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MICROPLASTIC: PREVELANCE, ABUNDANCE AND BACTERIAL INTERACTION AT THE LOCAL AND COASTAL REGION OF SAURASHTRA

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Abstract:

Microplastics represent one of the most pervasive and challenging environmental threats of the 21st century. Plastic was originally engineered for its durability and versatility; these same traits have led to a global accumulation of microscopic debris that now infiltrates every level of the ecosystem. Microplastic contamination has become a growing environmental issue due to its interaction with microbial communities. This study aims to evaluate the presence, abundance, and influence of microplastic on bacterial community from selected environmental samples. Here, microplastics were extracted using density separation and chemical digestion methods, followed by microscopic quantification and characterisation based on size and morphology. The microbial load associated with isolated microplastics was evaluated using the Standard Plate Count (SPC) technique for total viable microorganisms and the Most Probable Number (MPN) method for indicator bacteria. The total number of microplastics present in sample is comparatively more than SPC and MPN. A higher microbial population is anticipated on microplastic particles compared to the surrounding matrices. Further investigation will be conducted to study the type of micro plastics and their abundance.

Keywords: Microplastic contamination, Microbial load, SPC, MPN, Environmental sample.

1. Introduction

Microplastics are defined as plastic particles smaller than 5 mm, ranging from millimetre-sized fragments to nano-plastics that can penetrate biological barriers. While secondary microplastics are created when plastic packaging, synthetic textile fibres, wastewater-derived debris, and agricultural plastics break down through physical, chemical, and biological processes, primary microplastics are integrated into consumer and industrial products such as cosmetics and detergents, agriculture, and textiles. Their transport behaviour, environmental persistence, and interactions with biological systems are significantly influenced by key properties such as particle size, shape, and polymer composition (Chaudhary et al., 2025). After being released, these particles are carried by aquatic systems, ocean circulation, wind-driven movement, and atmospheric deposition across interconnected environmental compartments, allowing for dispersal to far-flung locations such as deep-sea sediments and polar environments (Meng et al., 2025; Lee et al., 2025).

Large amounts of plastic trash have accumulated in natural areas as a result of the quick growth of plastic production worldwide, ineffective waste management, and a lack of recycling capability. Microplastics persist over an extended period of time because plastics gradually break up into tiny pieces rather than fully mineralizing. Microplastics have become a notable indicator of anthropogenic change in natural systems due to their prolonged buildup (Meng et al., 2025; Shi et al., 2025). Microplastics have been found in almost every environmental matrix, including freshwater and marine systems, soils, sediments, and the atmosphere, according to recent studies. Because of their small size and low density, they may spread widely through the air, water, and biological movement, enabling transmission between aquatic and terrestrial environments. Additionally, evidence from sediment core records highlights the global spread of microplastics and shows that they have been present in natural systems for several decades (Meng et al., 2025; Zhang et al., 2025). In order to better understand their spread and assist efficient management tactics, recent advancements in detection and

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remediation techniques have been helpful (Shi et al., 2025).

Microplastics have a variety of ecological and biochemical impacts on ecosystems. Multiple trophic levels of species can easily consume them, which makes it easier for them to accumulate and move through food webs. Furthermore, adsorbed pollutants and chemicals linked to plastic can increase toxicity, cause oxidative stress, and disturb physiological processes. Key biogeochemical processes in aquatic and terrestrial settings are altered by microplastics, which also affect the makeup of microbial communities and the cycling of nutrients, especially carbon and nitrogen (Basumatary et al., 2025; Rillig et al., 2025).

Microbial attachment is facilitated by the physicochemical characteristics of microplastic surfaces, including hydrophobicity, surface imperfections, oxidation caused by weathering, and contaminant adsorption. The taxonomic makeup and functional characteristics of microbial communities that grow on microplastic surfaces—collectively referred to as the plastisphere—are different from those of microbial populations that live freely. Because of the selective requirements imposed by plastic-associated microenvironments, biofilm-forming and stress-tolerant species frequently dominate plastisphere assemblages. This suggests that rather than only serving as passive carriers, microplastics actively influence microbial ecology (Meng et al., 2025). Microbial survival, metabolic activity, and ecological function are significantly affected by these surface-mediated interactions (Shi et al., 2025).

In addition to their impact on the environment, microplastics also pose a risk to human health. Inhaling airborne particles, coming into contact with contaminated settings, and consuming contaminated food or drinking water are some of the ways that intake can happen. Although precise exposure thresholds in people are still unclear, experimental research has connected extended exposure to oxidative stress, inflammatory reactions, immunological disruption, and possible organ-specific consequences. Accurate risk assessment is nevertheless limited by ongoing difficulties in detecting nanoplastics and the absence of standardised analytical techniques (Zhang et al., 2025; Chaudhary et al., 2025).

The ecosystem and human health are at risk from wastewater contamination on a biological and physical level. Because microplastics are persistent and non-biodegradable, they contribute to physical pollution (Chaudhary et al., 2025). Elevated microbial loads also make waterborne diseases more likely. When high bacterial counts and microplastics coexist, the risk becomes more serious, especially when Most Probable Number (MPN) values surpass 1000 MPN/100 mL, which indicates poor water quality (WHO, 2017). These tainted water sources are frequently linked to illnesses like cholera, typhoid, dysentery, and diarrhoea. Microplastics also encourage the production of biofilms, serve as repositories for genes that cause antibiotic resistance, and carry dangerous bacteria (Amaral-Zettler et al., 2020). Consumption of tainted drinking water, irrigation of crops with contaminated wastewater, and bioaccumulation in the aquatic food chain are all ways that humans can be exposed.

Rose Bengal staining's affinity for biological materials rather than synthetic polymers has made it an excellent method for separating microplastics from organic detritus (Hidalgo-Ruz et al., 2012). Besides the Rose Bengal staining method, the sucrose gradient method for microplastic study can be used (Imhof et al., 2012; Claessens et al., 2013). The Most Probable Number (MPN) method is a common technique for identifying coliform contamination in water (Tripathi et al., 2025), whereas the Standard Plate Count (SPC) is frequently used to estimate viable heterotrophic bacterial populations (Fitri et al., 2020).

2. Materials and Methods:

Microplastics were extracted and identified using digestion, density separation, and Rose Bengal staining. Bacterial load was

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enumerated by the Standard Plate Count (SPC) method. Further, Coliform presence was estimated using the Most Probable Number (MPN) technique.

2.1 Materials:

In the presented study, all the chemicals used were procured from Rankem, India, while the bacteriological media used were purchased from Hi-Media, India.

2.2 Methods:

2.2.1 Sample collection

The samples for the study were collected from the different sites representing possibilities of finding microplastics. The sample sites selected for the study were – Sewerage cannel near Munjka village (for four months), and coastal regions of Saurashtra region (Somnath, Porbandar, Jamnagar and Kutch). Furthermore, the study was extended to understand the rate of plastic leaching, for which the three 1-litre glass beakers were taken – the first beaker was filled with soil, the second was filled with tap water, and the third one was filled with seawater. An equal number of plastic particles of different sizes (1 mm - 5 mm) were added to all the beakers and kept in the laboratory under natural conditions. The water was regularly refilled in the beaker to maintain the volume. The samples from these three beakers were taken at regular intervals and studied to reveal the abundance of the microplastic.

2.2.2 Prevalence & Abundance of microplastics analysed using the Rose Bengal staining method

In order to prevent contamination and physicochemical changes, wastewater samples were gathered in sterile glass vials. Chemical digesting technique, based on hydrogen peroxide oxidation, was used to eliminate organic debris in order to reduce polymer deterioration. The potassium hydroxide (KOH) method was used to digest organic matter. The digestion of organic matter was done according to the Foekema et al. (2013) and Dehaut et al. (2016) method, briefly, 1 L of sample was treated with 10% KOH and incubated at 60°C for 24 to 48 hours to ensure efficient destruction of biological materials. After digestion, saturated sodium chloride (NaCl) solution was added for density separation. The mixture was thoroughly mixed and left undisturbed for a full day to allow low-density microplastics to float (Thompson et al., 2004).

After being meticulously gathered and passed through a 0.45 µm membrane filter, the supernatant was allowed to air dry at ambient temperature. The filter was treated with Rose Bengal solution for five to ten minutes to distinguish organic waste, and excess stain was then gently washed off. Microplastic particles remained unstained and were seen as translucent. The Rose Bengal staining technique, which shows variations in staining behaviour and morphology, was used to differentiate microplastics from biological remains. While microplastics stay transparent, biological materials absorb the dye and turn pink to red. While biological remains exhibit uneven forms and tissue-like characteristics, microplastics have uniform colours, smooth edges, and no cellular structure. In microscopic examination, these features make it possible to distinguish between manufactured particles and organic waste.

2.2.3 Effect of microplastics on bacteria

2.2.3.1 Standard plate counts (SPC)

The effect of microplastics on bacterial load was studied by preparing serial dilutions from a sample, followed by spreading on an N-agar plate. A Nutrient Agar plate was inoculated with 0.1 mL of the diluted sample, followed by incubation at 37°C for 24 to 48 hours. The bacterial number was calculated by counting the colonies after incubation and using the formula.

CFU/mL = (Number of colonies × Dilution factor) / 0.1 (APHA, 2017).

2.2.3.2 Most probable number (MPN)

The Most Probable Number (MPN) method calculates coliform levels using MacConkey broth. A 50 mL flask and one set of 10ml (5 tubes) of double-stranded MacConkey broth,

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and the remaining 2 sets of 5ml each of (5 tubes) of single-stranded MacConkey broth. 50ml of sample in a flask and three sets of tubes with sample quantities of 10 mL, 1 mL, and 0.1 mL, respectively, were incubated at 37°C for 24 to 48 hours. Gas production was observed in Durham's tub, and the colour change showed a positive result. Coliform contamination was verified by subsequent subculturing, which produced gas and acid. A conventional probability table based on positive tube counts was used to determine the results, which were represented as MPN per 100 mL of water (Cappuccino & Sherman, 2014).

3. Results and Discussion:

3.1 Abundance and distribution of microplastics in different samples:

The abundance of microplastics was studied by the Rose Bengal staining method, which proved to be very effective by lowering the possibility of false-positive identification by selectively colouring organic matter. Rose Bengal staining improved microplastic detection overall (Thompson et al., 2004). Microscopically, biological materials and organic detritus showed brilliant pink to red colour after Rose Bengal staining, but manufactured microplastic particles were either barely colored or mostly unstained. The accuracy of visual recognition was increased by this contrast, which made it easier to distinguish between plastic particles and natural organic matter (Claessens et al., 2013). The majority of microplastics were found to be threads and fragments of different colours with varying sizes. Particles subjected to environmental conditions occasionally displayed mild surface staining as a result of biofilm attachment; however, the stain was not absorbed by the polymer core (Imhof et al., 2012).

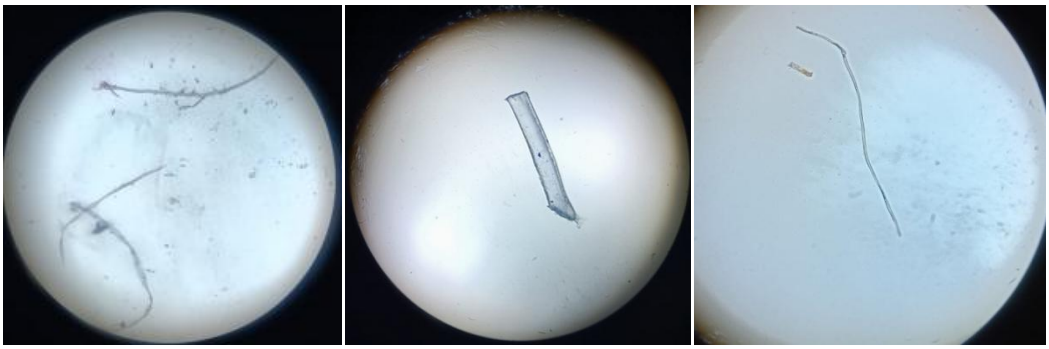


Figure 1: Microscopic observation of microplastic using Rose Bengal Staining method.

Microplastic analysis employs the rose bengal staining method to differentiate organic matter from plastic particles. The dye stains biological materials pink or red, enhancing visual identification under a microscope, and reduces misidentification in samples with significant organic detritus.

High microplastic abundance was shown by Rose Bengal staining, which successfully separated organic matter from plastic particles in a variety of samples. Microplastics remained in treated effluents despite treatment procedures, corroborating previous research on their ubiquity in diverse settings and capacity for microbial colonisation.

Ocean water had the greatest quantities of microplastics (1450 particles/L), followed by soil (1200 particles/L) and tap water (1150 particles/L). Amongst all the studied samples, high amounts of microplastics were found in the coastal region of Kutch (760 particles/L). Other sites with moderate microplastic concentrations were Somnath, and the sewerage canal near Munjka village. In

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general, seasonal samples were more impacted, demonstrating the environmental persistence of plastics despite limited treatment effectiveness.

3.2 Influcence of microplastics on bacteria

3.2.1 Spread plate count (SPC)

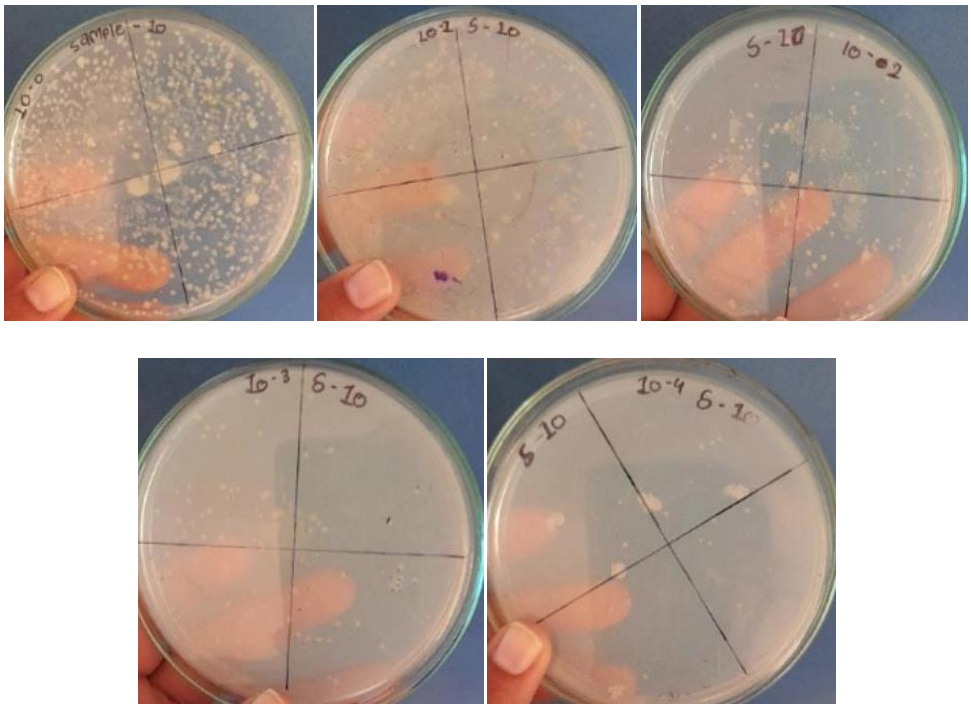


Figure 2: Bacterial colonies observed during Spread Plate Count.

The Standard Plate Count (SPC) is a microbiological technique that counts live bacteria to assess the microbial burden. Because organic materials encourage the growth of bacteria, high SPC values signify considerable organic contamination. Since a decrease in bacteria signifies successful germ eradication, SPC is useful for tracking the efficacy of treatment.

The SPC findings show a strong positive correlation between the concentration of microplastics and bacterial loads. Elevated SPC values, which peak at 2,975,000 CFU/mL, are correlated with higher microplastic counts, whereas lower counts result in lower SPC levels. This implies that microplastics can promote the adhesion and development of biofilms by bacteria.

The analysis of SPC results at several locations showed notable variations in pollution levels and bacterial load. The most polluted coastal site was Kutch, which had the greatest SPC (2,975,000 CFU/mL), followed by the sewage canal near Munjka village (January) at 2,209,000 CFU/mL. Somnath exhibited less contamination (30,000 CFU/mL), whereas Jamnagar (105,000 CFU/mL) and sewerage canal near Munjka village (October, 370,000 CFU/mL) had moderately high SPC levels. The least contaminated samples came from Chopati, Porbandar (4,000 CFU/mL). In comparison to untreated samples, this investigation demonstrates how well treatment systems work to lower microbial burdens in treated water.

3.3 Most Probable Number (MPN)

The Most Probable Number (MPN) method uses repeated dilution to identify gas and acid production in broth media in order to estimate coliform bacteria. Effective microbiological clearance is demonstrated by a decrease in

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MPN after treatment; results are given as MPN per 100 mL.

Microplastic abundance and MPN (coliform levels) had an inconsistent connection; some high microplastic samples showed elevated MPN values, whereas those with moderate or lower quantities also showed high coliform levels. This suggests that rather than being exclusively associated with microplastics, faecal contamination is influenced by several environmental factors, such as sewage inflow and outflow. MPN values were lowered by treatment procedures, although the presence of coliforms in treated samples indicates partial elimination. Thus, in addition to the effects of microplastics, a variety of environmental factors affect coliforms.



Figure 3: MPN analysis of sample collected form sewerage of Munjka village

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The highest MPN was observed as 1600 coliforms/ml at places like the sewerage canal near Munjka village. The comparative analysis of MPN values shows that the most contaminated samples in terms of faecal contamination was sewerage from Munjka village and Arabian Sea (Kutch) was the most polluted coastal region with MPN 280.

3.4 Site wise corelation of the outcomes:

The relationship between microplastics and bacterial populations in various environments was studied. High microplastic levels and a moderate to high bacterial load coexisted in Somnath, suggesting some positive correlation between microplastics and bacterial growth. Similarly, Chopati demonstrated that bacterial colonisation is supported by even very low plastic concentrations. The presence of microplastics and bacterial burden was positively correlated in Jamnagar, supporting the idea that microplastics served as colonisation sites. High microbial counts with high microplastic presence were found by Kutch, indicating increased microbial adhesion. At the sewage canal near Munjka village, seasonal increases in bacteria and microplastics were noted, highlighting the function of microplastics in sustaining biofilm ecosystems and the impact of human interferences. Overall, the results show that microplastics promote rather than prevent bacterial growth in a variety of samples.

Microplastics serve as hydrophobic, inert surfaces that microorganisms quickly colonise to create organised biofilms called plastispheres (Zettler et al. 2013). According to research, rather than actively encouraging or preventing bacterial development, plastics largely enhance microbial populations by providing surfaces for attachment (Oberbeckmann et al., 2015). Furthermore, it has been demonstrated that thick and metabolically active bacterial consortia that are different from the surrounding water populations are present in plastic-associated biofilms (Amaral-Zettler et al., 2020).

Higher concentrations of microplastics were consistently associated with higher SPC values in the current investigation, indicating that microplastics do not inhibit bacterial growth. Rather, they probably behave as physical

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substrates that promote the production of biofilms and bacterial adhesion. Therefore, rather than directly affecting growth dynamics, the observed positive association suggests that microplastics function as colonisation platforms and transporters, promoting bacterial survival and perhaps raising microbial burden in polluted settings.

3.4.1 Sewerage water sample:

We had taken sample from sewerage canal near Munjaka village for four times – September, October, November and January to check the seasonal difference.

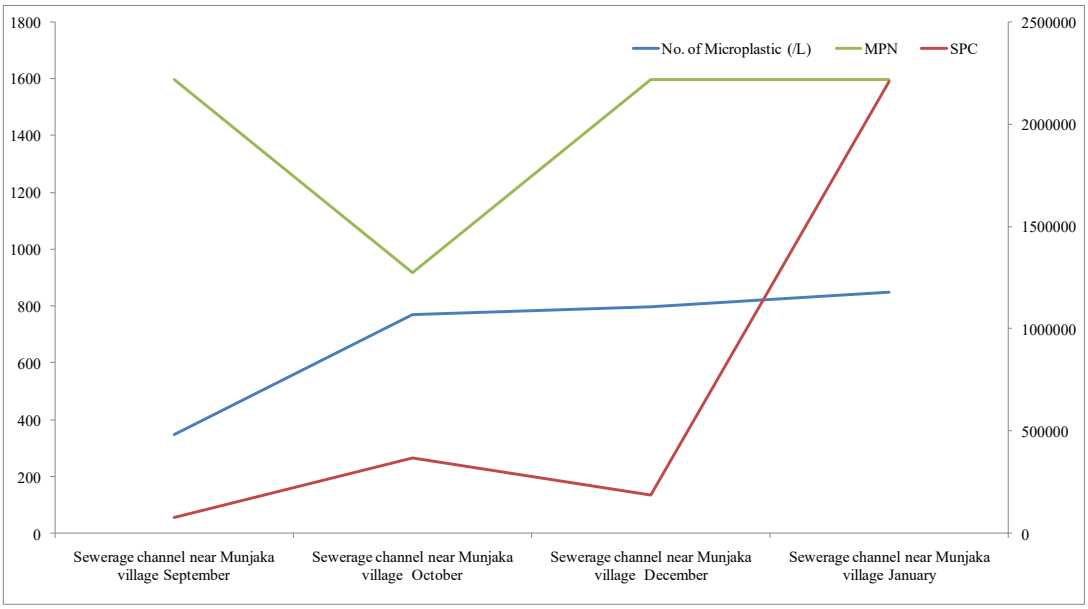


Figure 4: Comparative graph of SPC, MPN and number of microplastics from sewerage sample collected at different time.

The concentration of microplastics from the sewerage channel near Munjaka village rose gradually from 350 particles/L in September to 850 particles/L in January. There were significant swings in the Standard Plate Count (SPC), which increased generally and peaked in January at 2,209,000 CFU/mL. The Most Probable Number (MPN), on the other hand, fluctuated a little, falling in October but staying high at 1,600 overall. In general, there was an upward tendency toward January in both microplastic levels and bacterial load (SPC). Result is summarized in figure 4.

3.4.3 Coastal region:

The samples from different coastal regions of Saurashtra were studied which includes costal region of Somnath, Porbandar, Jamnagar and Kutch.

Porbandar (Chopati) recorded the lowest contamination levels, with 130 microplastics per litre, a Spread Plate Count (SPC) of 4,000 CFU/mL, and an MPN value of 45 MPN/100 mL. In comparison, the Kutch (Arabian Sea) sample showed the highest level of contamination, containing 760 microplastics per litre, a very high SPC of 2,975,000 CFU/mL, and an MPN of 280 MPN/100 mL. The samples from Somnath and Jamnagar exhibited moderate microplastic concentrations. Somnath water contained 510 microplastics/L, an SPC of 30,000 CFU/mL, and the lowest MPN value of 14 MPN/100 mL, suggesting relatively lower faecal contamination. Meanwhile, the Jamnagar sample showed 270 microplastics/L, an SPC of 105,000 CFU/mL, and an MPN of 45 MPN/100 mL. Overall, among all sampling

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locations, Kutch (Arabian Sea) exhibited the highest contamination levels for microplastic abundance, bacterial load (SPC), and coliform presence (MPN). Results are summarized in figure 5.

3.4.4 Demonstrating the number of microplastic by adding plastic pieces in lab:

Water samples from the coastal region, tap water, and soil were collected for the study. Known plastic fragments were added to each of these three sample types, and the number of microplastics was monitored monthly using the Rose Bengal staining method. This approach helped in estimating and comparing the presence of microplastics in different environmental samples over time.

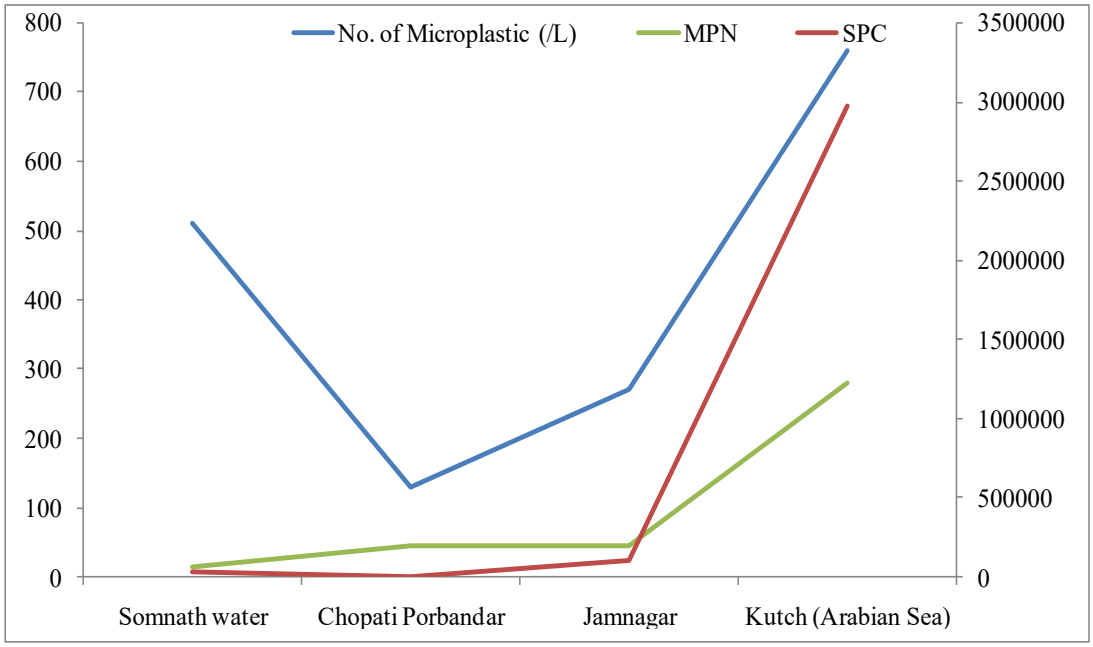


Figure 5: Comparative graph of SPC, MPN and number of microplastics from samples of costal region of Saurashtra

From September to January, the amount of microplastics in soil, tap water, and ocean water gradually increased. The highest levels of contamination were found in ocean water (from 440 to 1450 particles/L), tap water (from 600 to 1150 particles/L), and soil (from 570 to 1200 particles/L). The microplastic content increased in all samples, showing a consistent leaching of plastic samples, which leads to an increase in microplastics in different ecological niches. The above finding warns about the serious consequences of the plastic waste existing in nature. Result is summarized in figure 6.

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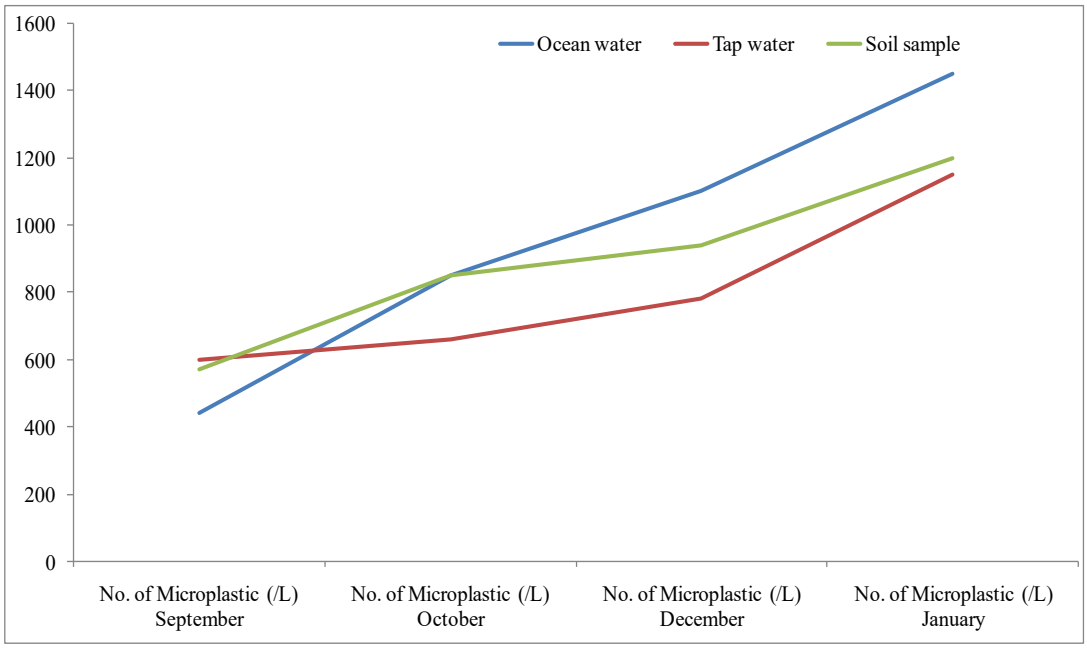


Figure 6: Comparative graph of number of microplastics from artificially createdpot

4 Conclusion:

The microplastics and abundance of bacterial population in soil and water (tap water and ocean water) were thoroughly investigated in this study. The findings consistently showed a positive correlation between plastic particles and total bacterial load, with higher microplastic concentrations being accompanied by higher Standard Plate Count (SPC) values. This positive correlation may be due to microplastics provides surfaces for microbial attachment and biofilm formation.

According to seasonal analysis, the concentration of microplastics in sewage water increased gradually between September and December, indicating either seasonal buildup or continuous environmental input.

Compared to SPC, there was less consistency in the relationship between microplastic abundance and coliform levels (MPN). Other samples showed variability, suggesting that faecal contamination may be impacted by other factors, such as sewage intake, runoff, and human activities, even though some samples with high microplastic counts also showed elevated MPN values. Overall, the results underscore the significance of microplastics for the environment and public health by indicating that they may serve as carriers of microbes and lead to increased microbial colonisation. To fully comprehend the ramifications of microplastic-associated microbial contamination, more research combining bigger datasets, in-depth statistical analysis, and molecular approaches is advised.

With a most probable number (MPN) of 14 and a standard plate count (SPC) of 30,000 CFU/mL, Somnath water has 510 microplastics/L, indicating low microbiological contamination and a moderate plastic presence. Chopati at Porbandar showed minimal plastic presence and moderate faecal contamination with 130 microplastics/L, an SPC of 4,000 CFU/mL, and an MPN of 45. Jamnagar found a significant bacterial load associated with plastic, with 270 microplastics/L, SPC of 105,000 CFU/mL, and MPN of 45. With an exceptionally high SPC of 2,975,000 CFU/mL and MPN of 280, Kutch (Arabian Sea) contained 760 microplastics/L, indicating serious contamination with a strong plastic-microbial interaction.

Microplastics and faecal contamination in water sample from sewerage canal near Munjaka village were found to be increasing

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seasonally: September had 350 microplastics/L, SPC of 79,000 CFU/mL, and MPN of 1600; October had 770 microplastics/L, SPC of 370,000 CFU/mL, and MPN of 920; December had 800 microplastics/L, SPC of 190,225 CFU/mL, and MPN of 1600; and January had the highest values with 850 microplastics/L, SPC of 2,209,000 CFU/mL, and MPN of 1600.

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