

## Unraveling the land-based discharge of microplastics from sewers to oceans – A comprehensive study and risk assessment in wastewaters of Goa, India

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### Abstract

Owing to their pervasive dispersion in the environment and their potential ramifications on both marine life and human health, microplastics (MPs) are of increasing concern. However, there is still a lack of research on the release of MPs from different land-based pathways like creeks, drainage outfalls, and conduits into coastal water systems in India. This study represents comprehensive research into the attribution of MPs in the estuarine system, specifically those emanating from wastewater sources in Panjim City, Goa, India. Urban wastewater collected from different locations in and around Panjim City exhibited values ranging from  $79 \pm 21$  to  $338 \pm 7$  MPs/L, with a prevalence of fibrous and black MP particles. The size range of the MPs at all sampling sites was 100-300  $\mu\text{m}$ . Analysis by  $\mu$ -FTIR revealed 35 distinct polymeric compositions in wastewater, with a dominance of polyacrylamide (PAM), polyvinyl chloride (PVC), and polyamide (PA). Additionally, primary and secondary MPs were studied to unravel the contributions from land-based sources. This included the quantification of MPs in ten samples from personal care products (PCPs) and twenty samples from washing machine effluents (WMEs). MPs in PCPs ranged from 1.8 to 1554 MPs/g. Microfibres and fragments were predominant in WMEs (3986 to 4898 MPs/L). This study suggests a strong relation between polymers found in wastewater effluent and those present in PCPs and WMEs. The identified polymers showed high polymer hazard indices (IV and V), posing a significant threat to the ecosystem and a potential risk to human health.

**Keywords:** microplastics, wastewater, sewage, personal care products, washing machine effluents,  $\mu$ -FTIR.

### **Abbreviations used in the text**

AIM: Advanced Imaging & Microscopic

ATR: Attenuated Total Reflectance

CPCB: Central Pollution Control Board

EVOH: Ethylene vinyl alcohol

GSPCB: Goa State Pollution Control Board

$\mu$ -FTIR: Micro-Fourier Transform Infrared Spectroscopy

HDPE: High-Density Polyethylene

LDPE: Low-Density Polyethylene

MPs: Microplastics

MLD: Million litres per day

NOAA: National Oceanic and Atmospheric Administration

PCPs: Personal care products

PAM: Polyacrylamide

PA: Polyamide

PBT: Polybutylene Terephthalate

PET: Polyethylene Terephthalate

PE: Polyethylene

PI: Polyimide

PMMA: Polymethyl methacrylate

PP: Polypropylene

PS: Polystyrene

PTFE: Polytetrafluoroethylene

PVAL: Polyvinyl alcohol

PVC: Polyvinyl chloride

PVP: Polyvinyl Pyrrolidene

SBR: Sequential batch reactor

STP: Sewage treatment plant

TPA: Tonnes per Annum

WMEs: Washing machine effluents

WSP: Water soluble polymer

WWTPs: Wastewater treatment plants

## 1. Introduction

The omnipresent occurrence, recalcitrance, and prolonged breakdown process of marine plastic debris have become a global concern. Annual plastic production has significantly increased over the past decades from 1.7 million tonnes in the 1950s to 367 million tonnes in 2020 (Plastics Europe, 2021). Despite the pandemic (COVID-19) outbreak, a negligible decrease (0.3%) in plastic production was observed compared to the year 2019 (370 million tonnes). Many studies have reported the ubiquitous presence of MPs in estuaries (Gupta et al., 2021), subtropical oceanic gyres (Law and Thompson, 2014), polar ice caps (Cole et al., 2014), and the deep ocean (Liu et al., 2019). Almost 80% of plastic litter originates from terrestrial sources (Andrady, 2011; Wagner et al., 2014) and ends up in the marine environment (Hurley and Nizzetto, 2018; Rochman, 2018). A recent study also highlighted “street runoff” as an important pathway of MP’s transport from terrestrial sources to marine ecosystems (Sugiura et al., 2021). In coastal cities, most wastewater from innumerable sources is directly discharged into the nearby coastal bodies, rendering them one of the substantial land-based point sources of MPs. Although treatment plants are able to trap larger-sized plastics (20 mm), current technologies are inefficient in retaining small-sized MPs (<1 mm) and ultimately find their way into the marine and estuarine aquatic bodies causing serious threat to marine ecosystems (Carr et al., 2016; Magni et al., 2015). Size plays an important role in the bioavailability of MPs, as micro and nanoplastics are more prone to be absorbed by lower trophic-level organisms (Moore, 2008). The small-sized MPs provide a larger surface area to volume ratio for the adsorption of harmful organic contaminants like persistent organic pollutants (POPs), and metals (Ashton et al., 2010; Cole et al., 2011).

So far, various researches have been carried out on MP occurrence along the estuarine, coastal, and marine environments in India. The majority of studies in India have been conducted on different matrices of water and sediment (Gupta et al., 2021), biota (Goswami et al., 2020; Nikki et al., 2021; Saha et al., 2021; Selvam et al., 2021) and beaches (Karthik et al., 2018; Maharana et al., 2020; Ranjani et al., 2022; Robin et al., 2020; Sruthy and Ramasamy, 2017; Tiwari et al., 2019). Contrarily, very few studies have addressed MPs in wastewater compared to other water bodies in the Indian context. Studies, such as sewage treatment plants (STPs) of Nagpur (Patil et al., 2023) and Bihar (Parashar and Hait, 2022) evaluated the presence of MPs in sewage and sludge in a secondary treatment-based STP system. Further, worldwide studies have investigated MPs in wastewater systems (Hu et al., 2019), influents, effluents, and different stages of WWTPs (Lares et al., 2018; Ruan et al., 2019; Talvitie et al., 2017), sewage

sludge (Gatidou et al., 2019), agricultural soil and farmland (Chen et al., 2020; Corradini et al., 2019), domestic, industrial, agricultural and aquaculture wastewaters (Wang et al., 2020), urban stormwater (Werbowski et al., 2021) and street runoff (Sugiura et al., 2021). Research related to the removal efficiency of MPs by WWTPs has also been reported (Carr et al., 2016; Mintenig et al., 2017). Nevertheless, this research pertains to the release of MPs transported by both treated (WWTPs and STPs) and untreated wastewater originating from various land-based sources, such as creeks, drainage outfalls, and conduits, and their entry into nearby coastal water systems lacks concerted information. Additionally, a land-based signature source-based study was carried out based on the comprehensive analysis of the primary MP contributors, specifically PCPs and WMEs which remains inadequately explored within the Indian perspective. Jhdnjdmjd

Despite the pressing need to safeguard an extensive range of aquatic systems from contamination, and to institute the recycling of treated sewage as a mechanism to alleviate the escalating strain on water reservoirs, the domain of sewage treatment and reuse continues to linger as an overlooked facet in India (CPCB, 2005). Indian water bodies receive millions of liters of wastewater poured into 14 (major), 55 (minor), and numerous smaller rivers from a myriad of terrestrial sources, *viz.* domestic, industrial, and agricultural (Singh et al., 2016). According to the CPCB report, India's metropolitan centers produce around 72,368 million L (MLD) sewage per day with 31,841 MLD of installed sewage treatment capacity and 26,869 MLD of operational capacity, of which 20,235 MLD is actually used to treat sewage while 52,133 MLD remains untreated (CPCB, 2021). For the State of Goa, the estimated sewage generation is 176 MLD, whereas the installed capacity is 66 MLD (37.5% of generated sewage) and 110 MLD (62.5% of generated sewage) remains untreated (CPCB 2021). In the Indian context, the existing capacity for wastewater treatment only accommodates a mere 10% of the total generated wastewater, leading to the discharge of the remaining untreated fraction into neighboring aquatic systems (Singh et al., 2016). This discrepancy between wastewater generation and treatment capacity is conspicuous, as highlighted by governmental sources (MoEF 2011-2012; Envis Centre 2012), and necessitates redressed to harness the potential of wastewater as a valuable resource (Urban Wastewater Scenario in India, 2022). Several studies suggest that in certain other countries, sewage is similarly discharged without undergoing treatment into surface water bodies. As an illustration, approximately 45% of China's population resides in rural regions devoid of sewage pipe networks (Cheung and Fok, 2017). Similarly, within the context of Antarctica, 52% of 71 research stations lack wastewater

treatment plants (WWTPs), resulting in a substantial release of MPs through wastewater discharges (Waller et al., 2017).

Therefore, studies on MPs associated with wastewater in urban agglomerations are of utmost importance as these MPs can be transported to estuarine and ultimately to the marine environment. Numerous studies have reported lethal ramifications by MPs due to accidental ingestion by diverse organisms inhabiting the aquatic ecosystem (Huang et al., 2021; Benson et al., 2022; Rakib et al., 2023). Once ingested, MPs can be hazardous resulting in digestive injuries and a decrease in predatory efficiency that can induce starvation (de Sá et al., 2015; Ferreira et al., 2018). In addition, they can be toxic through the transfer of chemical contaminants such as e.g., POPs and have adverse physiological effects on organisms (Besseling et al., 2013; Rochman, 2015). Further, estuarine biota like mussels, shrimp, and oysters are often served as seafood which is consumed directly posing potential implications for human health. Evidence has indicated the hostile behaviour of MPs in the human biological system e.g. human placentas (Ragusa et al., 2021), lung tissues (Jenner et al., 2022), sputum (Huang et al., 2022), human blood (Leslie et al., 2022) human colon (Bonanomi et al., 2022) and human heart (Yang et al., 2023) which may lead to complex physical and toxicological damage.

The novelty of the present investigation resides in its identification of MP hotspots within urban agglomerations, thereby bridging the knowledge gap concerning the origins of MP pollution. This study represents the illustration of investigating and categorizing MP concentrations within a diverse range of wastewater (both treated and untreated) sources. These discharges directly impact the nearby estuarine ecosystem situated in Panjim City, Goa, India. Panjim, being a relatively small urban area with a limited population, has experienced minimal alterations in its urban infrastructure. There have been no notable developments in industrial zones, and substantial changes in drainage and wastewater treatment systems have not transpired. Hence, this study plays a pivotal role in providing baseline data, which is of paramount significance for comprehending the impact of land-based MP sources on the aquatic ecosystems within the urban conglomeration. These water bodies eventually channel their effluents into the proximate Mandovi estuary. Hence, this research serves as the initial snapshot, offering insight into the attribution of MPs carried by wastewater and their land-based origins. Moreover, the study offers a comprehensive longitudinal evaluation incorporating the abundance, spatial fluctuations, and provenance of MPs stanching from urban wastewater systems which has not been widely addressed around the globe. Furthermore, both

primary and secondary MPs arising from PCPs and WMEs were studied to unravel the major contributors from land-based sources. Thus, the research endeavours to shed light on the distribution and prevalence of MPs within urban wastewater, addressing four primary objectives: 1) elucidating the significant roles of point and non-point sources as pathways for MP influx, 2) quantification and characterization of MPs in urban wastewater, 3) deciphering the conspicuous signatures of dominant land-based contributors of MPs in the wastewater, and 4) appraising the potential risks posed by the polymers identified in the study. Therefore, this study hypothesizes that the MP influx into coastal waters from land-based sources is high and that the hazardous polymers may pose a potential risk to human health. Consequently, this research serves as the initial snapshot, offering insight into the attribution of MPs and their primary origins originating from land-based sources. Given the prevailing landscape of plastic waste management in India, such research is both indispensable and emergent. Hence, this investigation will play a critical role in delineating effective strategies, particularly within urban sewage networks, to mitigate the prominent sources recognized as MP hotspots.

## **2. Materials and methods**

### *2.1 Study area*

The study has been carried out in the coastal state of Goa, India comprising of 105 km long coastline and a land area of 3,702 km<sup>2</sup> located on the central west coast along the Arabian Sea. The capital of Goa, Panjim is a small urban agglomeration that lies on the banks of the Mandovi estuary covering an area of 8.27 km<sup>2</sup>. The city is a hotspot for tourism and supports critical infrastructure for the vast tourism activity in the area, especially during the months of October- March (dry season). Though the population of Panjim is 70,991 as per the census (Public Works Department, 2019), the city has a high floating population since it receives international as well as domestic tourists. Total visitor arrivals escalated from 2.3 million in 2005 to 7.8 million in 2017, an increase of about 11% annually, demonstrating the sector's rapid growth. However, around the Panjim area, only one Sewage Treatment Plant (STP) was operational, which operates on a sequential batch reactor (SBR) process designed for 12.5 MLD capacities. It covers an area of 4 km<sup>2</sup> with a sewer network length of about 45 km and is situated at an elevation of 3.0 m above sea level. The treated wastewater (~6 to 7 MLD), is directed to the effluent pump. This is achieved by either diverting it to the creek (SW5) or releasing it directly into the Mandovi estuary, all under the supervision of GSPCB. For the present MP study, wastewater samples were collected from nine locations (SW1-SW9), in which one treated (SW1) and eight untreated (SW2-SW9) were selected (Table S1).

Nonetheless, among the nine designated locations, SW1 specifically denotes the collection of treated wastewaters from the STP, while the remaining eight sampling points (SW2-SW9) encompass untreated wastewater collected strategically from various locations across the area, chosen for their representativeness of Panjim City. Furthermore, the selected locations precisely represent the features of wastewater routes, covering drainage channels like direct outfalls such as SW2, SW6, SW7, SW8, and SW9. It also includes a creek (SW5) and flowing ravines or conduits (SW3 and SW4), all containing untreated wastewater from diverse sources within the city. Factors like domestic influence, tourism, market activity, and inputs from hotels and restaurants have collectively contributed to their selection as suitable sampling points for this study. Further, it was noticed that all 8 untreated wastewater outlets through sewage networks were directly discharging to the Mandovi estuary. The details of the sampling sites, number of samples, type, quality of wastewater, and description (SW1- SW9) were illustrated in Table S1. The study sites were mainly selected based on their functionality status located in and around Panjim city (Fig. 1). Thus, functional outfalls consisting of untreated wastewater were identified and selected for sample collection in this investigation.

## *2.2 Sample collection*

### *2.2.1 Collection of wastewater samples*

Sampling was conducted during the dry season in December 2018, across all nine chosen locations. Replicate sampling was employed, wherein triplicates gathered from six locations and duplicates from the remaining three, resulting in a total of twenty-four sample (Table S1). Moreover, Leslie et al. (2017) who collected duplicate suspended particulate matter samples when analysing MPs in canals in Amsterdam, the Netherlands, obtained a similar number of samples in a study. The rationale behind employing varying sample replicates was primarily influenced by specific challenges associated with accessing certain sampling locations. In the present study, the exclusion of depth measurements was deliberate, given that certain samples were collected from creeks, ravines, and conduits, specifically targeting surface wastewater with clean stainless steel bucket from 2-3 different points of same site. While, additional samples were directly procured from outlet points in distinct containers. The primary emphasis of this study was to quantify the MPs in wastewater (whether it is surface water of a stream or it is flowing water coming from an outlet). Further, the two or three replicates particularly covered the variation in space, but not in time. Other variables such as weather were supposed to contribute only to a minor extent to the variation. Further, during sampling the containers were rigorously rinsed three times with Milli Q before each collection. All

sample collection activities were optimized and carried out during low tide, which offered optimal exposure to the outfalls and facilitated the sampling process. Wastewater was sampled using a clean stainless steel bucket tied to a cotton rope, which was immediately transferred to pre-cleaned glass bottles. The glass bottles were transferred immediately to the laboratory and stored at 4°C until analysis (Jagadeesh and Sundaram, 2021).

### *2.2.2 Collection of personal care products and washing machine effluents for source identification*

For the assessment of major land-based sources of primary MPs, ten different types of commonly used brands of facial scrubs (C1-C7) and facewash (C8-C10) in duplicates were taken as source samples from different supermarkets in Goa (Table 1). The brand names of the purchased products were not mentioned in this study to avoid policy concerns. Two products (labeled as C11-C12) were selected as control samples, where the microbeads are non-polymer and organic origin as mentioned on the label of the product (ingredients).

An additional significant source of land-based MPs was incorporated into the source analysis, involving the collection of WMEs from both household and commercial laundry settings after washing various textiles. These textiles, included jackets, athletic wear, swimsuits, silk suits, cleaning cloths (from hotels and restaurants), and household linens, underwent laundering using both semi-automatic and automatic top-load washing machines, commonly used for domestic laundering practices. A total of 20 WME samples were collected, comprising 10 samples (1L each) from different domestic washing machines and 10 samples (1L each) from various commercial laundries (using front-load machines). The washing process in both cases was carried out at room temperature (30 °C), with a spinning duration of 15-20 minutes. All samples were directly collected through an outlet pipe into steel containers and transported to the laboratory for subsequent analysis.

## *2.3 Laboratory analysis*

### *2.3.1 Processing of wastewater samples*

The wastewater samples were collected and immediately brought back to the lab for further analysis. The collected wastewater was passed through a series of two steel stacked sieves of mesh sizes 5mm and 1mm (Nonka Rikaki Co. Ltd, Japan) attached to a steel receiver to allow visual sorting and identification of larger plastic particles. Subsequently, the samples retrieved in the receiver were separately treated by the method recommended by our previous studies (Gupta et al., 2021). Concisely, wet peroxide digestion by adding a specific volume (100 mL) of 30% H<sub>2</sub>O<sub>2</sub> was carried out to digest the organic matter. Thereafter, 5.3 M sodium



chloride (NaCl) was added for density separation. Also, recent studies have shown that NaCl is more effective in MP density separation than other available solutions (Chaudhary and Suthar, 2023; Cutroneo et al., 2021). Further, the entire solution was left overnight after sufficient stirring. The supernatant obtained was vacuum filtered with the help of a 5 µm filter paper (nitrocellulose filter, 47 mm diameter). The filter paper was sealed and dried in an oven at 40 °C and stored at 4 °C before further analysis.

### *2.3.2 Extraction of microplastics from personal care products and washing machine effluents*

In the laboratory, the extraction of MPs from PCPs was followed by the methods outlined by Lei et al. (2017) and Napper et al. (2015), with minor adjustments. Firstly, the contents in the PCPs were weighed and mixed with 1L boiling ultra-purified water (Milli Q) with continuous stirring until the soluble ingredients were completely dissolved. The resulting solution was filtered through a cellulose membrane filter with a 47 mm diameter (nitrocellulose filter) with a pore size of 5 µm. The filter paper was dried in an oven at 40-50 °C until fully dry. The mass of all particles captured on the filter was assessed by measuring the weight variation of the filter pre- and post-filtration, utilizing LC/GC electronic weighing scales. Subsequently, these filters were stored in petridishes for ensuing analysis.

The effluents collected from the washing machine were directly vacuum filtered through a cellulose membrane filter with pore size 5 µm, 47 mm diameter (mixed cellulose ester). Thereafter, the filter papers were stained using Nile Red Stain dried in an oven, and stored in petriplates for further stereoscopic and compositional analysis (Sturm et al., 2023).

### *2.4 Observation and identification of microplastics*

Filtered samples (treated and untreated wastewater, WMEs, and PCPs) in 5 µm filter paper and in 300 µm nylon mesh net were examined visually under a stereo-zoom microscope (Model: Nikon SMZ18) attached to a digital camera (Nikon DS-Ri2) with a zoom ratio of 18:1 and zoom range of 0.75-13.5x. Each item was photographed and measured at its largest dimension with the specific size scale mentioned, using the stereomicroscope Nikon SMZ18 coupled with the imaging software NIS-Elements D (NIKON, H600L, Japan). A visual assessment was conducted to identify the morphological characteristics of plastics like particles. The targeted morphological characteristics recorded are abundance (total count), size (20-100 µm, 100-300 µm, 300-1000 µm, and 1000-5000 µm), shape (fiber, fragment, film, and beads), and colour of plastic like particles in each sample.

## *2.5 Identification of the polymeric composition of microplastics*

All smaller-sized MPs (<1mm) were examined using a micro-Fourier Transform Infrared Spectroscopy (FTIR- Affinity-1 Shimadzu, Japan) coupled with an Automated Infrared Microscope (AIM-8800) to identify the polymer compositions. FTIR absorption spectra were calculated as an average of 100 scans in the mid-infrared range 700-4000  $\text{cm}^{-1}$  at a resolution of 8  $\text{cm}^{-1}$ . Since particles are mostly thick, opaque, and colorful, and adhered to the nitrocellulose filter paper, high sensitivity diffuse reflection mode was applied. The polymer characteristics were identified based on the absorption frequencies for specific chemical bond types present in the relevant polymer samples. More specifically by comparing sample micro-FTIR spectra against known reference spectra from the database Shimadzu ATR polymer library with a matching score of >60% (Rathore et al., 2023).

Particularly, PCP samples (microbeads) and particles (larger sized >1 mm) were identified using FTIR coupled with attached ATR (Attenuated Total Reflectance) diamond crystal. The IR spectrum was recorded at an average of 45 scans in the mid-infrared range 400-4000  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$ . The polymers were matched and identified by comparing the IR spectrum against the known spectrum with >60% matching score from the Shimadzu ATR of the polymers library (Maharana et al., 2020; Rathore et al., 2023).

## *2.6 Quality assurance and quality control (QA/QC)*

All glassware used in the experiment was rinsed thoroughly with three solvents (methanol, acetone, and hexane) and covered with aluminum foil before use. During sample purification and filtration, clean aprons made up of natural fabric (non-shredded polymer) were worn. We tried limiting the use of plastic materials during the entire study. Ultrapure water (18.2-ohm resistivity, Milli-Q) was used for rinsing the leftover samples in the bottles during filtration to reduce the loss of MPs. In order to reduce the risk of contamination by airborne particles/MPs, the ultrapure water was filtered with a 0.8  $\mu\text{m}$  filter paper before commencing the experiment. After every sample, the filtration cup was washed and thoroughly rinsed with ultrapure water before starting the next sample. The experiment was performed in a pre-cleaned sterile laminar flow hood and all the samples were checked for airborne contamination mainly microfibers leading to overestimation of MPs by running blanks before filtration of samples (Saha et al., 2021).

Recovery tests were also performed to determine the extraction efficiency of the amended method by spiking fragments (n=12) with known polymers. The spiked polymers included 2 HDPE (high-density polyethylene), 3 PP (polypropylene), 2 PVC (polyvinyl chloride), 3 PA (polyamide), and 2 PS (polystyrene) particles of different shapes and sizes (~300-500µm), which followed the entire analytical procedure. As a prior quality control, the procedural blank (without sample) was also subject to the extraction method to quantify the background contamination of MPs. Out of 12 particles, 10 were identified using a stereozoom microscope which were further characterized using µ-FTIR. The confirmed polymers found were 2 HDPE, 2 PP, 2 PVC, 2 PA, and 2 PS thus the recovery rate acquired was 83% for the optimization of MP extraction and analytical method (Gupta et al., 2021).

### *2.7 Microplastic polymer hazard assessment*

To evaluate the potential risks of MPs in wastewater, we have considered both the concentration and the chemical composition of MPs (Lithner et al., 2011; Xu et al., 2018a). The polymer hazard assessment of MPs was calculated using the following formula used in several worldwide studies (Ranjani et al., 2021; Yin et al., 2021; Adji et al., 2022):

$$PHI = \sum P_n \times S_n \quad (1)$$

where 'PHI' is the calculated polymer hazard index caused by MP, 'P<sub>n</sub>' is the percentage of specific polymer types collected at each sampling location, and 'S<sub>n</sub>' is the hazard score of polymer types of MPs derived from (Lithner et al., 2011). The score further indicates Level V (>1000) might be carcinogenic; Level IV (100–1000) cause an allergic skin reaction; Level III (10–100) can be toxic if inhaled, swallowed, or in contact with skin; Level II (1–10) can cause respiratory irritation, skin irritation, serious eye irritation, severe skin burns, or eye damage; and Level I (0-1) less harmful to humans. In addition, to evaluate the hazard level of MP contamination, the PHI was calculated for each studied location.

### *2.8 Statistical analysis*

The concentrations of MPs at various sampling locations were reported in the format of mean ± SD. To scrutinize the spatial variations in the concentration of MPs in wastewater, one-way analysis of variance (ANOVA) was performed. The threshold level of statistical significance for ANOVA is p < 0.05. Principal Component Analysis (PCA) was utilized to illustrate the variation in MPs of diverse sizes and shapes found in wastewater collected from multiple sampling points. PCA proves to be a useful approach in simplifying complex datasets by reducing their dimensionality, thereby improving interpretability, while preserving a

substantial amount of the information within the data. Moreover, the analysis is organized into distinct groups that help clarify the associates between various shapes and sizes.

### 3. Results

#### 3.1 Occurrence and microplastic concentration in wastewater

A significant presence of MPs was detected in all wastewater samples within the range of  $79 \pm 20.7$  to  $338 \pm 7.1$  MPs/L. In terms of spatial distribution, a significant difference was observed among all sampling locations ( $F=15.27$ ;  $p=0.001$ ). As illustrated in Fig. 2, the highest concentration was noted at SW3, with a mean value of  $338 \pm 7.1$  particles/L, followed by SW2 ( $273 \pm 178$ ) and SW7 ( $203 \pm 85$ ) particles/L. Similarly, SW5 ( $188 \pm 38$  particles/L) and SW4 ( $160 \pm 6$  particles/L) exhibited a relatively higher abundance of MP contamination as compared to treated wastewater. For location SW1 ( $122 \pm 23$  particles/L), though the occurrence of MPs indicates a reduction from aforementioned sites, but the final concentration of MPs remains a cause of concern. However, the lowest MP concentration was observed at SW9 ( $112 \pm 18$ ), SW6 ( $106 \pm 8$ ), and SW8 ( $79 \pm 20.7$  particles/L), representing the outfall points along the coastal waters of nearby Mandovi estuary.

#### 3.2 Morphological characteristics (shape, colour, and size) in wastewater

Microplastics were categorized as primary and secondary plastics based on morphology by means of visual identification in stereozoom microscopy (Figs. 3 a, b, c, and d). Fibers (range 42.6 - 72.6%; avg 60.2%) were found numerically dominant at all the stations (Fig. 4a), followed by films (range 9.1 - 40.7%; avg 25.3%) and fragments (range 5.1-16.6%; avg 12.1%), while beads were a minority (range 1.3 - 4.0%; avg 2.4%). Also, the wastewater samples exhibited diverse colorations, with predominant being black (46.4%), red (32.5%), green (10.1%), transparent (5.3%), and blue (2.8%). Collectively, these colors constituted 97.1% of the overall MP content across all samples. Other colors such as brown, orange, yellow, white, and purple accounted for (2.9 %) of the total (Fig. 4b). In terms of size composition, as depicted in Fig. 4c, prevalence of MP particles in the 100-300  $\mu\text{m}$  size range comprised maximum fraction (61%), followed by 300-1000  $\mu\text{m}$  (29%), 1000-5000  $\mu\text{m}$  (6%), and 20-100  $\mu\text{m}$  (4%) across all locations.

In the present study, we utilized a PCA biplot to establish relationships and correlations between the characteristics of MPs (specifically, shape and size) and various sampling sites. The PCA biplot, representing both shape and size, illustrates a cumulative variance of 75.06%

and 87.76%, respectively, as depicted in Fig 5a and b. Notably, in the PCA plot, fibers and films (shape) along with the size categories of 100-300  $\mu\text{m}$  and 300-1000  $\mu\text{m}$  are clustered in the left quadrant, closely associated with the PC 2. Conversely, beads and fragments (shape) along with the size categories of 1000-5000  $\mu\text{m}$  and 20-100  $\mu\text{m}$  are grouped in the right quadrant, linked with PC 1. Examining specific stations, it is evident that fibers predominate in sites SW1 and SW7, while films are more prominent in SW2, SW3, and SW4, indicating a localized distribution of these MP shapes. Similarly, in terms of size, smaller-sized MPs (100-300  $\mu\text{m}$ ) are concentrated in site SW6, while 300-1000  $\mu\text{m}$ -sized MPs prevail in SW1 and SW2. The findings derived from PCA offer valuable insights into the spatial distribution of MPs across various locations. This might contribute to an understanding of the land-based sources of MPs by elucidating the specific contributions of distinct shapes and sizes of MPs observed in the study area.

### *3.3 Polymeric composition in wastewater*

A total of 1580 particles were scrutinized, of which 800 particles (constituting 50.63%) were successfully identified via  $\mu$ -FTIR as synthetic polymers. Intriguingly, it unveiled a diverse assemblage of polymeric composition ( $n=35$ ) within the smaller-sized MPs (Table S3a). In totality, a consolidated array of polymer types, predominantly polyacrylamide (PAM -18.3%), PVC -13.5%, polyamide (PA- 10.3%), ethylene vinyl alcohol (EVOH - 8.5%), styrene isoprene (8.2%), polyimide (PI 7.6%), polybutadiene (6.5%), polyethylene (PE - 5.1%), PP - 4.7%, polyvinyl alcohol (PVAL) & polytetrafluoroethylene (PTFE) (3.9% each), polyester (3.4%), and polycarbonate (PC - 3%), collectively constituting 97% of the total composition were recorded. Other polymers exhibited a less pronounced presence, accounting for 3% of the overall composition (Table S3a). The station-specific distribution of polymeric constituents is presented in Fig. 6a.

### *3.4 Identification and quantification of microplastics from personal care products and washing machine effluents*

Different types of beads with distinct morphological characteristics (shape, colour, and size) were identified in 10 PCP samples. We have found microbeads with round, spherical, filamentous, distorted, long fibrous, cluster forms which can agglomerate. Similarly, beads with vivid colours (*viz.* red, green, brown, red, and white) were also observed (Figs. 3 e, f, g, and, h). The geometric means of the mass and abundance of microbeads were  $1.15 \pm 0.12$ - $30.03 \pm 12.4$  g/L and 1.8 - 1554 particles/g, respectively. The size of the microbeads derived

varied from 320-1600  $\mu\text{m}$  (Table 1). In terms of polymer composition, PE was the major polymer found in the PCP samples followed by polyacrylate, vinylidene chloride/acrylonitrile copolymer, acrylonitrile film, polyvinylidene fluoride, and ethylene /propylene copolymer. Besides this, two non-polymer PCP samples (C11 and C12) were also analysed as control and remained undetected as plastic polymers.

A consequential number of MPs were also identified in both laundry and household washing machine effluents (Figs. 3 i, j, k, and l). The fibers were found predominantly (83% and 74%), followed by fragments (17% and 26%) in laundry and household samples, respectively (Fig. S2 a and b). The average abundance of MPs varied between 3986 - 4898 MPs/L, whereas the size of the MPs in laundry and household effluents was found to be 20-3669  $\mu\text{m}$  and 50-2280  $\mu\text{m}$ , respectively. The colours of the MPs detected in WMEs were mainly blue (36.07%) and red (22.95%), followed by black, green, grey, transparent, and others (Fig. S2b). The polymeric compositions of MPs detected in laundry samples (Fig. 6b) were mainly PA (37%), polyester (28%), and rayon (11%) followed by PAM (10%), PVAL (8%), and others (6%). Similarly, the household effluents were dominated by PA (35%) making up the major proportion followed by PAM (18%), rayon (15%), vinyl alcohol/vinyl butyryl copolymer (9%), PVAL and polyester (10%) each and others (3%) comprising distinct varieties of polymers found in varied proportion (Fig. 6c).

### *3.5 Polymer risk assessment in wastewaters*

According to the hazard ranking model used the obtained polymer hazard index (PHI) values reveal that the chemical compounds in plastics are perilous. Based on formula (1), the risk values associated with individual polymers as well as in different locations were obtained. the risk levels were found to be increased with the presence of dominant polymers found in this study. The toxicity scores of dominant polymers such as PAM(2240), PVC (10551), polybutadiene (20001), and styrene isoprene copolymer (2788) Further, polyester (1117) and PC (610), exhibited an elevated hazard index V and IV whereas the least was obtained for PA (63), PE (11) and PP (1) portraying hazard index III, II and I respectively at all locations. Interestingly, the category labeled as "other" polymers, even though they constituted a smaller proportion (%), displayed notably high hazard scores. For instance, acrylonitrile butadiene styrene (ABS) (6552), Poly methyl methacrylate (PMMA) (1021), polyvinyl pyrrolidone (PVP) (20001), PS (1628), PU (3844), acrylonitrile film (11521), and epoxy resin (7139) exhibited hazard indices several orders of magnitude higher, falling into the hazard index categories V and IV, in stark contrast to the hazard scores of other polymers. (Table S3 a).

The calculated PHI values in this study revealed a significant hazard index ( $V$ ,  $>1000$ ), underscoring the presence of potentially hazardous polymers within the wastewater samples. Analysis of PHI values across different sampling locations (Table S3 b) revealed an uneven pattern, with the highest values observed in SW4 (10,78,028.1), SW5 (9,38,492), and SW3 (9,27,344), followed by SW1 (8,43,113.3), SW7 (8,03,826.2), SW2 (7,95,710.6), SW9 (7,94,421), SW8 (7,52,304.6), and SW6 (7,06,160.3). These results provide critical insights into the distribution of hazardous polymers in wastewater within the studied urban agglomeration.

## **4. Discussion**

### *4.1 Abundance and spatial distribution of microplastic in wastewaters*

The notable influence of STPs and wastewater originating from both point and non-point sources in shaping estuarine systems can be recognized as a pivotal factor driving plastic and MP contamination within the marine realm. The present assessment offers a glimpse into the repercussions curtailing from treated (STP) and untreated effluents, on the persistent occurrence and copious profusion of MPs within the estuarine environment. The abundance of MPs at the SW1 (treated wastewater) site, clearly notifies the inefficiency of the treatment plant to eliminate smaller-sized plastic particles, which can easily pass through the STP screening filters. The size of secondary screens used in the studied STP is nearly 6mm and fails to trap MPs  $<5$ mm which is consistent with the study of Stuetz and Stephenson, (2009). Also, the values of MPs established in this study were nearly comparable to the values reported for the Indian STPs (Parashar and Hait, 2022; Patil et al., 2023) (Table 2). A significant amount of MPs was retrieved from SW2 ( $273 \pm 178$  MPs/L), which was adjacent to the Panjim ferry jetty. Initially, triplicates were collected from this location (Table S1) wherein, two samples were fetched from the direct outfall where untreated wastewater was continuously flowing, as shown in Fig S1. This direct outfall (SW2) was linked to drainage networks from various commercial establishments, market areas, and households, that discharge into the Mandovi River aligning with a report by GSPCB (Anon 2019). Consequently, a third sample was collected at the identical site (SW2), slightly distant from the direct discharge point where effluent and estuarine water conjoin. Thus, this particular sampling location showcased a significant presence of MP pollution, stemming from a combination, of both land-based and sea-based sources. These sources encompass the effects of maritime vessel activities, including the movement of cargo and passengers, as well as the potential contribution of MPs from vessel coatings, unauthorized waste disposal, and recreational activities such as boat excursions and

casino operations as reported in our previous study by Gupta et al. (2021). Hence, the cumulative influence of all these factors contributes to the heightened abundance of MPs observed at this specific site.

Subsequently, the highest particle load of  $338 \pm 7$  MPs/L was observed in the SW3 location which can be attributed to several factors. Firstly, it is likely a result of the flowing ravine, which serves as a direct conduit for untreated sewage from households and commercial complexes in Panjim city. Furthermore, SW3 was located in an area that harbours a large number of hotel industries, restaurants, and local shops. These establishments may contribute to the discharge of solid waste, particularly plastics, directly into the ravine which could considerably add to the MP contamination in this location. Additionally, the fact that SW3 is an open drainage channel, the atmospheric deposition of MPs could be one of the sources of contamination in this particular location. In the case of SW4, it also exhibited a remarkably high concentration of MPs,  $160 \pm 6$  MPs/L. This specific site is characterized by being a stagnant ravine with open channels that serve as conduits for stormwater drainage and intercepting wastewater streams. The elevated levels of MPs on this site can be ascribed to various influences. One significant source of MPs in SW4 is the result of road-related activities. These include tiny plastic fragments produced from the abrasion of vehicular tires, rubber waste, thermoplastic coatings used in road markings, and road dust which can find their way into this ravine due to its proximity to roads aligning with the findings reported by Mak et al. (2020). In addition, exposure to pollution sources associated with road-related activities such as population density, and heightened traffic volume (especially during the tourist season, which was considered during the sampling), can contribute to the elevated proportion of MPs in this location. A recent study by Franco et al. (2023) also highlighted that tourism and recreational activities around the area can alter the presence of MPs and increase their discharge and contamination in the receiving water system.

The geographical location SW5, once characterized by its scenic creek, has unfortunately transformed into a ravine due to the inflow of untreated sewage and the indiscriminate dumping of solid waste by residential and commercial complexes. This issue was brought to light in a local news report, revealing the discharge of raw sewage into the creek by tanker operators transporting waste from other areas of the city (Anon 2020). Additionally, SW5 receives treated wastewater from SW1, potentially introducing a notable quantity of MPs into the locale. Regarding sites SW6, SW7, and SW8 corresponding to effluent outfalls along the Mandovi, these locations witness direct discharge, particularly of untreated wastewater,



influenced by waste from various sources in the Panjim area. Finally, location SW9 corresponds to a segment of another creek that is equipped with direct outlets connected to channels releasing untreated sewage from the pump house (Anon 2023) in the nearby estuary. The justification of the observed variations in MPs from these direct outfalls poses challenges, indicating that anthropogenic activities and community practices in Panjim City significantly contribute to the presence of MP pollution in wastewater.

The abundance of MPs in untreated wastewater samples allows for a numerical comparison with influents from previous studies, as presented in Table 2 for legitimate global comparisons. However, such comparisons face challenges due to various factors, including diverse sampling techniques, sampling times, employed methodologies, population density, sewage production, and collection methods (Chen et al., 2020; Underwood et al., 2017), as well as variations in wastewater types (Mahon et al., 2017). The spatial discrepancies in MP abundance may also be contingent on the population served within the city (Lares et al., 2018; Ziajahromi et al., 2017). These considerations collectively underscore the complexity of making accurate cross-study comparisons. Nevertheless, the findings presented herein aims to depict the implications of MPs for both the local population and the environment.

#### *4.2 Physical characteristics of microplastics in wastewater (shape, colour, and size).*

The shapes and size of MPs can play a vital role as the toxicity varies according to the morphology of the particle which can be easily ingested/engulfed by a wide range of organisms. Discrete shapes of MPs classified as fibers, fragments, films, and beads were observed. Shape-wise, the spatial distribution of MPs showed a similar trend of occurrence at all wastewater stations. The dominance of fibrous MPs (60.2%) in wastewater can be attributed to the loss of textile fibers most likely from (synthetic) clothing and carpets, during domestic washings. The presence of fibers in all the samples has been extensively documented which mainly corresponds to the laundry source as consistent with other studies by De Falco et al. (2018) and Zambrano et al. (2019) (Table 2). Subsequently, some underestimated sources of MP fibers in the environment from wet wipes and sanitary towels flushed out into the toilets could be one of the major sources of microfibers in the wastewater as observed by Briain et al. (2020). Spatially, PCA analysis results regarding the shape of MPs (Fig 5a, Table S2a) indicate a notable concentration of fibers at SW1. This concentration signifies the presence of fibers in this specific location, which is associated with treated wastewater from SW1 (STP). This finding strongly implies that the fibers likely originate from various sources, including households and other diverse contributors to wastewater, making the effluents from the STP a

significant contributor to MP pollution in the nearby estuarine environment. Films (25.3%) were the second most copious shape of MPs comprising a curved, twisted structure, and are generally associated with commercial activities (Helm, 2017). It contributes to secondary MPs mainly stem from the breakdown of larger plastic particles like plastic bags, tires, cigarettes, tobacco packing, and packaging films (Estahbanati and Fahrenfeld, 2016; Hoellein et al., 2014) due to various physical processes *viz.* photodegradation, oxidation, and chemical degradation (Andrady, 2011). Similarly, the PCA plot (see Fig. 5a, Table S2a) indicated a notable presence of films in SW2, SW3, and SW4. This occurrence might be attributed to the proximity of these sites to the ferry point and market area, as previously discussed. Additionally, sites SW3 and SW4 represent a ravine/conduit (refer Table S1), which receives untreated sewage from various sources and experiences improper disposal of single-use bags and food packaging due to human activities. These factors could serve as plausible explanations for the observed elevated values of films at these specific locations. Fragments (12.1%) were found in lower concentrations compared to fibers and films. Probably, as the wastewater undergoes several treatment stages in STP a large number of fragmented MPs precipitated in the secondary treatment system (Sun et al., 2019). Fragments are known to be exposed to shear stress forces such as liquid flow conditions in water streams, pipes, or drains contributing to the generation of secondary MPs and nanoplastics (Enfrin et al., 2019). In this study, a lower percentage (2.4%) of beads was observed, attributed to increased photodegradation and oxidation during the dry season sampling in December compared to the wet season. This observation aligns with Gupta et al.'s (2021) earlier findings of decreased bead concentration in the dry season. The dry season conditions contribute to plastic corrosion, possibly transforming microbeads into fibrous or irregular fragments. The study's results provide evidence of fibrous and fragmented microbeads originating from personal care products (PCPs), suggesting that the aging process alters the morphology and dimensions of microplastics (MPs). Due to their small size and high surface area, microbeads tend to adsorb contaminants and undergo biofouling, affecting buoyancy and potentially explaining the observed decrease in bead proportion in wastewater samples.

The colour of MPs can influence their visual resemblance to natural prey items in the marine environment. In this study, black (46.4%), red (32.5%), green (10.1%), transparent (5.4%), and blue (2.8%) accounted for >97% of the total amount. Black was predominant, probably sourced mainly from electronic waste and abrasion of tire wear and tear on roadway surfaces or shredding of used tires for recycling processes (Parker-Jurd et al., 2021). Red, green, blue, and transparent particles might originate from packaging materials, textiles (Xu et

al., 2018b), PCPs (Nel et al., 2019), and other applications. Similar color compositions of MPs were obtained in various global wastewater studies (Table 2).

In terms of size, small-sized particles have been widely observed in several wastewater systems. The size can play a significant role on the fate of MPs, as it is speculated that small-sized MPs can be ingested by biota once transported to the marine environment. Further, size helps in determining the dynamics of plastic transportation from various sources. In the present study, 100-300  $\mu\text{m}$  and 300-1000  $\mu\text{m}$  sized MPs prevailed, probably due to physical actions like abrasion between the particles, and fragmentation leading to a higher abundance of smaller-sized particles. This finding was in line with another study in which the estimated size of MPs in wastewater (>95%) ranged from 20-300  $\mu\text{m}$  (Conley et al., 2019). Location-wise, the outcomes of the PCA analysis (refer to Fig. 5b, Table S2b) revealed a heightened prevalence of MPs in the size range of 100-300  $\mu\text{m}$  in SW6. This observation can be linked to the fragmentation of larger plastics during the transportation of wastewater through the sewer system, as suggested by Franco et al. (2021). Notably, the size category of 300-1000  $\mu\text{m}$  was predominant in SW1 and SW2, providing further confirmation that both treated and untreated wastewater significantly contributes to the presence of smaller-sized MPs in the nearby estuarine environment, thereby posing a potential threat to the aquatic ecosystem. In addition, the 1000-5000  $\mu\text{m}$  sized MP fraction was relatively skewed towards a smaller fraction which may be correlated with the higher degradation rates in the dry season resulting in smaller size MPs. Conversely, the size range 20-100  $\mu\text{m}$  (4%) was inherently small in proportion. This observation is substantiated by (Yan et al., 2020) who affirm that the aging process induces a modification in the form of MPs, leading to their conversion into rod-shaped plastic fibers. These altered attributes can enhance their conducive to traveling a longer distance, consequently underpinning the observed decline in the prevalence of particles within the range of 20-100  $\mu\text{m}$  in the present study. The chemical composition of particles will be discussed in the next section 4.3.

#### *4.3 Polymeric characterization in wastewater: Source estimates*

The presence of polymers in estuarine systems, originating from wastewater (land-based sources), are indicative of MP pollution in the estuarine system. PAM, a prevalent polymer, serves as a flocculating agent in WWTPs/STPs and also widely used in food packaging, drug treatment, textiles, biomedical and industrial sectors, as well as cosmetics (Kadajji and Betageri, 2011; Novillo et al., 2020). PVC, the second most dominant polymer, is primarily utilized in pipes, construction, clothing, furniture, electronics, and healthcare

applications (Xu et al., 2020). PA and polyester find extensive usage in textiles, carpets, packaging, fishing gear, household goods, consumer goods, sports, and tourism equipment (Mărieș and Abrudan, 2018; Nelms et al., 2018). Polymers like styrene isoprene and polybutadiene, employed in tire and rubber manufacturing (Hüffer et al., 2019), while PI used in automotive, bar code seals, heaters, stencils, loudspeaker components, and pressure-sensitive tapes (McKeen, 2017). EVOH is used in packaging materials, medical, and pharmaceutical applications as an oxygen barrier film (Goswami et al., 2020). PE particles are utilized in the manufacturing of microbeads from PCPs. Other detected polymers (e.g., PP, PVAL, PMMA, PVP, polyacetal, polyvinylidene, ABS, polyvinyl acetate, PU, polyphenylene oxide) have diverse applications in PCPs, detergents, food packaging, medical equipment, and electronics.

#### *4.4 Source apportionment of microplastics in wastewater: To comprehend land-based sources and their impact on the environment*

##### *4.4.1 Characterization of microbeads in personal care products*

The abundance of MPs (1.8-1554 particles/g) in the present study from 10 major brands of face wash and face scrub was in agreement with previous studies like Conkle et al., (2018) and Godoy et al. (2019), where 123-3730 particles/g in cosmetics samples. Table S4 summarizes various PCP sample categories and test results from previous studies. With respect to morphological characteristics, the size range found in the present study (300-1600  $\mu\text{m}$ ) aligned with the study conducted by (Yaseen et al., 2022) who reported particle sizes from 224 to 1882  $\mu\text{m}$  (Table S4). Subsequently, the distinct shapes of microbeads found in the present study (irregularly shaped like filamentous, distorted, long fibrous type and many formed clusters and agglomerates) were supported by studies that stated that microbeads are most likely to have irregular shapes rather than spherical ones (Möhlenkamp et al., 2018). In terms of polymeric composition, studies have been published stating PE as a major contributing polymer type which is in agreement with our current study (Conkle et al., 2018; Guerranti et al., 2019). Other dominant polymers in PCP samples in this study were polyacrylate, vinylidene chloride/acrylonitrile copolymer, acrylonitrile film, polyvinylidene fluoride, and ethylene /propylene copolymers. Besides, the relative contents of MPs in PCPs consumed in Asia are minimal as compared to the USA where it is maximum (Sun et al., 2020).

Remarkably, an intriguing revelation from this study is that among the 35 identified polymers in wastewaters, almost 50% polymers (e.g. PVP, PVAL, PAM, polyacrylate, PMMA

etc.) are prominently employed in the formulation of PCPs. Additionally, MPs with size <1mm in wastewater also stands in the similar size range of microbeads present in PCP. Hence it is noteworthy that smaller-sized MPs (microbeads), can intrude into marine and estuarine ecosystem through waste water and play a pivotal role in enhancing the bioavailability and potential toxicity of these MPs to marine life.

#### *4.4.2 Characterization of microplastics in washing machine effluents*

In washing machine effluents, the MPs (3986 - 4898 MPs/L) were consistent with the study by (Tang et al., 2020) who estimated 2900 to 7100 n L<sup>-1</sup> in laundry wastewater. A study by Browne et al. (2011) demonstrated that a garment can shed >1900 fibers per wash, eventually making laundering a major contributor of MPs in nearby ecosystem. Apart from fibers, other shape-like fragments were also found in this study which were rarely reported in the previous studies. This could be the result of some fragmentation of buttons, small shiny disc-shaped beads, and sequins which are mostly made of plastics like PVC, available in a wide variety of colors. It has been further estimated that some of the polymers examined in WMEs were mainly used in detergent formulation (e.g., acrylic acid, maleic acid, polyethylene glycol, vinyl alcohol, propylene oxide, PVP, and PVAL). However, these polymers are not naturally biodegradable and, therefore persist in oceans, lakes, and other water depositories posing a threat to marine life (Yangxin et al., 2008). Additionally, microfibers released from WMEs via sewage networks, when ingested by aquatic organisms, persist longer in the gut, leading to entanglements in the convoluted fish gut (Goswami et al., 2020). This can result in changes to their growth rate, energy reserves, and nutritional depletion, impacting fish yield and ecosystem functioning.

#### *4.6 Exposure and risk assessment associated with polymers: An incipient threat*

There are numerous reports on the risks of plastics, along with other additives, which may be hazardous for ecosystems particularly humans (Araujo et al., 2002). Specific polymers such as PAM were found to be predominant among all polymers. PAM as such, however, does not pose a threat directly to the environment. However, PAM is known to secrete the secondary metabolite acrylamide (monomer), which is considered genotoxic, carcinogenic, and neurotoxin in nature (Udovicki et al., 2022). Other polymers such as PVC, styrene isoprene copolymer, and polybutadiene were also found to be posing chemical threats as these are classified under hazard level (V), which outlines the substance to be carcinogenic, mutagenic,

persistent, and toxic for reproduction, and hazardous to the ozone layer. Surprisingly, polymers such as epoxy resin, acrylonitrile film, PU, ABS, PMMA, and PVP also fell under hazard level IV which further suggests that these polymers though present in small proportions (“others category”), can pose a serious threat to the ecosystem (Servien et al., 2023) due to their high hazard score index. Also, polymers with low hazard values (III, II, and I) found in the study cannot be ignored as their release into the environment can prove to be detrimental to aquatic species as well as human health. Similarly, it is crucial to highlight that the PHI values observed among different sampling locations in this study consistently exceeded a hazard level (V) threshold of >1000 (Table S3b). This finding aligns with studies conducted by Ranjani et al. (2021) and Gupta et al. (2023), which focused on coastal regions along the west coast of India, revealing similar high PHI values (>1000). These elevated PHI values were attributed to the presence of MPs with notably high hazard scores of V and IV in the present study (Table S3b). This highlights the significance of our findings, indicating that even in cases where MP abundance may be relatively low, the PHI values exhibit high values due to the presence of exceptionally hazardous polymers found in wastewater. Hence, it is imperative to conduct an inclusive assessment of the risks posed to both human health and the environment.

It is worth pointing out that the derived outcomes postulate a discernible connection between the signature of polymeric compositions present in wastewater with those observed in PCPs and WMEs. Therefore, it is advisable to channel future endeavours into the establishment of household-level MP treatment technologies. Similar to other countries like the UK, USA, Canada, Netherlands, and others, India could enhance implementations to ban or restrict the use of plastic microbeads in cosmetic products. These regulatory innovations and policies carry the potential to play a fundamental role in mitigating MP pollution at its origin, thereby ensuring the protection and preservation of our ecosystems.

## **5. Conclusion and way forward**

This research represents the investigation of MP levels in wastewaters and their potential risks to human health within the relatively modest expanse of Goa, India. The discharge of MPs into the coastal waters assumes the role of a precursor for the presence of MPs in the coastal environment of Goa. The prevalence of MPs detected in wastewater varied from  $79 \pm 21$  to  $338 \pm 7$  MPs/L with MPs sized 100-300  $\mu\text{m}$ , black-colored, and fibres (60.2%) emerging as the prevailing traits. Regarding the polymer attribution in wastewater, some prominent polymers e.g. PVP, PVAL, PAM, polyacrylate, PMMA, polyvinyl fluoride, polyurethane, polyvinyl acetate, PE, polyphenylene oxide, acrylonitrile film, polyethylene

glycol showed a clear signature of the contribution of PCPs since they contain the same polymers. Similarly, microfibrils recovered from WMEs share similarities with microfibers (PAM and PA) identified in wastewater samples. This underscores the role of wastewater as a distinct point source for impelling estuarine contamination encircling the environs of Panjim City. With respect to PHI values in wastewater, all sampling locations are categorized as 'high risk' (i.e., PHI >1000) with high polymer hazard. The chemical risks were mainly posed by polymers like PVC, PA, polybutadiene, polyester, PMMA, and PC. The finding confirms land-based origins for MP pollution in wastewater and their potential risks to the ecosystem and human health, also confirming our hypothesis covering a knowledge gap about the pathways from source to sink. This study was first to provide a snapshot information that illustrates the substantial influence of MPs in wastewater and their transmission to receiving coastal waters. Hence, this study has the capacity to make a meaningful contribution to a particular objective outlined in Sustainable Development Goal 6 (specifically SDG 6.3). This goal aims to comprehend global wastewater generation, reduce pollution, eliminate dumping, minimize the release of hazardous substances, cut the proportion of untreated wastewater, and significantly enhance global recycling and safe reuse efforts. Nevertheless, future studies await exploration, which includes maintaining consistent temporal and seasonal monitoring. This could be achieved by incorporating additional replicates to cover a larger geographical area, thereby facilitating longer and continuous studies. Thus, prudent strategies for management and effective mitigation measures are imperative to curtail the impacts of land-based MP pollution before it infiltrates the marine ecosystem.

#### **Credit authorship contribution statement**

**Chayanika Rathore:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization, writing – original draft, writing – review & editing. **Mahua Saha:** Supervision, Conceptualization, Writing – review, and editing. **Jacob de Boer:** Writing – review, and editing. **Aniket Desai:** Software illustration, writing – review & editing, **Priyansha Gupta:** Sampling and Formal analysis, **Akshata Naik:** Formal analysis, **Haritha Yespal Subha:** Sampling and Formal analysis.

#### **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Figures

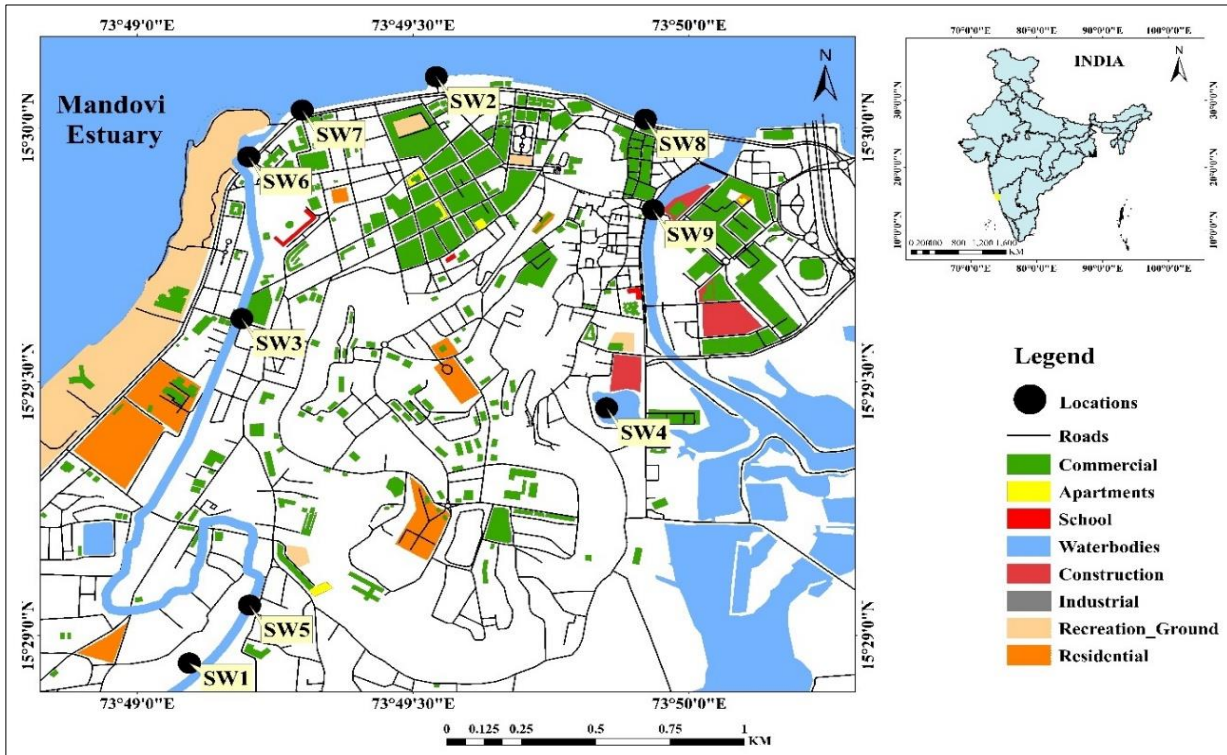


Fig.1. Study area showing the various wastewater sampling locations (black dots) including treated (SW1) and untreated (SW2- SW9) effluents around Panjim city, Goa.

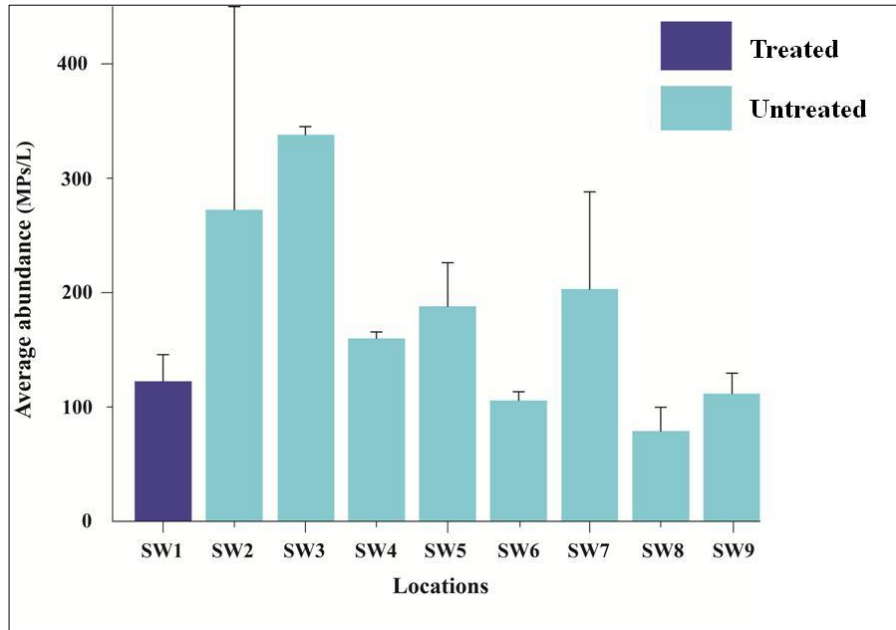


Fig. 2. Mean abundance (average  $\pm$  standard deviation) of MPs found in wastewater (SW1: treated and SW2-SW9: untreated) among all sampling locations.

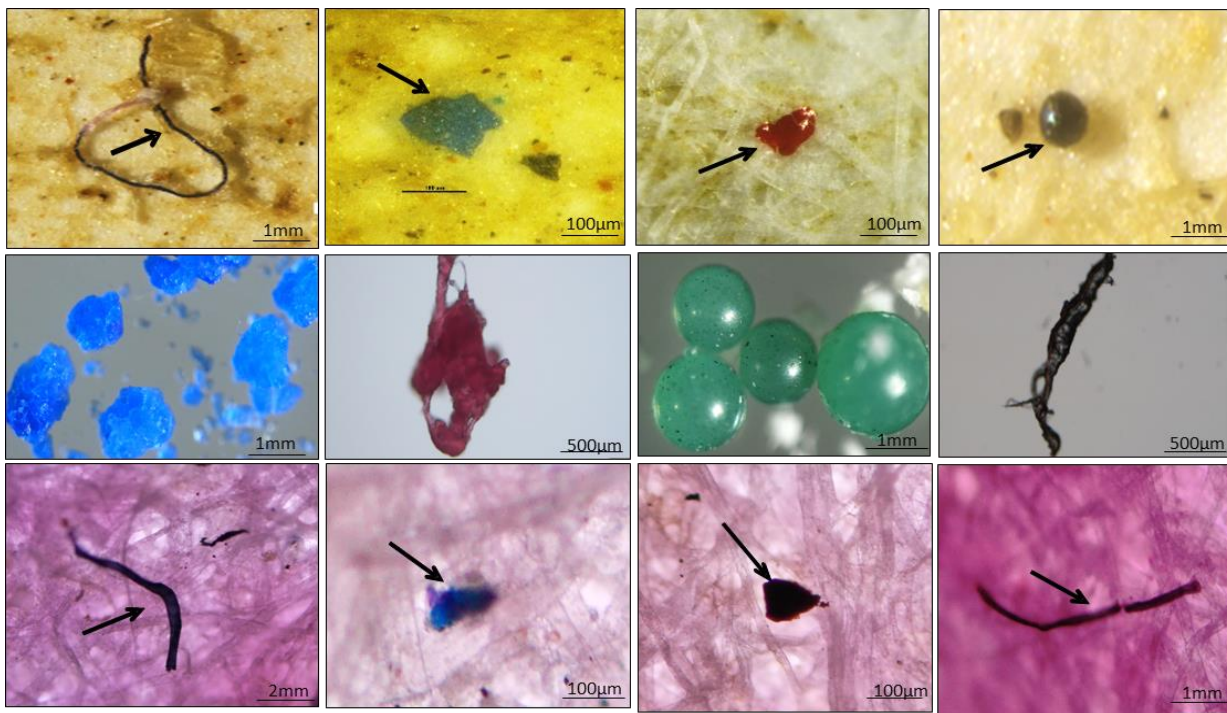
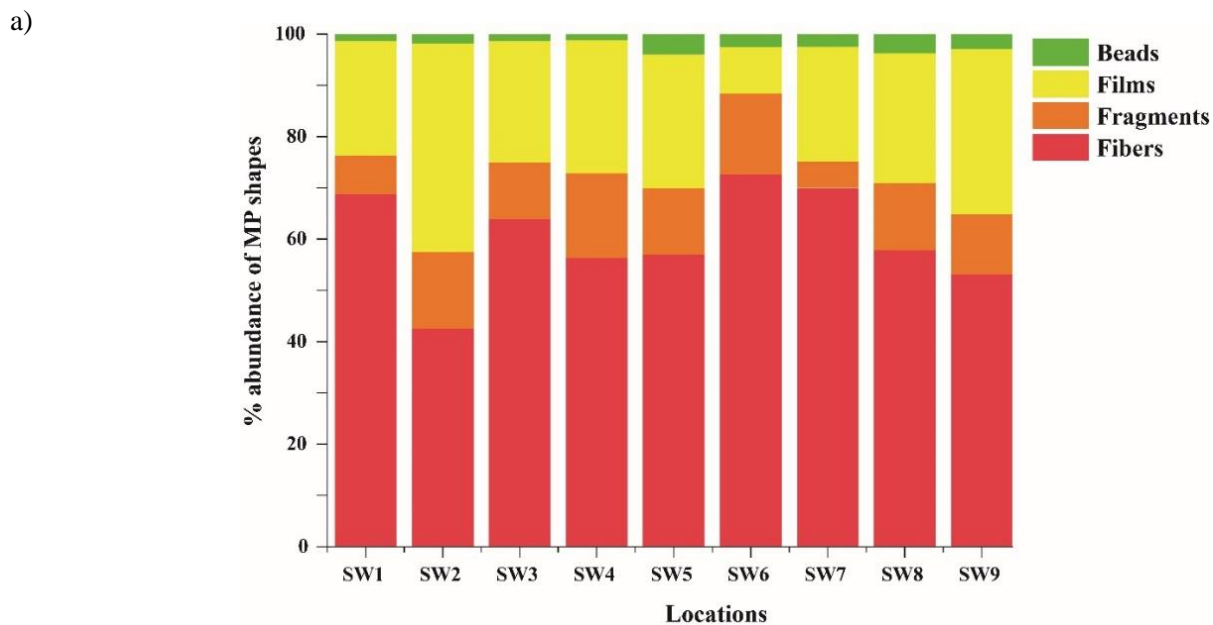
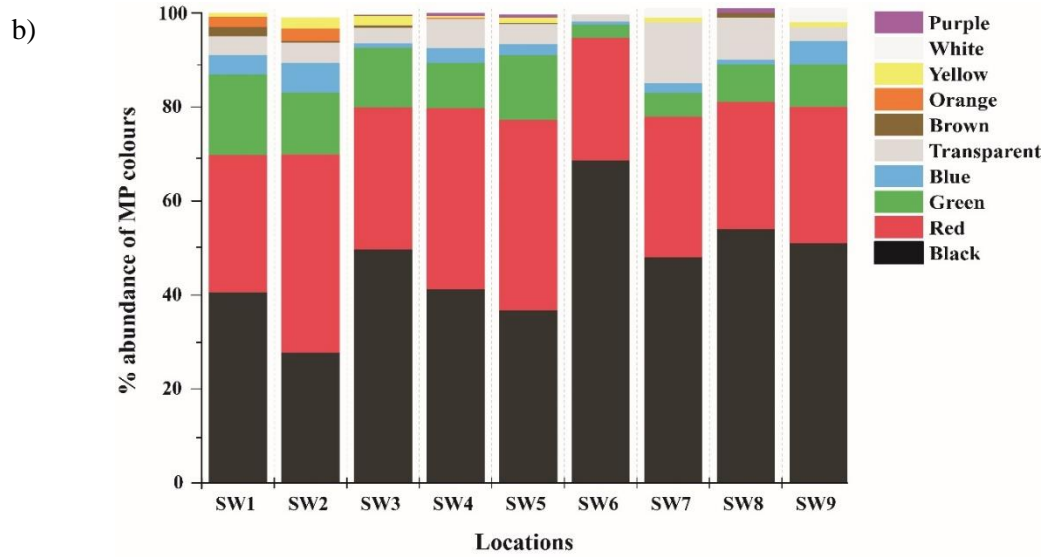


Fig.3. Stereoscopic images showing different morphology of MPs where (a) represents fiber, (b) fragment (c) film, and (d) bead in wastewater samples; (e and f) represent distorted microbeads (g) spherical microbead, and (h)- fibrous microbead in PCPs; (i and j) represent microfibrils and fragment from laundry, and (k and l) fragment and microfiber from household WMEs.





c)

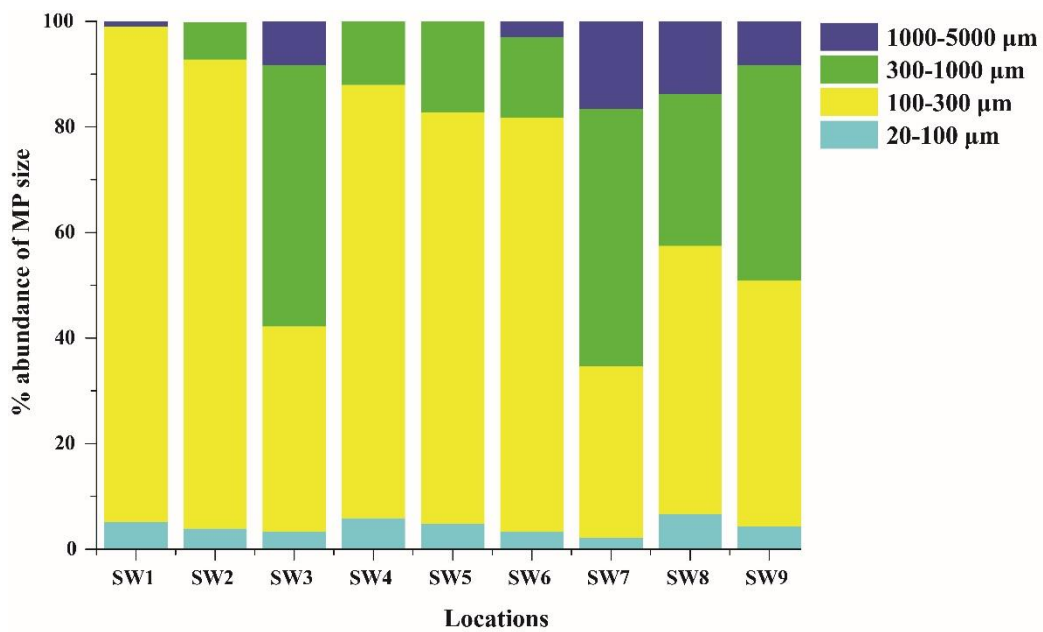


Fig.4. Percentage numerical abundance of MPs depicting (a) shape, (b) color, and (c) size among all locations.

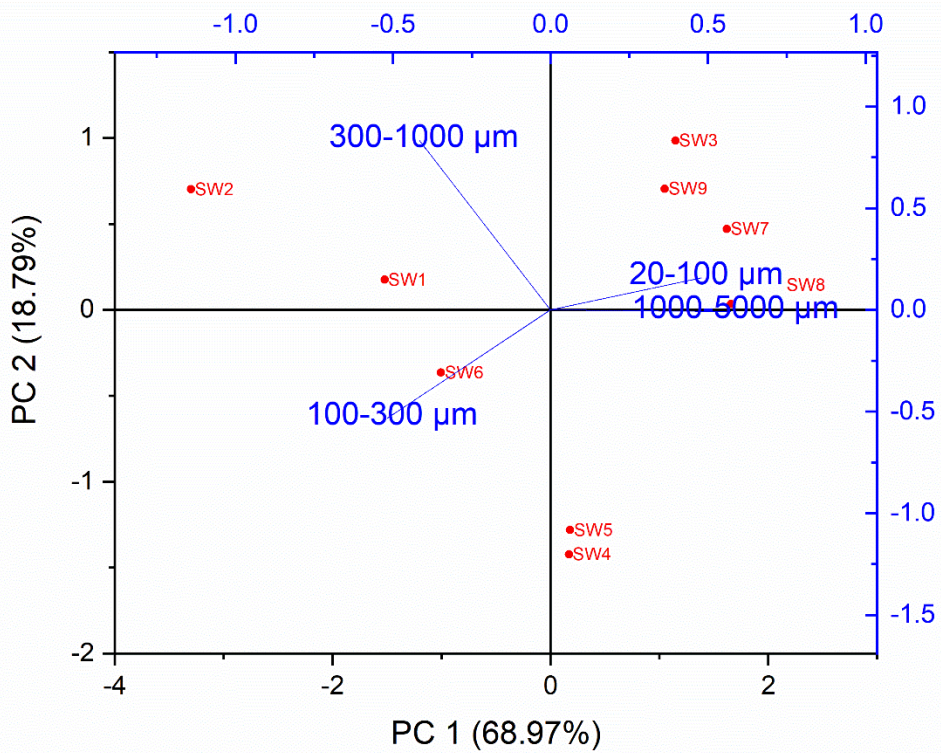
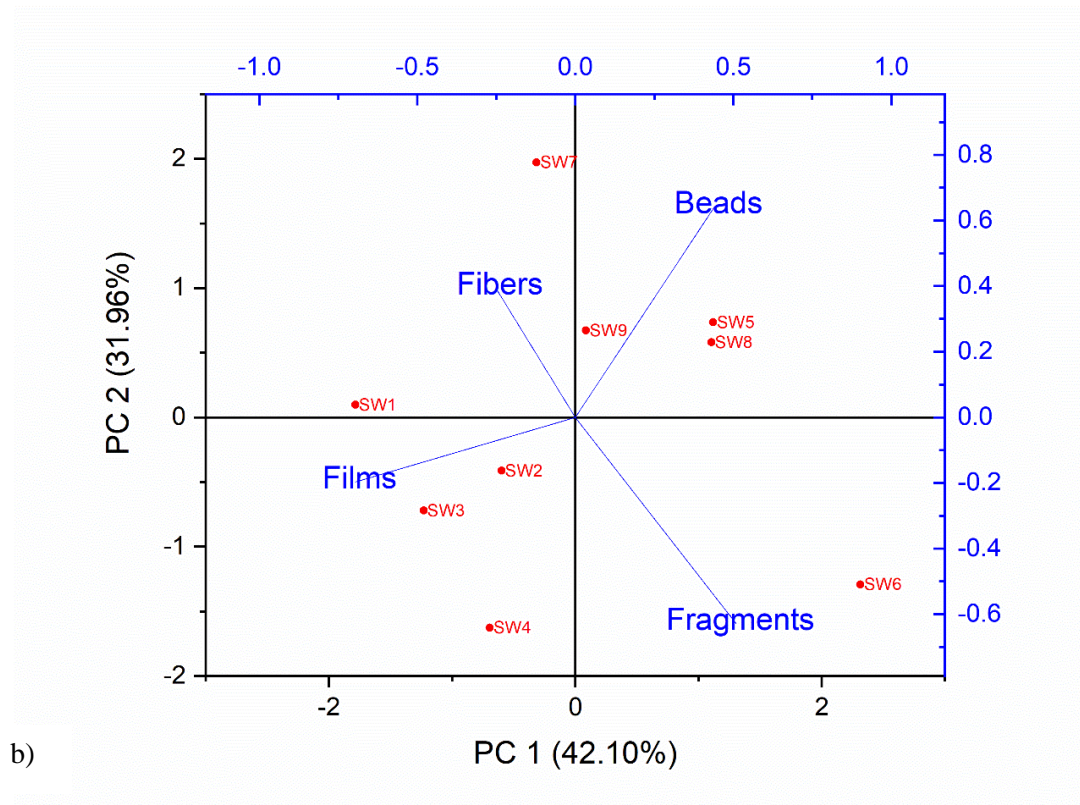
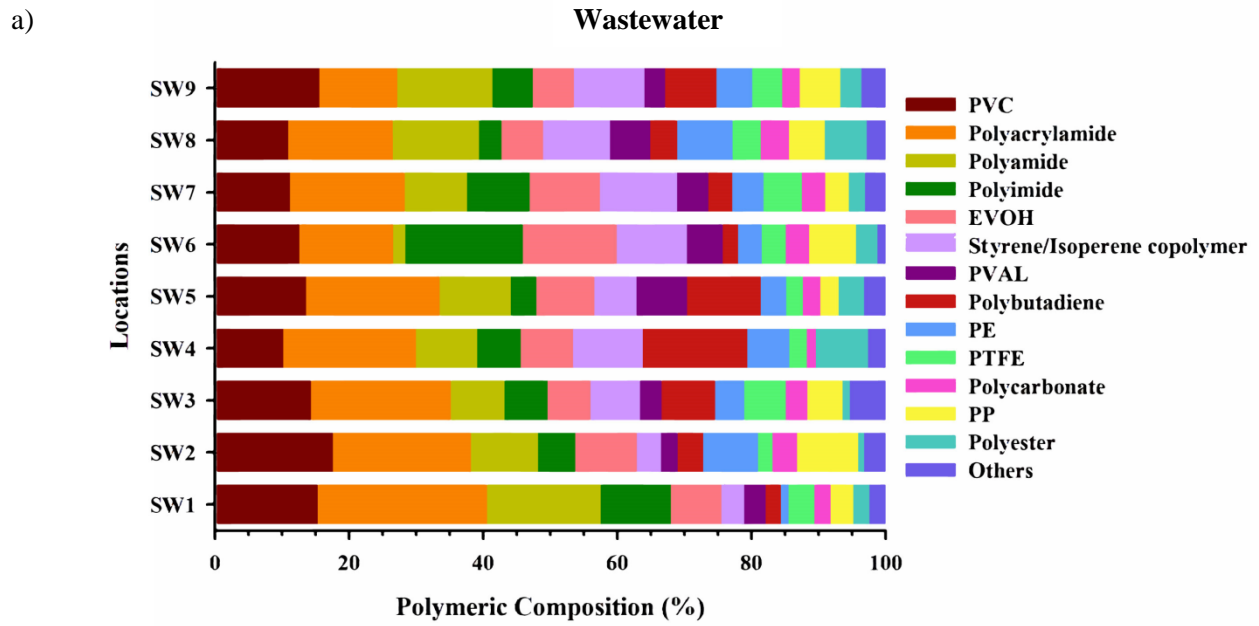
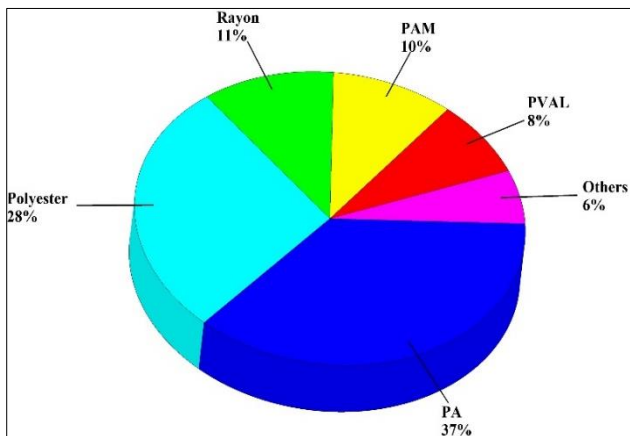


Fig. 5. Principal Component Analysis of pairwise log ratios biplot depicts a) different shape b) size fractions of MPs distributed among all studied locations.



b)

### Laundry



c)

### Household

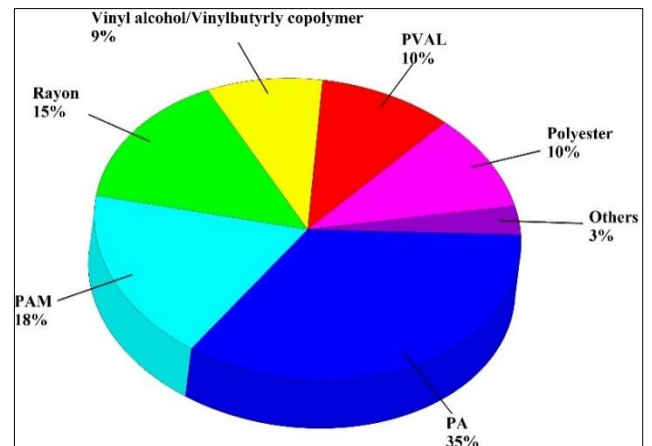


Fig.6. MPs percentage depicting polymeric composition for (a) wastewater, (b) laundry, and (c) household washing machine effluents (WMEs).

Table 1

<b>Sample code</b>	<b>Sample intake(g)</b>	<b>Total weight (g)</b>	<b>Colour</b>	<b>No.of microbeads</b>	<b>microbeads/g</b>	<b>Shape</b>	<b>Mean size(<math>\mu\text{m}</math>)</b>	<b>Polymer type</b>
<b>C1a</b>	50	1.2	Green	98	2.0	Spherical	1300	Polyethylene
<b>C1b</b>	50	1.1	Green	101	2.0	Spherical	1300	Polyethylene
<b>C2a</b>	120	14.3	Colourless	Not countable	Not countable	Distorted	700	Polyvinylidene Fluoride
<b>C2b</b>	120	13.2	Colourless	Not countable	Not countable	Distorted	700	Polyvinylidene Fluoride
<b>C3a</b>	50	1.2	Reddish Brown	89	1.8	Spherical	1100	Polyethylene
<b>C3b</b>	50	1.3	Reddish Brown	96	1.9	Spherical	1000	Polyethylene
<b>C4a</b>	50	1.3	Green	178	3.6	Spherical	1400	Polyethylene
<b>C4b</b>	50	1.7	Green	175	3.5	Spherical	1400	Polyethylene
<b>C5a</b>	50	5.8	Brown	3320	66.4	Distorted	800	Ethylene / Propylene Co-polymer
	50	5.3	White	3330	66.6	Distorted	500	Polyethylene
<b>C5b</b>	50	5.2	Brown	3308	66.2	Distorted	800	Ethylene / Propylene Co-polymer
	50	5.3	White	3310	66.2	Distorted	500	Polyethylene

<b>C6a</b>	100	0.1	Dark Green	N.C	N.C	Fibrous	700	Acrylonitrile Film(ZEXLON)
<b>C6b</b>	100	0.0	Dark Green	N.C	N.C	Fibrous	700	Acrylonitrile Film(ZEXLON)
<b>C7a</b>	50	NIL	Colourless	N.C	N.C	Not detectable		No polymer detected
<b>C7b</b>	50	NIL	Colourless	N.C	N.C	Not detectable		No Polymer detected
<b>C8a</b>	45	38.8	Bright Orange	69930	1554	Spherical	300	Vinylidene Chloride/Acrylonitrile Co-Polymer
<b>C8b</b>	45	21.3	Bright Orange	68945	1532	Spherical	300	Vinylidene Chloride/Acrylonitrile Co-Polymer
<b>C9a</b>	4	32.1	Colourless	4428	1107	Spherical	1500	Polyethylene
<b>C9b</b>	4	16.1	Colourless	4432	1108	Spherical	1500	Polyethylene
<b>C10a</b>	40	8.6	Red	N.C	N.C	Fibrous	1100	Polyethylene
	40	8.6	White	N.C	N.C	Spherical	1600	Polyacrylate
<b>C10b</b>	40	10.4	Red	N.C	N.C	Fibrous	1000	Polyethylene
	40	10.4	White	N.C	N.C	Spherical	1600	Polyacrylate



Table 2  
Comparison of MPs in effluents/wastewaters reported worldwide with the present study.

Study area	Type of wastewater (particles/L)	Dominant shapes	Colour	Size ( $\mu\text{m}$ )	Polymer type	References
Sewage treatment plant urban Nagpur, India	Influent: $1860 \pm 265$ , effluent: $148 \pm 51$	Fragments, fibers and films	Black, transparent, red, green, blue, orange	108-4494	LDPE, PP, PS, PVC, PVA, PU, CA, RY	(Patil et al., 2023)
Sewage treatment plant Bihar, India	STP system: $201 \pm 19$ (2021), $197 \pm 22$ (2022)	Fragments and fibers	Black, white, transparent, blue, red, green, yellow	<50-500	LDPE, PVC	(Parashar and Hait, 2022)
San Francisco Bay	Stormwater runoff: 1.1-24.6	Fibers and fragments	-	125-500	PE, PET, PP, PAM, PU, PS, PC, PVA, PA, ABS, PMMA, PBT, cellulose acetate, polybutyl acrylate, polyacrylonitrile, polyvinyl butyl	(Werbowski et al., 2021)
Urban wastewater, estuarine water, street runoff, Tokyo, Japan	Wastewater-Influent: 420, effluent: 8.7; estuarine water-1.4-2.3, street runoff: 81-292	Fibers	-	10- 200 $\mu\text{m}$	PE, PP, PS, PEPD, PEP, PVA, PET, AS.	(Sugiura et al., 2021)

Copenhagen, Denmark	Influent: 2223.0-18,285, Effluent: 35.0- 214	Fibers and fragments	-	10-500	Acrylate, PE,PP, VAC-acrylic copolymer, PVC, PS, EVA, PA, PVA SAN	(Simon et al., 2019)
Viikinmaki, Finland	Influent: 610, effluent: 3.5	Fibers, fragments, flakes, films and spherical	Blue, black, red, yellow, green, white, brown, transparent	20->300	Polyacryl, Polyester, PE	(Talvitie et al., 2017)
Eastern China	Drainage system and STP effluent: 130 ± 30 to 8500 ± 1241	Granular fiber and film			PE, PP, PET, PAN	(Chen et al., 2020)
WWTPs Luthunia	Influent-1964±50 – 2982± 54 Effluent-744±13 - 1244±21	Fragment	Black, white and transparent		PET, PS and PP	(Uogintė et al., 2022)
Wastewaters of Latvia and Lithuania	Lithuina- 33.75 ± 40.08 Latvia 11.67 ± 12.58	Fibers, Fragments and pellets	Blue and Black	<250-5000	PET, PVC, nylon, PS and PP	(Pashaei et al., 2023)
Urban wastewater of Goa, India	Wastewater: 79±21- 338±7; STP:122.±23	Fibers, films, fragments, and beads	Black, red, green, transparent blue, and others	20-5000	PAM, PVC, PA, EVOH, styrene isoprene, polybutadiene, PVA, PE, and others	Present study

## Supporting Information



Fig. S1: Sample collection representing few sampling sites (SW1, SW2, SW3, SW4, SW7 and SW8) selected for this study (SW- Sampled wastewater)

Table S1

A detailed description of sampling locations, type, and quality of wastewater collected.

Location ID	Sample ID	Type of wastewater collected	Quality	Lat/Long	Description
SW1	S1 S2 S3	STP	Treated	15°28'56.70"N 73°49'5.66"E	The only STP functional in Panjim area.
SW2	S4 S5 S6	Direct outfall (opening near Panjim Ferry jetty)	Untreated	15°30'6.05"N 73°49'32.50"E	Outfall connecting household, market and other commercial establishment drainage channels.
SW3	S7 S10	Nallah (a flowing sewage channel)	Untreated	15°29'37.44"N 73°49'11.37"E	Nallah connects the creek which receives raw sewage and waste from various parts of the city.
SW4	S11 S12	Mala (a stagnant conduit /nallah)	Untreated	15°29'26.88"N 73°49'51.01"E	A conduit is under the influence of sewage and garbage. Mostly, water remains stagnant. Also, receives runoff from the Altinho Hill.
SW5	S14 S15	Creek (a flowing wastewater channel)	Untreated	15°29'3.56"N 73°49'12.21"E	This beautiful creek in the past has now turned into a nallah since it has been receiving untreated sewage and solid waste from all over the city.
SW6	S16 S17 S18	Direct outfall along Mandovi estuary	Untreated	15°29'56.57"N 73°49'12.11"E	Outfall connecting drainage and sewage outlets from various household, market, and other

					commercial buildings drainage.
SW7	S19 S20 S21	Direct outfall along Mandovi estuary	Untreated	15°30'2.09"N 73°49'17.93"E	Outfall connecting drainage channels from the household, market, and other commercial buildings drainage.
SW8	S22 S23 S24	Direct outfall along Mandovi estuary	Untreated	15°30'1.01"N 73°49'55.24"E	Outfall connecting drainage channels household, market, other commercial buildings drainage. Also, creeks and conduit channels are connected to the outfall.
SW9	S25 S26 S27	Direct outfall (part of creek)	Untreated	15°29'50.30"N 73°49'56.08"E	The creek has few functional discharge points that connects households, and markets drainage channels and also receive untreated sewage and solid waste from varied channels.

Table S2 a

PCA details depicting the correlation between variables in different shape variations observed along all studied wastewater samples.

Shape Loading Plot	Eigen Value	PC1	PC2	Locations Score Labels	PC1	PC2
		46.53%	28.12%		46.53%	28.12%
		Loading Plot	Loading Plot		Scores	Scores
Fibers	1.86123	-0.69115	-0.28051	SW1	-1.03832	-1.36574
Fragments	1.12482	-0.05991	0.90352	SW2	2.04187	1.24428
Films	0.96466	0.69734	-0.12299	SW3	-0.70731	-0.05142
Beads	0.0493	0.18011	-0.29973	SW4	-0.10233	1.46222
				SW5	0.32321	-0.11949
				SW6	-2.66328	0.71623
				SW7	1.02637	-1.69993
				SW8	0.20367	-0.02244
				SW9	0.91613	-0.16372

Table S2 b

PCA details depicting the correlation between variables of different size fractions observed along all studied wastewater samples.

Shape Loading Plot ( $\mu\text{m}$ )	Eigen Value	PC1	PC2	Locations Score Labels	PC1	PC2
		76.63%	20.24%		76.63%	20.24%
		Loading Plot	Loading Plot		Scores	Scores
20-100	3.06522	-0.31707	0.92166	SW1	-1.47704	0.17465
100-300	0.80941	-0.55924	-0.01987	SW2	-2.27679	-1.12618
300-1000	0.0904	0.5556	0.18065	SW3	1.94551	-0.1782
1000-5000	0.03497	0.52728	0.3428	SW4	-1.53525	0.54362
				SW5	-0.99886	0.11804
				SW6	-0.37017	-0.68624
				SW7	2.80002	-1.06405
				SW8	0.63189	1.7303
				SW9	1.28068	0.48806

Fig. S2: Morphological characteristics of MPs (%) where (a) depicts shapes and (b) depicts colors in WMEs.

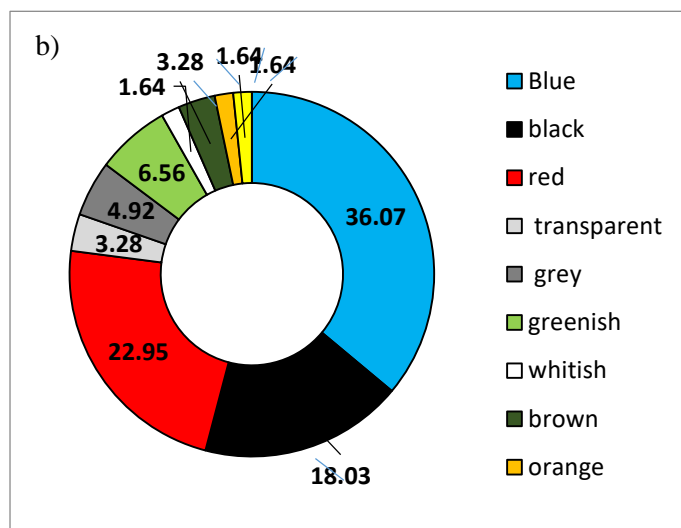
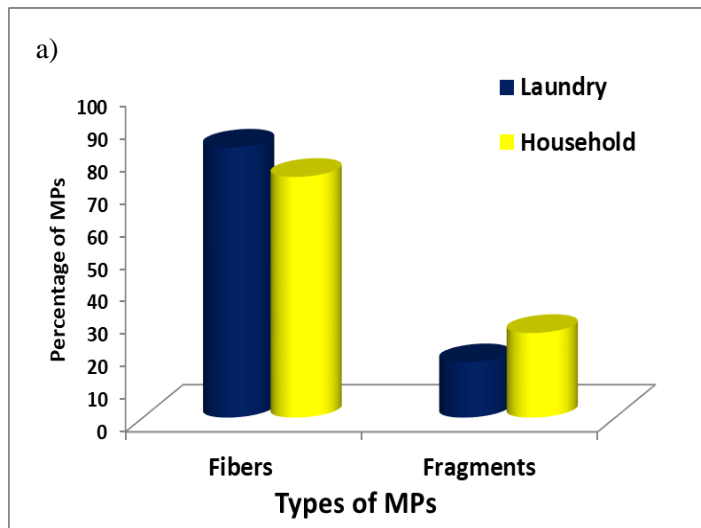


Table S3: (a) Polymer types detected in wastewaters i.e. percentage of polymers found in this study (n=35) with respective hazard score, PHI values, and Hazard score level.

Sr.no	Polymers	Hazard Score* ( S <sub>n</sub> )	% of polymers found in the study (P <sub>n</sub> )	PHI of polymers( (P <sub>n</sub> * S <sub>n</sub> )	Hazard score level
1	Polyvinyl Chloride (PVC)	10551	13.5	142438.5	V
2	Polyacrylamide (PAM)	22240	18.3	406992	V
3	Polyamide (PA)	63	10.3	648.9	III
4	Polyimide (PI)	0	7.6	0	-
5	Ethylene vinyl alcohol (EVOH)	0	8.5	0	-
6	Styrene/isoprene copolymer	2788	8.2	22861.6	V
7	Polyvinyl Alcohol (PVAL)	0	3.9	0	-
8	Polybutadiene	20001	6.5	130006.5	V
9	Polyethylene (PE)	11	5.1	56.1	II
10	Polytetrafluoroethylene (PTFE)	0	3.9	0	-
11	Polycarbonate (PC)	610	3.0	1830	IV
12	Polypropylene (PP)	1	4.7	4.7	I
13	Polyester	1117	3.4	3797.8	IV
14	Acrylonitrile butadiene styrene (ABS)	6552	0.2	1310.4	IV
15	Polymethyl methacrylate (PMMA)	1021	0.2	2204.2	IV
16	Polyvinyl pyrrolidone (PVP)	20001	0.1	2000.1	IV
17	Polystyrene (PS)	1628	0.1	162.8	III
18	Polyvinyl acetate	1	0.1	0.1	I
19	Polyvinylidene fluoride	111	0.2	22.2	II
20	Polyphenylene Oxide	400	0.1	40	II
21	Polyurethane (PU)	13844	0.2	2768.8	IV
22	Vinyl pyridiodene/vinyl copolymer	0	0.1	0	-
23	Methyl vinyl ether/maleic acid	0	0.2	0	-
24	Acrylonitrile film	11521	0.1	1152.1	IV
25	Polyethylene glycol	0	0.2	0	-



26	polyacrylate	0	0.1	0	-
27	polyetherimide	0	0.2	0	-
28	polybutylene terephthalate	0	0.1	0	-
29	polysulphone	0	0.1	0	-
30	epoxy resin	7139	0.2	1427.8	IV
31	rayon	0	0.2	0	-
32	polyphenylene sulfide	897	0.1	89.7	II
33	Polyether ether ketone	0	0.1	0	-
34	Polyethylene terephthalate (PET)	4	0.1	0.4	I
35	Polyacrylic acid	230	0.1	23	II
	<b>Overall</b> = $\sum P_n \times S_n$			<b>719838</b>	<b>V</b>

\*Hazard score derived from Lithener et al. (2011).

The sum of the percentage of these polymers comes to 3% so in the manuscript, it has been mentioned as “others” due to its lower proportion.

Table S3: (b) PHI values and hazard score levels in wastewater samples among different locations.

Locations	PHI Values	Hazard score level
SW1	843113.3	V
SW2	795710.6	V
SW3	927344	V
SW4	1078028.1	V
SW5	938492	V
SW6	706160.3	V
SW7	803826.2	V
SW8	752304.6	V
SW9	794421	V

Table S4 : Literature showing different PCPs abundance, size, and polymeric compositions reported globally.

Type of samples	No of MPs	Size range (µm)	Polymer types	References
Microbeads	4594 and 94,500 per use	164-327	PE	(Napper et al., 2015)
Microbeads	9906± 791 pieces/g	332-1015	PE and LDPE	(Cheung and Fok, 2016)
Facial cleansers Shower gel	25.04+10.69 mg MP/g	313+130 422+185	PE	(Lei et al., 2017)
Microbeads in facial scrubs	20,860 particles/g.	85 to 186	PE or LDPE, PVC	(Cheung and Fok, 2017)
Facial cleaner/scrub	11,776 ± 138 to 36,636 ± 285	3 and 145	LDPE	(Praveena et al., 2018)
	19,543 ± 873 to 52,342 ± 2954	10 and 178	LDPE and PP	
Cosmetics	164 to 31266 particles/g	< 300	PE	(Conkle et al., 2018)
Microbeads	50391 particles/g	24-800	PE, LDPE, wax, PVC,	(Guerranti et al., 2019)
Facial Scrubs	123-3730 particles/g	5 -2188	PE	(Godoy et al., 2019)
Facewash/Scrub	1.8- 1554 particles/g	320-1600	PE, PolyvinylideneFlouride, Acrylonitrile film, Ethylene / Propylene Co- polymerandVinylidene Chloride/Acrylonitrile Co-Polymer	Present Study