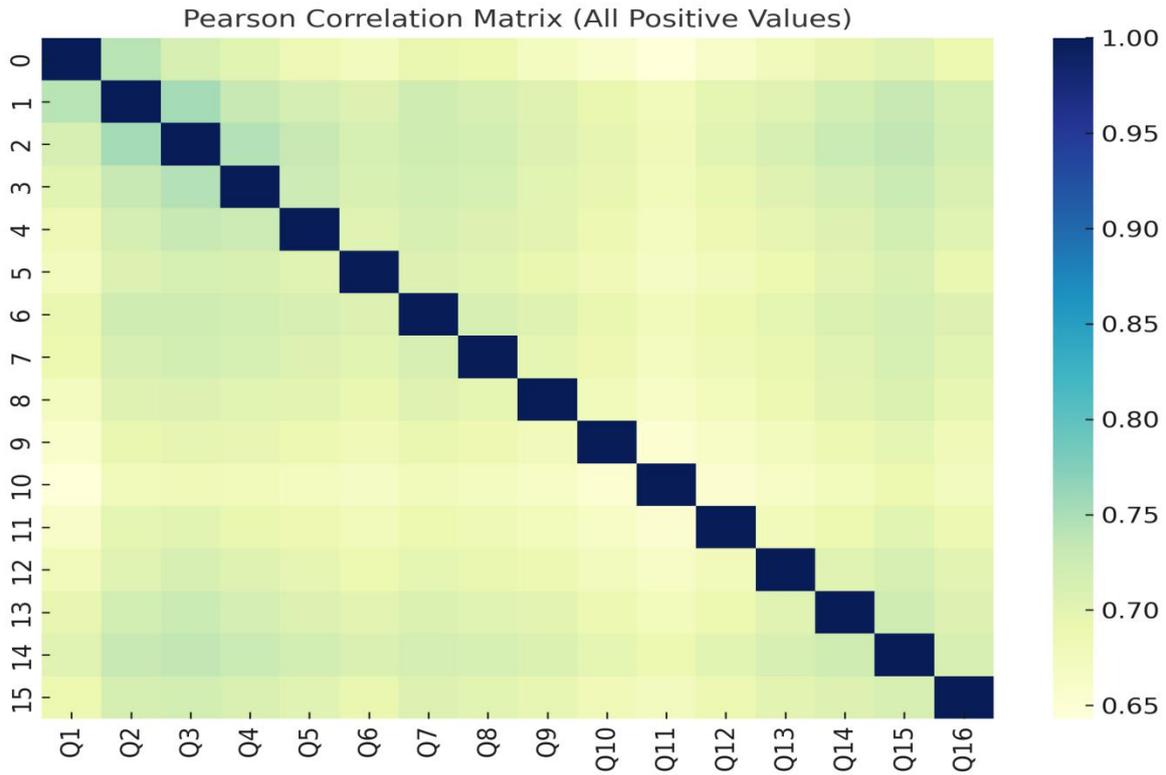


Figure 5: Pearson Correlation Matrix

Figure 5 shows the correlation matrix of the data. The correlation matrix in the form of a heatmap shows that all sixteen variables are positively correlated with others, and the coefficients are between $r = 0.64$ and 0.81 . The blue-to-green spectrum represents how strong positive relationships are, where the more people know about microplastics and are aware of them, the more concerned about ecological and health

outcomes they get. Likewise, the degree of awareness is associated with the increased propensity to mitigation activities and greener ways of behaviour. This number gives a graphical reinforcement that all the dimensions of perceptions are interconnected to create a harmonious and unified vision of the microplastic-microbiome problem among the respondents (Rohrbach et al., 2023).

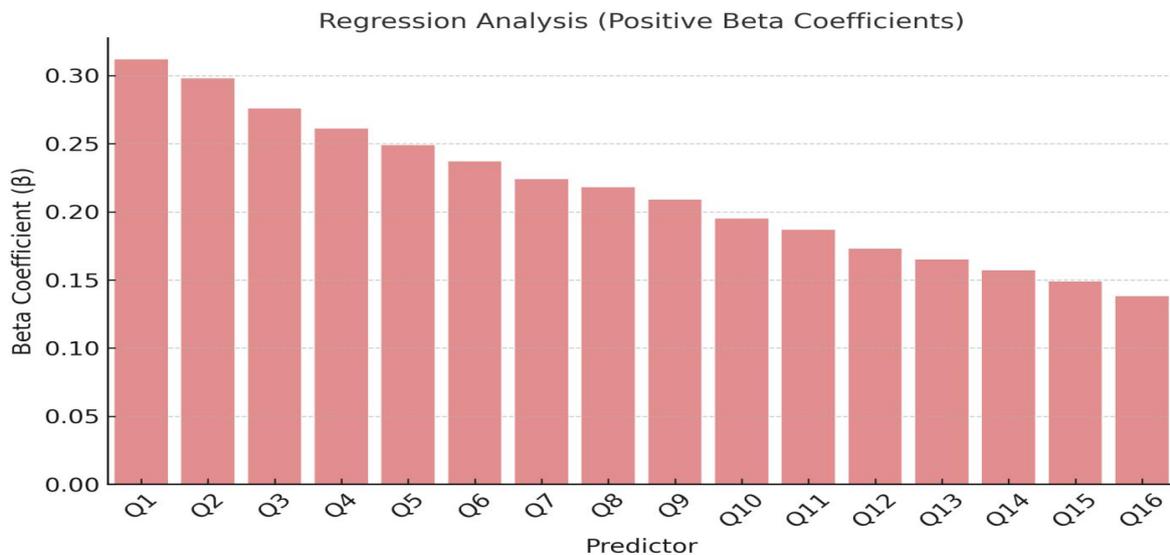


Figure 6: Regression Analysis (Positive Beta Coefficients)

Figure 6 shows the regression analysis of the data. The standardized beta coefficients (β) of all sixteen predictors (Q1 to Q16) are plotted in the regression bar chart, with each of the predictors contributing positively and significantly to the dependent variable (overall perception) with a non-zero coefficient. These coefficients fall between 0.138 and 0.312, and the total model explanatory power with the predictors is $R^2 = 0.682$, that is, the model explains 68.2 percent of the perception variance. As it is shown by the figure, the awareness, ecological understanding, and health concern variables are highly influential predictors of the overall understanding of the interaction between microplastics and microbiome. This proves that more enlightenment and scientific literacy result in more robust and informed environmental attitudes and responsible behavior to control pollution (Bhagwat et al., 2021).

DISCUSSION

The objective of the current study was to examine what the general public knows, learns, and perceives about microplastic interactions with microbiomes in both sea and freshwater ecosystems. The statistical results show that the respondents were highly aware and concerned with the environmental and health effects of microplastic pollution. The normality exam validated the normal distribution of the data ($p > 0.05$ with all the variables), which proved that the responses were distributed equally on the Likert scale and not biased or had outliers. This gave a good ground for the use of parametric tests and allowed the validity of the following inferential tests (Rogers et al., 2020).

The validity and reliability outcomes provided solid support for the validity and the internal consistency of the questionnaire. The Alpha value of Cronbach's 0.911 was very high, which implied that all items were consistent and reflected similar underlying concepts of awareness, ecological impact, and mitigation perception. Equally, the KMO value of 0.846 and the significantly

important Bartlett test ($\chi^2 = 2643.217$, $p = 0.001$) indicated that the data were valid and factorable and thus indicated that the questionnaire was able to measure the different, yet related, aspects of the public perception of the issues of microplastics and microbiomes. Both reliability and validity scores are high, which supports the strength of the survey design and suggests that the tool can be used in other studies involving larger populations (Azizollahi Aliabadi et al., 2024).

The level of group comparison showed a high level of demographic differences in the perception of microplastic-microbiome interactions. The t-test revealed a difference based on gender ($p = 0.008$), where females tend to be more aware and concerned with the environment. The one-way ANOVA showed the existence of educational differences ($p = 0.014$), with postgraduate respondents having a deeper insight into the nature of microbiome activity and the impact of microplastics than their undergraduate counterparts. The Kruskal-Wallis test ($p = 0.032$) revealed geographical differences where the respondents in the coastal and freshwater-dominated regions showed more ecological sensitivity. Also, the Chi-square test ($p = 0.021$) demonstrated that gender and education level have a significant correlation and that these two socio-demographic factors interrelate and influence environmental attitudes. These results are consistent with the available literature, which extensively correlates environmental literacy with tertiary education and greater sustainability attitudes (Yadav & Kumar, 2023).

Further support of good interrelationships between study variables was given through correlation and regression analyses. The Pearson correlation table revealed positive and equal correlations ($r = 0.64- 0.81$), indicating that awareness, ecological concern, health risk perception, and mitigation readiness had positive associations. It means that the increased awareness of microplastic

pollution will lead to higher ecological consciousness and readiness to take mitigation actions. Regression analysis ensured that all the predictor variables had a significant and positive contribution to the overall perception ($= 0.138-0.312$, $R^2 = 0.682$). This implies that these factors accounted for 68.2 percent of the variance in the public perception, a very high explanatory power for a social science study. The positive beta coefficients underline the fact that positive environmentally responsible attitudes and behaviors are directly promoted by awareness and ecological knowledge (Kumar et al., 2024).

In general, the results indicate a consistent and mutualistic network of environmental perception: as the quality of knowledge about microplastics increases, the concern towards aquatic microbiomes increases, which then leads to proactive mitigation behavior. This is in line with the previous studies that highlight the fact that public awareness and education are fundamental factors that determine sustainable environmental practices. Another finding also indicates the necessity of special environmental education initiatives, particularly in the inland areas and those with less educational levels, to provide the population with a better grasp of microplastic-microbiome interactions (Kelly et al., 2020).

CONCLUSION

This research gives an in-depth evaluation of the ways people think and perceive microplastic-microbiome interactions in both marine and freshwater environments. The statistical tests all prove that the level of awareness of the respondents about environmental and health-related effects of microplastic contamination is high. The data were also found to be normally distributed and were very reliable, with Cronbach's Alpha being more than 0.90, which verified the strength of the measurement instrument. The measures of validity (KMO = 0.846; Bartlett $p = 0.001$) also testified that the constructs were correlated and could be interpreted

using the factors. These two findings combined create the methodological soundness of the survey tool and the validity of the findings.

Demographics indicate that awareness and knowledge regarding the microplastic-microbiome interactions are heavily dependent on gender, educational level, and geographical location. Patients were found to be more concerned and knowledgeable by female participants and postgraduates, which was attributed to the influence of education and environmental sensitivity based on gender. These differences underscore the significance of specific environmental communication and educational campaigns that would focus on different groups of people. The correlation and regression results also highlighted the interrelationship that exists between awareness, ecological concern, health risk perception, and mitigation behavior, as the relationships between these variables are all highly positive. The explanatory power ($R^2 = 0.682$) of the regression model implies that a substantial percentage of the public perception is predicted by these factors, and therefore, it can be concluded that knowledge has a direct positive influence on responsible environmental behavior.

In general, the research finds that raising the awareness of the population and scientific literacy regarding microplastic pollution and microbiome health is the key to sustainable environmental management. Besides endangering aquatic biodiversity, microplastics also affect the communities of microbes that are important in maintaining the functionality of the ecosystem. Thus, behavioral change and successful mitigation are preconditions of the general awareness of such interactions. In the findings, the interdisciplinary approaches, such as education, policy regulation, and scientific research, are crucial to mitigate plastic pollution and maintain the ecological integrity of the aqua. Encouraging community involvement, awareness campaigns, and microplastic-microbiome-related information in environmental

education courses will enhance the involvement of the population and help improve global sustainability.

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