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Multidisciplinary analysis of microplastic dynamics: from sources to environmental fate in urban rivers during floods

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PhD Thesis

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Multidisciplinary analysis of microplastic dynamics: from sources to environmental fate in urban rivers during floods

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ABSTRACT

Microplastics (MPs) are emerging contaminants of increasing concern in freshwater environments, yet a comprehensive understanding of their mobilization, retention, and transport across spatial scales is still lacking. This thesis addresses the issue through an integrated multi-scale approach, combining controlled hydraulic experiments, analytical method development, and spatially explicit modeling.

At the experimental scale, flume tests were conducted to investigate the role of riparian vegetation in retaining MPs during high-flow events. Artificial flexible vegetation was used to simulate different canopy structures. Results show that particle retention is strongly influenced by density, shape, and vertical position in the water column, with retention rates exceeding 90% for dense, disk-shaped MPs. These findings contribute to a mechanistic understanding of MP-vegetation interactions under turbulent flow conditions.

At the analytical scale, a method combining Pressurized Liquid Extraction (PLE) and double-shot Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) was optimized for the quantification of polymer-specific MPs (PE, PET, PP, PS) in complex matrices such as wastewater and stormwater. The protocol demonstrated consistent recovery rates (43–58%) and revealed high concentrations of MPs in real samples, up to 749 $\mu\text{g/L}$ of PE in untreated effluents. This method enhances the reliability and reproducibility of MP quantification in environmental monitoring.

At the basin scale, a spatially distributed model was implemented to estimate MP emissions and delivery from multiple sources across the Arno River catchment. The model integrates emission factors from literature with the Revised Universal Soil Loss Equation (RUSLE) and a calibrated Sediment Delivery Ratio (SDR) based on the Index of Connectivity (IC). Results highlight agriculture as the dominant source (up to 1253 t/year), followed by wastewater treatment plants and combined sewer overflows. Landscape connectivity and land use patterns were found to strongly control transport efficiency.

Overall, this thesis provides a coherent multi-level framework for investigating microplastic pollution, bridging local-scale processes with catchment-scale assessments. The integrated approach proposed here offers new tools for quantifying MP fluxes and supports the development of evidence-based strategies for environmental risk assessment and mitigation.

RESUMÉ

Mikroplast (MPs) er fremvoksende forurenende nja af stigende bekymring i ferskvandsmiljøer, men en omfattende forståelse af deres mobilisering, tilbageholdelse og transport på tværs af rumlige skalaer mangler stadig. Denne afhandling adresserer problematikken gennem en integreret multiskala tilgang, der kombinerer kontrollerede hydrauliske eksperimenter, analytisk metodeudvikling og rumligt eksplicit modellering.

På det eksperimentelle niveau blev der udført kanaltests for at undersøge den ripariske vegetations rolle i tilbageholdelsen af MPs under højvandshændelser. Kunstig fleksibel vegetation blev anvendt til at simulere forskellige kronestrukturer. Resultaterne viser, at partikeltilbageholdelsen er stærkt påvirket af densitet, form og vertikal position i vandsøjlen, med tilbageholdelsesrater på over 90 % for tætte, skiveformede MPs. Disse resultater bidrager til en mekanistisk forståelse af interaktioner mellem MPs og vegetation under turbulente strømningsforhold.

På det analytiske niveau blev en metode, der kombinerer væsketrykekstraktion (PLE) og dobbelt-skud pyrolyse-gaskromatografi/massespektrometri (Py-GC/MS), optimeret til kvantificering af polymerspecifik mikroplast (PE, PET, PP og PS), i komplekse matricer som spildevand og regnvand. Protokollen viste konsistent genfindning (43–58 %) og afslørede høje koncentrationer af MP'er i reelle prøver, op til 749 µg/L PE i ubehandlede spildevand. Metoden forbedrer pålideligheden og reproducerbarheden af MP-kvantificering i miljøovervågning.

På oplandsniveau blev der implementeret en rumlig model model til at estimere MP-udledninger og transport fra flere kilder i Arno-flodens opland. Modellen integrerer emissionsfaktorer fra litteraturen med den reviderede universelle jordtabsligning (RUSLE) og et kalibreret sedimentleveringsforhold (SDR) baseret på et konnektivitetsindeks (IC). Resultaterne fremhæver landbrug som den dominerende kilde (op til 1253 t/year), efterfulgt af renseanlæg og kombineredekloakoverløb. Landskabets konnektivitet og arealanvendelsesmønstre viste sig at have en stærk indflydelse på transporteffektiviteten.

Samlet set giver denne afhandling en sammenhængende multiskala ramme til undersøgelse af mikroplastforurening, der forbinder processer på lokalt niveau med vurderinger på oplandsniveau. Den integrerede tilgang, der foreslås her, tilbyder nye værktøjer til kvantificering af MP-fluxer og understøtter udviklingen af evidensbaserede strategier for miljø risikovurdering og afbødning.

1 Chapter 1: General introduction and objectives

1.1 Background

The problem: plastic pollution and the rise of microplastics

Microplastic pollution has emerged as one of the most pervasive and least understood forms of environmental contamination, particularly in urban freshwater systems where both sources and pathways remain poorly characterized. Since 1950, global plastic production reached approximately 9.5 billion metric tons, with around 7 billion metric tons becoming plastic waste, the vast majority of which has accumulated in landfills or the environment (Houssini et al., 2025; UNEP, 2023). Compared to larger plastic debris, MPs are considered more hazardous because they are less visible and can be more readily ingested across a broad range of species (Corinaldesi et al., 2021; Jeong et al., 2024; Koelmans B et al., 2019).

While microplastics have been widely documented in marine ecosystems, posing risks to various organisms (Amelia et al., 2021; Auta et al., 2017; Cole & Galloway, 2015; Marcharla et al., 2024), evidence of their presence is also rapidly growing in freshwater and terrestrial environments (Allen et al., 2019; Dris, Imhof, et al., 2015; Gasperi et al., 2018; Horton, Walton, et al., 2017a; Wong et al., 2020).

A better understanding of how microplastics are generated, transported, and retained in urban catchments is crucial to assess their environmental fate from source to sink, including terrestrial runoff, freshwater systems, and ultimately marine environments. Despite the assumption that over 80% of marine plastic originates from land-based activities (Geyer et al., 2017; Jambeck et al., 2015; T. Wang et al., 2024), the specific sources and pathways remain poorly characterized (Kunz et al., 2023; Rochman, 2018), due to analytical limitations (Lehner et al., 2019). Alarming, plastic emissions to freshwater and soils may be up to 23 times greater than those to oceans (Horton, Svendsen, et al., 2017). Therefore, the study of urbanized watersheds, which are major hubs for plastic emissions due to dense populations and industrial activities, is vital for source identification and mitigation.

In terms of composition, a limited number of polymer types dominate the market. Specifically, polyethylene (PE in its high, low, and linear low density forms), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), and polyurethane (PUR) account for the vast majority of all plastics produced to date. In the case of fibers, polyethylene terephthalate (PET, a type of polyester) is the main material, particularly in textiles (Geyer et al., 2017). These plastics are typically composed of about 93% resin and 7% additives by weight, including plasticizers, flame retardants, and colorants. Among the most commonly reported polymers found in environmental studies are PE, PP, PS, PVC, and PET (Koelmans B et al., 2019; Rochman et al., 2013), which have been repeatedly identified in water, sediment, air, and biological samples due to their widespread use and poor degradability.

The accelerating demand for plastics has led not only to an increase in total production but also to a rising prevalence of small plastic particles in the environment. These fragments, which originate either from direct manufacture or environmental degradation of larger plastic items, are now widely recognized as persistent contaminants (Rochman et al., 2019). The ongoing accumulation of these particles in aquatic and terrestrial systems is exacerbated by their resistance to degradation and their widespread dispersal.

MPs can originate through different pathways, broadly classified into primary and secondary categories. Primary MPs are intentionally produced at small sizes for specific uses, such as polyethylene microbeads in cosmetics, polyester or acrylic abrasives in industrial applications, or pre-production pellets ("nurdles"). In contrast, secondary MPs result from the progressive breakdown of larger plastic debris in the environment via chemical, physical, and biological processes (Andrady, 2011; Gigault et al., 2016; J. Song et al., 2024). Weathering, including UV exposure, mechanical abrasion, thermal fluctuations, and microbial degradation, alters polymer structure and leads to fragmentation (Pfohl et al., 2022; Sorasan et al., 2022; Zhao et al., 2024).

The potential ecological and biological impacts of microplastics (MPs) have been the subject of growing concern in recent literature. A number of ecotoxicological studies have demonstrated that these particles can interact with a broad spectrum of aquatic organisms, often leading to adverse physiological and behavioral effects (M. Liu et al., 2023; O'Connor et al., 2020; Ugwu et al., 2021; Yuan et al., 2019). In Arctic and sub-Arctic regions, for example, benthic organisms have been found to ingest MPs ranging from 0.1 to 1.5 mm, with fibers making up the majority of ingested material and playing a dominant role in trophic transfer (Fang et al., 2018).

MP ingestion has been widely reported across trophic levels, from microalgae and macroinvertebrates to benthic organisms in polar regions. While some effects appear reversible at current concentrations, chronic exposure and accumulation in food webs raise concerns (Parker et al., 2022; Windsor et al., 2019).

Beyond direct physical effects, one of the most debated risks of MPs is their potential role as vectors for environmental contaminants, including organic pollutants, heavy metals, and pathogens (Curren & Leong, 2019; Teuten et al., 2007, 2009; Vethaak & Leslie, 2016). Once ingested, plastics may release sorbed chemicals into the tissues of exposed organisms, although in some contexts they may also reduce contaminant bioavailability (Beckingham & Ghosh, 2017). While recent findings suggest that the chemical transfer from MPs is often negligible compared to other exposure routes (Koelmans et al., 2016; Beckingham & Ghosh, 2017), elevated concentrations in pollution hotspots may significantly alter this balance (Chen et al., 2018; Diepens & Koelmans, 2018).

Importantly, health risks may stem both from the particle itself (e.g., inflammation, physical obstruction) and from the associated chemical burden. Differentiating between these mechanisms is critical to risk assessment (Besseling et al., 2017; Koelmans et al., 2017). Inhalation or ingestion of MPs may trigger tissue inflammation, leaching of additives or monomers, or translocation to distant organs, depending on particle morphology and composition (Chang et al., 2020; Wright & Kelly, 2017; Ziajahromi et al., 2016).

However, several research gaps remain. The role of particle shape (e.g., fibrous vs. fragment) and size in driving toxicity is still poorly understood (Koelmans et al., 2019). Likewise, the extent of human exposure to MPs through food and beverages, and the resulting health implications, remain largely uncertain.

Challenges in detection and quantification

Understanding the potential risks associated with microplastics is intrinsically linked to our ability to detect and characterize them accurately in environmental samples. However, the small size, diverse polymer composition, and complex nature of most matrices pose significant analytical challenges. A range of analytical techniques is employed, each with specific benefits and limitations depending on the nature of the sample and target particles (Di Fiore et al., 2024; Sharma et al., 2024).

Among the most widely used are vibrational spectroscopy methods, including Fourier Transform Infrared Spectroscopy (FTIR) and Raman spectroscopy, which enable polymer identification with limited sample destruction. However, FTIR typically has a lower detection limit of ~20 µm, while Raman spectroscopy can analyze smaller particles but is more susceptible to fluorescence interference, especially in complex samples (Araujo et al., 2018; Azari et al., 2024; Koelmans et al., 2019). As particle size decreases, the accuracy of both techniques declines, and misidentification with natural materials becomes more likely (Azari et al., 2024; Ballent et al., 2016; Eriksen et al., 2013).

Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) is a destructive method that allows quantification of specific polymers through thermal degradation of samples. It is particularly valuable in complex matrices, such as sludge and soil, but cannot provide information about particle size or morphology (Fuller & Gautam, 2016; Gies et al., 2018; Silva et al., 2018).

Moreover, matrix interference is a major obstacle, especially in samples rich in organic matter like wastewater, sediments, or stormwater runoff, where naturally occurring substances can hinder or confound plastic detection (Lykkemark et al., 2024; Worek & Styszko, 2025).

Currently, there is no universally accepted analytical protocol for MPs, leading to poor comparability across studies. As a result, recent research encourages combined analytical approaches and calls for the development of certified reference materials and interlaboratory harmonization (Burns & Boxall, 2018).

To support the accuracy and reliability of these analytical techniques, appropriate sample preparation is essential, particularly for separating MPs from complex environmental matrices. Sample preparation for MP analysis typically involves two key steps: (1) separation of plastic particles from mineral or organic solids, and (2) removal of natural organic matter (NOM) such as biofilms or plant debris.

Density separation is the most common method for isolating MPs, especially using saturated NaCl solutions (density $\sim 1.2 \text{ g/cm}^3$), due to their low cost and low toxicity (Crutchett & Bornt, 2024; Mattsson et al., 2022).

Organic matter digestion is often performed using hydrogen peroxide (H_2O_2), acids/bases (e.g., HCl, NaOH, KOH), or enzymatic treatments. The critical requirement is to eliminate organic materials without degrading plastic particles (Bergmann et al., 2015; Pfeiffer & Fischer, 2020; Renner et al., 2018). Harsh reagents or sonication may damage polymers, so wet peroxide oxidation (e.g., 30% H_2O_2 with Fe^{2+} catalyst) is frequently preferred due to its lower impact (Tagg et al., 2015; Tan et al., 2022). Protocols for water, sediment, and sludge have been widely published (Bayo et al., 2020; Crichton et al., 2017; Y. K. Song et al., 2015).

Newer methods include pressurized liquid extraction (PLE) (Dierkes et al., 2019; Fuller & Gautam, 2016), oil extraction (Scopetani et al., 2020), and procedures excluding sieving to prevent fiber loss. These methods are designed to handle sample sizes up to 5–10 g and particle sizes down to 1 μm , with minimal chemical treatment (Lares et al., 2018; Mahon et al., 2017; Talvitie et al., 2017).

Alternative digestion methods have been evaluated as well. KOH (10%, 24h, 60°C) has shown good tissue digestion without degrading most plastics (Dehaut et al., 2016), although Fenton's reagent has recently emerged as the most effective for organic-rich samples (R. R. Hurley et al., 2018). Adjusting pH and temperature is critical for safe and effective oxidation (Simon et al., 2018; Thiele et al., 2019).

Fate, transport and mitigation in urban watersheds

While methodological advances have improved the detection of microplastics in a variety of matrices, identifying their presence is only the first step in assessing their environmental impact. To understand how these particles move through and persist within the environment—especially in urban contexts—it is necessary to examine their transport dynamics and fate across hydrological networks. Urban watersheds represent critical nodes in the environmental cycle of microplastics, acting simultaneously as emission hotspots and dynamic transport corridors. Due to high population densities, extensive impervious surfaces, and diversified waste streams, they contribute significantly to environmental plastic pollution (Horton, Walton, et al., 2017a; Shao et al., 2024; Stovall & Bratton, 2022).

Plastic particles enter rivers through both point sources (e.g., WWTP effluents, sewer overflows) and non-point sources (e.g., urban runoff, atmospheric deposition, degradation of litter). MPs may be flushed from roads and surfaces during rainfall, with stormwater acting as a key transport vector, particularly during first-flush events (Do et al., 2023; Dris, Gasperi, et al., 2015). Rainfall-induced erosion can also mobilize MPs from terrestrial deposits into aquatic systems (Hanvey et al., 2017; Rehm et al., 2021).

Transport within river networks is controlled by hydrological conditions (flow velocity, turbulence, discharge), plastic particle properties (density, shape, size), sediment interactions, and biological reactions, such as biofilm colonization (Y. Fan et al., 2022; Mennekes & Nowack, 2023). The behavior of MPs can also change over time due to fragmentation, adsorption of pollutants, and aggregation with organic matter, altering their transport and deposition dynamics (Besseling et al., 2019).

Despite progress, mass-balance estimates of MP fluxes in river systems remain rare, and current understanding of their fate is incomplete. Limited data exist on in-stream retention, vegetative trapping, and re-release

mechanisms, all of which are essential for accurate modeling and mitigation (Berg et al., 2025; Ghinassi et al., 2023; Martuscelli et al., 2025).

Mitigating MPs pollution in urban catchments requires a comprehensive and multi-layered strategy, combining upstream prevention, technological innovation, and monitoring. Since these pollutants originate from diverse and diffuse sources, no single intervention can be fully effective (Hartmann et al., 2019; Imbulana et al., 2024; Rochman et al., 2013).

At the source, limiting the production and release of plastic, especially single-use products and synthetic fibers, is the most direct mitigation pathway. Policies such as bans, taxes, or product redesign can significantly reduce the volume of plastics entering urban systems (Geyer et al., 2017; Rochman, 2018).

Upgrades in wastewater treatment technologies are also effective. Tertiary processes like ultrafiltration, ozonation, or UV treatment can remove up to 95–99% of MPs under optimized conditions (Carnevale Miino et al., 2024; Iordachescu et al., 2024; Iyare et al., 2020; W. Liu et al., 2021), but due to high effluent volumes, even high-performing WWTPs may discharge millions of MP particles per day (Murphy et al., 2016). Removal efficiency is also influenced by polymer type and particle size (Iyare et al., 2020; F. Liu et al., 2019).

Stormwater management strategies such as vegetated retention basins, constructed wetlands, and permeable surfaces can help intercept MPs before they enter water bodies, especially during first-flush events (Dris et al., 2017; H. C. Lu et al., 2022). Riparian vegetation can further reduce MP transport during moderate flows by enhancing sedimentation and filtering particles (Gallitelli et al., 2020; Martuscelli et al., 2025).

A critical bottleneck in mitigation efforts is the lack of standardized protocols for sample collection and analysis, which hinders cross-study comparisons and long-term monitoring (Lehner et al., 2019). Harmonizing methodologies, developing certified reference materials, and encouraging inter-laboratory calibration are urgently needed (Renner et al., 2018).

Finally, raising public awareness remains essential. Reducing synthetic textile use, improving waste sorting, and discouraging littering can reduce diffuse sources at the individual level (M. Wagner et al., 2014).

Despite notable advances in detection methods and modeling, significant gaps remain. In particular, understanding the fate and retention of plastics within stream networks, quantifying MPs exposure, and separating methodological biases from true environmental signals are ongoing challenges (Burns & Boxall, 2018; Lehner et al., 2019).

Flood-induced mobilization of microplastics

Flood events, both fluvial and pluvial, play a pivotal role in the mobilization, redistribution, and flux amplification of MPs in urban catchments (Chen et al., 2024; Ockelford et al., 2020; Rolf et al., 2024). These hydrological extremes trigger sudden changes in flow regimes, sediment dynamics, and surface runoff connectivity, ultimately altering the balance between retention and transport of MPs across environmental compartments (Lechthaler et al., 2021). Despite this critical influence, the role of floods in shaping MP pathways remains underexplored in most studies and poorly represented in existing models.

Pluvial floods, typically generated by intense rainfall over urbanized areas, can rapidly mobilize MPs from impervious surfaces, road dust, synthetic turf, and degraded litter into combined sewer systems or directly into receiving waters. During such events, combined sewer overflows (CSOs) and stormwater discharges act as pulse emissions of MPs, bypassing wastewater treatment plants and delivering highly concentrated loads to rivers within minutes to hours (Nguyen et al., 2024). In contrast, fluvial floods, driven by river discharge exceeding bankfull conditions, primarily influence in-stream processes. These include scouring of bed sediments, lateral erosion of floodplains, remobilization of previously trapped MPs within riparian vegetation or depositional zones, and long-distance downstream transport due to enhanced turbulence and sediment entrainment (Ghinassi et al., 2023; He et al., 2021; Martuscelli et al., 2025).

Recent evidence highlights the intensity of these mechanisms. A flood event in the Meuse River in 2021, induced by extreme rainfall, accounted for over one-third of the annual plastic flux in less than a week, with peak discharges increasing transport rates by two orders of magnitude (T. H. M. van Emmerik et al., 2023). Similarly, controlled experiments have shown that even moderate storm events can remobilize significant amounts of MPs previously retained in riverbed sediments, effectively shifting rivers from temporary sinks to net sources of plastic pollution (Ockelford et al., 2020). These remobilized loads often exhibit different particle morphologies and polymer compositions, suggesting selective entrainment processes influenced by particle density, shape, and biofilm interactions (Lahon & Handique, 2023).

Urbanization further exacerbates these flood-driven dynamics. In highly urbanized sub-catchments, flood-triggered MP concentrations have been reported to increase 5-8 fold, particularly downstream of dense residential and industrial areas (de Carvalho et al., 2022). Time-resolved sampling in Central Europe demonstrated that episodic surges in microplastic concentrations consistently coincide with flash floods, especially after dry periods, a phenomenon known as the “first flush effect” (Balla et al., 2024). These findings underscore the temporal variability and unpredictability of MP transport under flood regimes, with potential implications for exposure hotspots and downstream accumulation zones.

Tackling plastic pollution requires a coordinated response across disciplines, from analytical chemistry and environmental modeling to infrastructure planning and regulation. Moving forward, efforts should prioritize source reduction, the optimization of treatment systems, and the implementation of harmonized monitoring protocols to achieve effective mitigation.

With urbanization projected to intensify in the coming decades, proactive strategies will be crucial to prevent plastic pollution from becoming an even greater environmental burden (Stokal et al., 2021; Xu et al., 2020).

Addressing these limitations requires the integration of hydrological variability into MP modeling, particularly through spatially distributed approaches that can simulate event-based transport and source connectivity. This thesis contributes to this emerging field by incorporating hydrological drivers into both laboratory-scale experiments and GIS-based transport models. In particular, the role of flood events is considered in the design of retention tests with flexible riparian vegetation, and in the definition of delivery coefficients for catchment-scale flux estimation.

Building on these foundations, the thesis addresses three complementary aspects of the microplastic pollution cycle in urban freshwater systems: (1) the physical retention mechanisms of MPs in vegetated river stretches, (2) the analytical challenges and optimization strategies for reliable polymer detection, and (3) the spatial modeling of MP transport from terrestrial sources to aquatic sinks. These components, though distinct, form an integrated investigation that spans from experimental hydraulics to analytical chemistry and spatial modeling, offering a multiscale, interdisciplinary perspective on microplastic behavior in catchments.

1.2 Knowledge Gaps Addressed by This Thesis

To partially address the current knowledge deficiencies outlined in the previous sections, this doctoral thesis investigates distinct but complementary aspects of MP behavior and management in freshwater systems. Through a combination of experimental, analytical, and modeling approaches, it aims to enhance our understanding of MP retention, quantification, and catchment-scale transport. Given the breadth and complexity of processes influencing MP dynamics, ranging from hydrological and morphological conditions to particle-specific properties and methodological limitations, it was necessary to define a set of focused research questions that guided the development of the individual studies. In particular, the work presented here is unified by a spatial and conceptual progression: from the fine-scale mechanisms of particle retention within vegetated river reaches, to the analytical optimization of polymer-specific detection protocols, and ultimately to the integration of these insights into spatially distributed models for source apportionment and flux estimation. Based on this structure, the thesis addresses the following critical knowledge gaps:

1. **Limited understanding of microplastic retention mechanisms in flexible riparian vegetation.**
While riparian areas have been identified as potential buffers against MP transport, empirical quantification of this function remains scarce. Existing studies often neglect the effects of foliage distribution or do not consider the influence of particle-specific properties. This thesis fills that gap by designing controlled laboratory flume experiments capable of quantifying MP retention across vertical layers of foliage and for different particle types.
2. **Lack of robust, polymer-specific quantification methods for microplastics in complex environmental matrices.**
Many analytical protocols suffer from low recovery rates, particularly for certain polymers like polystyrene (PS), and are often affected by matrix interferences and inconsistencies in calibration. This thesis responds to this challenge by developing and validating a Py-GC/MS method coupled with pressurized liquid extraction (PLE), enabling reliable quantification of four polymers (PE, PET, PP, PS) in wastewater and CSO samples, with explicit consideration of recovery efficiency and carry-over.
3. **Disconnect between analytical MP data and spatial modeling approaches.**
Most spatial models treat MP emission or concentration data as externally sourced, often ignoring how these values were generated and the associated uncertainties. Here, experimentally validated emission factors derived from robust analytical protocols are directly coupled with sediment connectivity metrics (IC and SDR), allowing for a more realistic and internally consistent GIS-based modeling of MP delivery to river systems.
4. **Underrepresentation of multi-source contributions at the catchment scale.**
Microplastic transport models often focus on single sources (e.g., urban runoff), overlooking the interaction between diffuse and point sources such as agricultural land use, roads, CSOs, and wastewater treatment plants. This thesis proposes a spatially explicit modeling framework that integrates these diverse inputs, distinguishing among source types and transport pathways, and estimating their relative contributions to riverine MP loads at the river basin scale.

1.3 Structure of the Thesis

This doctoral thesis is organized into three main chapters, each corresponding to a distinct yet complementary study addressing a specific facet of the microplastic pollution problem in freshwater systems. While each chapter was designed to be self-contained and suitable for publication, the work as a whole reflects a coherent trajectory from process-scale understanding to analytical innovation and spatial modeling.

- **Chapter 2** investigates the capacity of riparian vegetation to act as a physical barrier to microplastic transport. Through a series of flume experiments with synthetic flexible foliated vegetation, this study quantifies retention efficiency using particles with varying densities and shapes,. The outcomes include the development of new retention indicators and offer insights into the role of riparian vegetation in mitigating MP fluxes under different hydrodynamic conditions.
- **Chapter 3** focuses on the analytical challenges posed by complex environmental matrices. A novel protocol is presented that couples pressurized liquid extraction with Py-GC/MS to enable accurate quantification of target polymers (PE, PET, PP, PS) in wastewater and CSO samples. The method is validated against multiple performance metrics including recovery, matrix effects, and reproducibility, providing a robust tool for polymer-specific MP quantification.
- **Chapter 4** presents a GIS-based modeling framework to estimate the emissions and transport of microplastics from heterogeneous sources within an urbanized river catchment. The model leverages connectivity indices and sediment delivery functions to simulate source-to-river dynamics, explicitly incorporating the polymer-specific emission factors based on literature data, assigned to each source type in the catchment. The spatial outputs offer a synthetic overview of critical source areas and potential delivery pathways.

The thesis concludes with a synthesis chapter that discusses the overarching contributions of the three studies, highlights key findings, and outlines avenues for future research and policy development.

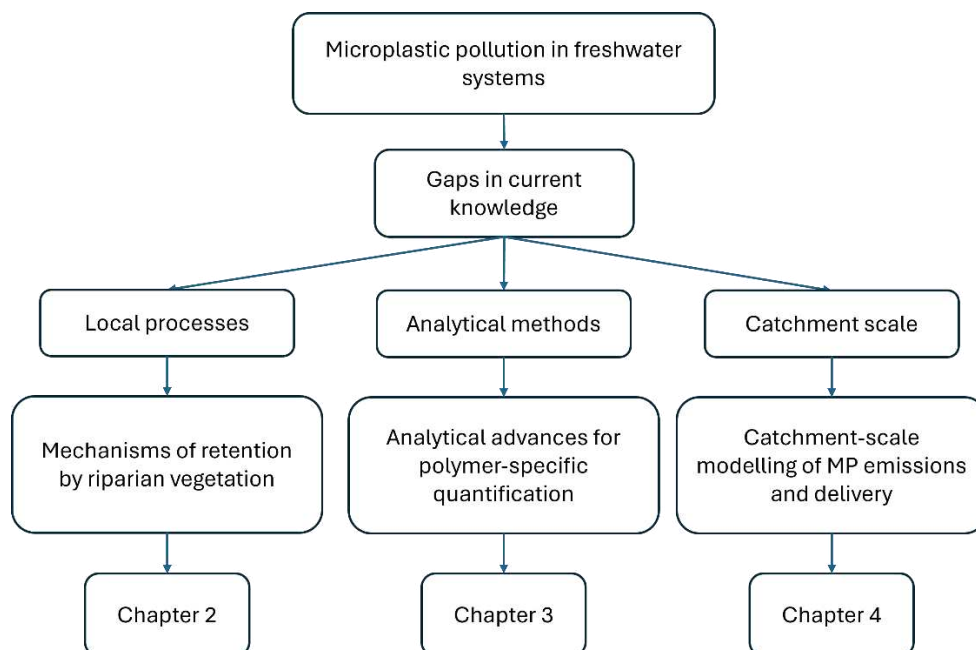


Figure 1. 1 - Conceptual overview of the thesis organization. The diagram illustrates how the research addresses current knowledge gaps in freshwater microplastic (MP) pollution through three complementary approaches

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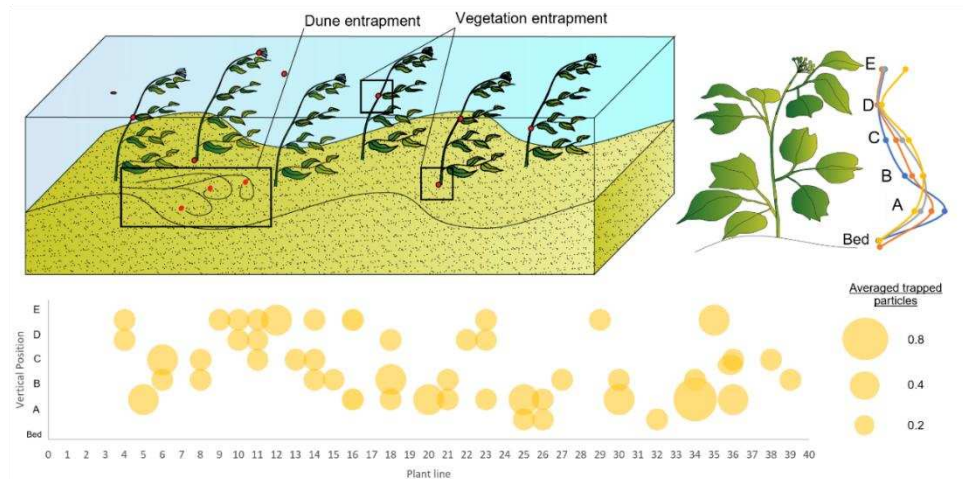
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2 Chapter 2: Riparian vegetation as a natural barrier: experimental analysis of plastic particle retention in a vegetated reach

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Abstract

Plastic pollution is a growing environmental concern, with rivers acting as critical pathways for plastic particles entering marine ecosystems. In river corridors, riparian vegetation has been identified as a potential natural barrier for plastic particles while transported downstream. Knowledge of the trapping dynamics appears fundamental for understanding the role of riparian barriers in mitigating plastic pollution. However, to date the effects of the foliage distributions of riparian flexible plants and of turbulence on the plastic particle retention dynamics have not yet been studied. This work aims to fill these knowledge gaps including also the influence of the particle characteristics (i.e., density, size, and shape) and of the vegetation structure (i.e., number of branches and leaves). Laboratory experiments were performed in steady flow conditions over a fixed dune bed, using just submerged flexible foliated plastic plants. Results show that particle retention is highly influenced by density and shape. Among the tested disk-shaped particles, those with higher density (1.4 g/cm^3) exhibited retention rates up to 93%, largely at the channel bed structures. At equal density (1.04 g/cm^3), irregular-shaped particles were retained at nearly twice the rate of disk-shaped ones (20% vs. 9–10%), with vertical dispersion influenced by turbulence. Turbulent Kinetic Energy (TKE), expressed as dimensionless TKE/U^2 values, showed a non-linear relationship with retention: values below ~ 0.0015 were associated with enhanced trapping, while higher turbulence reduced retention, likely by promoting resuspension and weakening particle adhesion. Vertical profiles of TKE/U^2 varied with leaf configuration, suggesting that vegetation structure modulates turbulence intensity along the canopy. These findings provide a quantitative framework to evaluate microplastic trapping efficiency in vegetated riparian zones, offering insights into natural and engineered nature-based solutions for mitigation of plastic pollution in aquatic systems.

Keywords: Microplastics, riparian vegetation, plastic retention, Leaf area index, Turbulent kinetic energy, trapping efficiency, disk-shaped particle

Highlights:

- Riparian vegetation traps microplastics, acting as a natural retention barrier.
- Particle shape, size, and density dictate retention patterns in vegetated flows.
- Denser particles (1.4 g/cm^3) show 93% retention, lighter ones disperse more.
- Vegetation structure govern microplastic trapping, shifting retention along the canopy.
- Lower TKE increases retention, reducing particle mobility.

2.1 Introduction

In recent years, microplastics (MPs) have emerged as one of the most pervasive and persistent pollutants (Bhardwaj et al., 2024; Jain et al., 2023; Stapleton & Hai, 2023). MPs are defined as particles in the range size 1 μm to 5 mm (Frias & Nash, 2019; Horton, Walton, et al., 2017b; Thompson et al., 2004).

The extremely large production and consequent use of plastic polymers is pushing the presence of this contaminant in all types of environments (Geyer et al., 2017; Rizzo et al., 2021; Schwarz et al., 2023; Williams & Rangel-Buitrago, 2022). Once into the environment, plastic litter is transported and involved in natural processes. Considering the water cycle, these elements are usually retained in different matrixes during transportation, such as soil, river sediments and aquatic vegetation (Free et al., 2014; Mancini et al., 2023; T. van Emmerik & Schwarz, 2020; Vermaire et al., 2017).

Recently, rivers are taking more interest in the scientific community showing that they are responsible for the largest part of the microplastics reaching the ocean (Meijer et al., 2021). Rivers are often receiving human-related water sources such as the effluents of wastewater treatment plants (WWTP) and of combined sewer overflows (CSOs) (Coyle et al., 2020; W. Fan et al., 2023; Ziani et al., 2023). In addition, MPs can reach the river corridors due to surface runoff from land field or roads, bringing MPs originated in agriculture or produced by tires abrasion (Nizzetto, Bussi, et al., 2016; Quilliam et al., 2023; Tamis et al., 2021; S. Xiao et al., 2024). Once in rivers, MPs are transported through complex and dynamic processes, such as interaction with river sediment, vegetation, and biota. (Adomat & Grischek, 2021; D'avignon et al., 2022; Frei et al., 2019; Gao et al., 2024; Gerolin et al., 2020).

The fate of microplastics is influenced by river hydraulic conditions, bed morphology and vegetation presence (Frei et al., 2019; X. Lu et al., 2023; McIlwraith et al., 2024; Summers et al., 2024). Microplastics can either remain suspended in the water column, or settle into sediment bed, and can be transported downstream (Lenaker et al., 2019; Pasquier et al., 2023). The MP characteristics, i.e. shape, density, roughness and possible biofouling, concur in determining if the particles are deposited, transported in suspension or as bed-load, if they can be easily retained by vegetation or boulders, or ingested by organisms (Fox et al., 2024; McIlwraith et al., 2024; Patidar et al., 2024; Salehi et al., 2024).

Particularly the role of riparian vegetation on the MPs transportation along rivers has recently been highlighted, suggesting the importance of vegetated patches in trapping microplastic. However, to date, the trapping mechanism is not yet fully understood, showing the need for more comprehensive studies to better understand the variability of this process (Battulga et al., 2019; Bruge et al., 2018; Kiessling et al., 2019; T. Van Emmerik et al., 2020).

(Cesarini & Scalici, 2022a) found that shrubby zones are effective in trapping plastics from urban sources, highlighting the role of riparian vegetation as a natural filter. Similarly, (Gallitelli et al., 2024) developed a protocol using a three-dimensional vegetation index to link vegetation structure (i.e., density, branches, species diversity) with plastic trapping efficiency. While both studies emphasize the role of vegetation, a more detailed understanding of how different vegetation types and structures influence plastic retention is needed, as well as the effects of environmental variables like seasonal flooding. Some experimental studies in laboratory settings (Baki, 2022; Ijaz et al., 2023) have examined the impact of turbulence and vegetation density on microplastic retention. (Ijaz et al., 2023) found that, under a specific geometric

configuration, large roughness elements, such as vegetation and boulders, increased turbulence and thus enhanced the retention of microplastics. (Baki, 2022) showed that higher vegetation density reduces flow velocity and increases turbulence, leading to greater microplastic retention. Both studies identified a need for improved predictive models to account for hydraulic factors such as the Turbulent Kinetic Energy (*TKE*) and the flow condition in natural systems.

Regarding the seagrass vegetation type, characteristic of a subaqueous environment, (Wilkie et al., 2012) found that low-density seagrass enhances particles retention in low-flow conditions. (McIlwraith et al., 2024) studied how the shape of MPs affects their retention in vegetated habitats: fibers were more likely trapped in denser vegetation patches, while flakes and fragments settled into sediments. It is still not completely understood the role of the particle adhesion mechanism and of the plant structure and density on microplastic retention (de Smit et al., 2021; Mendrik et al., 2024).

De Smit et al. (2021) and Mendrik et al. (2024) demonstrated that dense benthic vegetation structures, such as coral reefs and seagrass beds, enhance microplastic retention by reducing flow velocity and promoting particle interception and settling. Although these systems operate under different hydrodynamic conditions compared to riparian zones, their use of structurally complex elements to influence flow dynamics offers a useful parallel. In both marine and riparian contexts, physical complexity—such as the three-dimensional arrangement of stems, fronds, or branches—plays a key role in altering flow paths, creating low-velocity zones, and promoting deposition. These analogies suggest that insights from benthic environments can inform our understanding of retention processes in vegetated river corridors, while also highlighting the need to adapt such findings to the specificities of unidirectional and highly variable fluvial flows. Stride et al. (2023) demonstrated that neutrally buoyant microplastics in submerged vegetated flows exhibit transport dynamics comparable to solutes, with longitudinal dispersion primarily governed by vegetation height and flow velocity. Their findings suggest that vegetation structure plays a pivotal role in shaping MP transport pathways and support the applicability of solute-based models for predicting microplastic behavior in vegetated aquatic environments (Stride et al., 2023).

Valyrakis et al. (2024) demonstrated that dense riparian vegetation significantly reduces the downstream transport of floating macroplastics, emphasizing the importance of low-turbulence conditions and showing limited influence of particle size under certain flow conditions. These insights mirror sediment transport principles long established in the fluvial hydraulics literature (Bagnold et al., 1966; Engelund & Fredsee, 1976; Van Rijn, 1984), and point to the need for studies that categorize MPs transport and retention using analogous frameworks as recently proposed by Born et al. (2023) and Cowger et al. (2021) and (Waldschläger et al., 2022) for plastic particles.

Previous studies highlighted the role of vegetation in trapping microplastics transported by riverine and marine flows, demonstrating that vegetated environments enhance microplastic deposition compared to bare beds (de Smit et al., 2021; H. Liu et al., 2024). However, while several studies have investigated MP retention in submerged vegetation such as seagrass or coral-like structures, the mechanisms driving retention in riparian vegetation remain less understood. In particular, no prior studies have systematically explored the role of foliage structure in flexible riparian plants combined with the behavior of disk-shaped plastic

particles—a shape commonly derived from fragmented packaging films and frequently found in riverbanks. This represents a critical gap in understanding the retention dynamics in fluvial systems where vegetation can act as a natural filter for plastic pollution.

This research directly addresses these gaps by investigating how realistic flexible riparian vegetation interacts with microplastics particles under controlled hydrodynamic conditions. An experimental campaign was conducted in a hydraulic flume to examine the retention of plastic particles in a patch of flexible vegetation considering various foliage configurations and varying MPs properties.

Additionally, while most previous researches have focused on fibers or spherical microplastics, this study specifically investigates disk-shaped particles, which closely resemble the most common microplastic debris found in the environment, typically originating from the fragmentation of larger plastic objects, such as packaging materials or plastic films. Despite their environmental prevalence, the retention behavior of these microplastics types in vegetated flows remains poorly understood, making their inclusion a novel contribution to existing knowledge.

The objectives of this study are to: (i) quantify the microplastic retention efficiency of flexible riparian vegetation under controlled flow conditions, (ii) assess how particle properties (density, size, shape) and foliage structure influence retention patterns, and (iii) evaluate how turbulent kinetic energy (TKE) modulates retention efficiency and vertical particle distribution.

This study provides the first experimental evidence of how flexible riparian vegetation, under controlled hydraulic conditions, modulates microplastic retention as a function of both vegetation architecture and particle characteristics.

2.2 Materials and methods - Methodology

2.2.1 Retention efficiency

In this work, the retention coefficient (K) is considered as the percentage of MPs trapped by vegetation during their transport by water flow within the river channel. Consequently, the retention coefficient can be assumed as a direct estimation of the retention efficiency of vegetation in rivers (Ijaz et al., 2022, 2023; Mendrik et al., 2024). A higher value of K corresponds to an increased retention efficiency, indicating that a larger proportion of microplastic particles is retained within the channel. This parameter serves as a fundamental indicator of the capacity of vegetative structures to act as barriers or filters for MPs. In particular, the retention coefficient is defined as:

$$K = \frac{MPs_{Trapped}}{MPs_{Released}} \cdot 100$$

where $MPs_{trapped}$ is the number of particles trapped in the hydraulic channel and $MPs_{released}$ is the number of particles released at the beginning section of the hydraulic channel.

The retention coefficient highlights the interplay between fluid dynamics, vegetation characteristics, and microplastics characteristics. The evaluation of this coefficient allows the prediction of how changes in flow conditions, particle properties, or vegetation density can alter the retention behavior, potentially reducing the downstream transport of plastic pollutants in aquatic systems.

The value of the retention coefficient depends on a range of hydrodynamic and structural parameters. Hydrodynamic factors, such as water velocity, turbulence, and flow regime, influence the interaction between MPs and vegetation. Physical parameters, on the other hand, include the physical characteristics of both the microplastic particles (e.g., size, density, and shape) and the vegetation (e.g., stem density, flexibility, and spatial arrangement). These factors determine the probability of particle entrapment and, consequently, the magnitude of K .

2.2.2 The Buckingham π theorem

To better understand the parameters controlling this process, the Buckingham π theorem has been applied. The retention coefficient can be expressed as a function of various parameters, including the Froude number (Fr), the Reynolds number (Re), the Leaf Area Index (LAI) defined as the ratio of the total one-sided leaf surface area to the ground surface area, and the key physical properties of the system, the averaged flow velocity U , the water density ρ_w , the water level h_w , the height of the plants h_c , the diameter and the density of particles released in the channel, respectively ϕ and ρ_p , and the turbulent kinetic energy TKE .

Considering the Buckingham π theorem we can define the dependence of K as:

$$K = f(h_c, \rho_p, h_w, \rho_w, \phi, U, TKE, Fr, Re, LAI) \quad (1)$$

The formula was modified based on the considerations of constant flow rate and section, such that U could be retained constant. Considering the flow as fully turbulent, the influence of Reynolds number is negligible. The parameters h_c and Fr were omitted from the analysis as they were consistently maintained at fixed values during the experiments.

Based on these considerations, the equation (1) could be rewritten as follows:

$$K = f\left(\frac{TKE}{U^2}, \frac{\rho_w}{\rho_p}, \frac{\phi}{h_w}, LAI\right) \quad (2)$$

By considering the relationship between these variables, we can investigate the retention of plastic particles by vegetation. Factors such as the dimensionless TKE , the ratio of water to particle density ρ_w/ρ_p , the ratio of particle diameter to the height of the water ϕ/h_w , and the LAI play a key role in determining the likelihood of retention.

2.2.3 Experimental setup and scenarios

Experimental tests were performed at the Hydraulic Laboratory of the University of Florence, Italy. A rectangular cross-sectional flume, 0.44 m wide and 10.5 m long, was used for the experimental activity. An overview of the flume used for the experiments is provided in Supplementary Material Fig. S2.2. Tests were carried out considering a quasi-uniform flow and fixed bed conditions. Fig. 2.1 shows a sketch of the experimental design: the central part of the flume was covered with an 8 m pattern of fixed dune configuration (see Fig. 2.1A) over which eighty plants were installed (see Fig. 2.1B). The dune-bed configuration is adopted from previous experiments conducted by Artini et al., 2025, carried out in the same flume and hydraulic laboratory at the University of Florence. While that study did not investigate microplastic transport, it employed the same vegetation and channel bed layout to assess its influence on flow velocity patterns (Artini et al., 2025). The fixed dunes model was 3D printed

in an expanded polystyrene and installed at the bottom of the channel. The single dune average height is 0.04 m and the average wavelength is 0.7 m.

Water discharge was set equal to 36.4 l/s and maintained constant during the experiments; a weir at the end of the flume allowed imposing the downstream boundary condition. We used 10 water gauge sensors to measure the water surface and flow meters for evaluating the water discharge.

A flow rate of 36.4 l/s was selected and maintained constant during the experiments, to ensure consistency with previous experiments (Artini et al., 2025), enabling the reuse of detailed hydrodynamic measurements (e.g., TKE profiles) acquired under identical conditions. A weir at the end of the flume allowed imposing the downstream boundary condition. This discharge produced a mean flow depth of approximately 23 cm, resulting in just-submerged conditions for the artificial plants (23 cm in height), and corresponded to subcritical ($Fr \approx 0.3$) and fully turbulent ($Re \approx 90,000$) flow conditions representative of lowland rivers. Although the dune-shaped bed was fixed and constructed from polystyrene, the chosen discharge reflects typical values observed in mobile-bed conditions for similar dune geometries interacting with vegetation, thus maintaining hydraulic realism and ensuring compatibility with established flow-dune-vegetation configurations (Artini et al., 2024, 2025; Baas, 1994; Simons et al., 1966; Van Rijn, 1948).

Preliminary qualitative tests were conducted under identical hydraulic conditions but without vegetation, to verify the role of plants in particle retention. In these initial runs, high-density particles ($\rho = 1.4 \text{ g/cm}^3$) tended to settle within dune troughs, while particles with lower densities were not retained and exited the flume shortly after release. Although no formal quantification or repetitions were performed, these exploratory trials supported the assumption that vegetation presence was essential for microplastic trapping under the tested conditions.

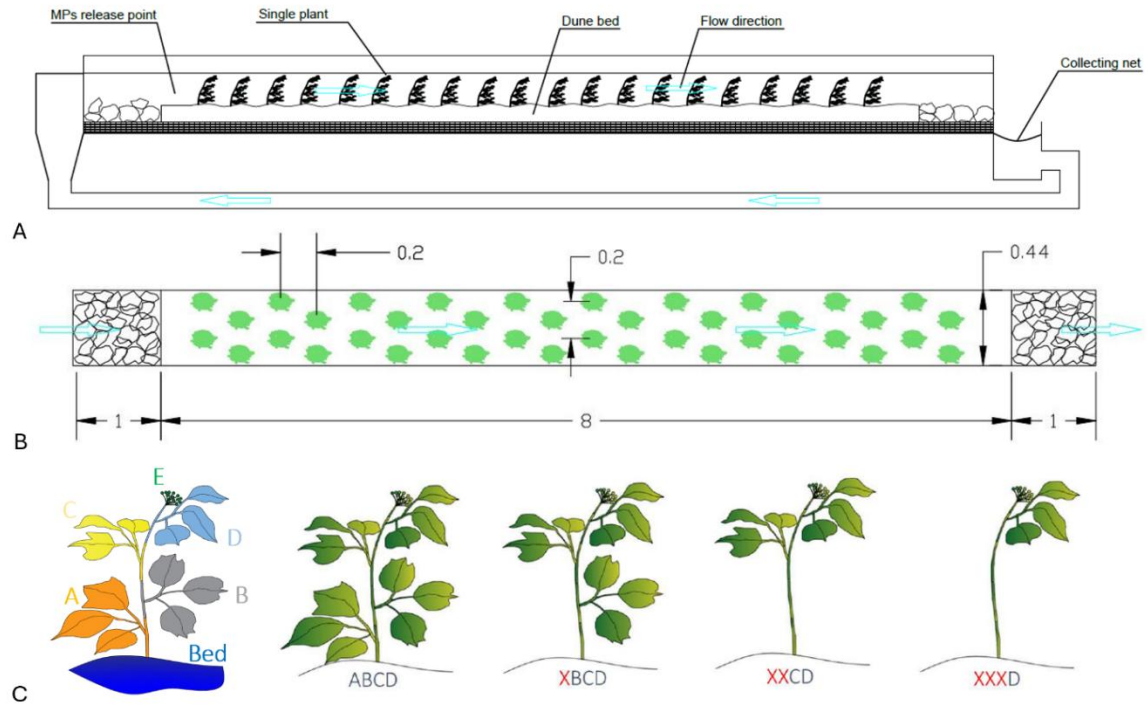


Figure 2. 1 - (a) Side view of the 10.5 m long flume showing the MPs release point, the vegetated section with artificial plants over a fixed dune bed, and the downstream collecting net. (b) Top view of the vegetated area (8 m), with 80 plants arranged in a staggered grid at 20 cm spacing. (c) Vertical segmentation of the plant into six trapping zones (Bed, A–E), and four foliage configurations (ABCD, XBCD, XXCD, XXXD) obtained by progressively removing lower branches

The plants used in the experiments are an artificial reproduction of white poplar (*Populus alba*) at juvenile age (Supplementary Material Fig. S2.1). The selection of *Populus alba* is ecologically motivated by its widespread presence in European riparian zones. It typically grows in floodplains and along riverbanks, tolerates fluctuating water levels, and contributes to bank stabilization. Its ability to thrive in diverse soil types and its role in stabilizing riverbanks make it a representative species for studying vegetation–flow interactions in riparian ecosystems (De Rigo & Bosco, 2011; Miletić et al., 2024; Reisner et al., 2007). The plants were arranged in a staggered pattern. The spacing between the plants was set at 20 cm in both directions. This corresponds to a vegetation density per unit meter of approximately $\lambda=0.25\text{ m}^{-1}$, calculated using the standard definition $\lambda=D/xi^2$, where D is the frontal plant diameter and xi is the plant spacing. The vegetation density per unit meter used in this study ($\lambda=0.25\text{ m}^{-1}$) is consistent with values reported for natural riparian corridors with low-stature or juvenile tree vegetation (Poggi et al., 2004; Tanino & Nepf, 2008; Valyrakis et al., 2024; Yagci & Strom, 2022), supporting the ecological relevance of the experimental setup.

The artificial plants and the set-up used in this work have been previously adopted in hydraulic flume experiments to investigate vegetation–flow interactions whose hydrodynamic characteristics were known (Artini et al., 2025; Jalonen et al., 2013; M E et al., 2008; Schoneboom & Aberle, 2009). Each plant has a total height of 23 cm and include a plastic-coated steel wire stem (diameter $\approx 3\text{ mm}$), four horizontal branches ($\sim 6\text{ cm}$ each), and twelve leaves made out of fully flexible dyed textile, fixed with flexible joints allowing rotation and partial streamlining under flow. The canopy width was approximately 15–18 cm (see Fig. 2.1C).

The plants have a bending stiffness of $E = 6965 \text{ N/mm}^2$ falls within the range of natural vegetation, supporting the mechanical realism of the model (ME et al., 2008; Schoneboom and Aberle, 2009b).

The plants used in the experiments had a modulus of elasticity (E) of 6965 N/mm^2 , which is within the typical range observed for natural vegetation (Wessolly and Erb, 1998), supporting the mechanical realism of the model (ME et al., 2008; Schoneboom and Aberle, 2009b). Flexural rigidity was evaluated by mounting each plant on a deformable steel beam instrumented with strain gauges. Under flow, the plant acted as a cantilever, generating bending moments that were used to calculate both the drag force and the effective lever arm of the applied force. This setup allowed for a detailed quantification of plant flexibility in response to hydrodynamic loading (Schoneboom et al., 2008).

For the experiments, four different foliage configurations were used and obtained by removing one or more branches from the plants. Each setup has a different one-sided leaf area A_L and refers to the surface area of a single side of a leaf, and consequently a different LAI (Table 2.1).

To investigate the vertical distribution of the trapped MPs at the plant location six possibilities were considered: Bed is the area on the riverbed around the plant and corresponds to the zero elevation level; A, B, C, and D identify, from bottom to top of the plant, the branch with all the leaves and part of the trunk at that level; and E indicates the area of the flower on the top of the plant. Based on the known geometry of the artificial plants (Jalonen et al., 2013; Schoneboom & Aberle, 2009), the vertical boundaries of the trapping positions are defined as follows: A = 0–5 cm, B = 5–10 cm, C = 10–15 cm, D = 15–21 cm, and E = 21–23 cm. Particles were considered to be retained at a given position if they were found within the corresponding vertical range, regardless of whether they were deposited on the trunk, branches, or leaves. In the experiments, four plants foliage scenarios were considered by varying the vertical leaf mass distribution (Fig. 2.1), while the position of the plants were maintained fixed. During the experiments the flexible plants were maintained in just submerged conditions.

Table 2. 1 - Plants foliage configurations and specific parameters. A_L is the one-sided leaf area, A_s is the sum of the stem frontal area and blossom frontal area, $A_{\text{tot}} = A_L + A_s$ (Jalonen et al., 2013). All plants shared the same stem and branch geometry with only the foliage configuration varying across treatments (ABCD, XBCD, XXCD and XXXD). Identical artificial models were used to ensure consistency in structural dimensions.

Plant set-up				
Leaf set-up	$A_L [\text{cm}^2]$	$A_s [\text{cm}^2]$	$A_{\text{TOT}} [\text{cm}^2]$	$LAI [-]$
ABCD	373.6	20	393.6	0.93
XBCD	237.8	20	257.8	0.59
XXCD	128.5	20	148.5	0.32
XXXD	66	20	86	0.17

Seven different types of particles were used in the experiment. The plastic particles used in the experiments are 2-dimensional particles with a diameter (ϕ) of a few millimeters. This is in line with recent study results whereby the most common plastic litter found trapped in riparian area are 2D plastic pieces (Cesarini & Scalici, 2022a). The particles are divided into groups with

different diameters, shape and density (Table 2.2). Images illustrating the disk-shaped and irregular particles used are included in the Supplementary Material (Fig. S2.3 a-g).

Particles were manually released one by one at the zero elevation level ($z = 0$ cm), along the centerline of the initial section of the dune pattern. This position was selected to represent microplastics previously deposited on the riverbed or on the riparian surface—such as those originating from atmospheric deposition or accumulated during earlier flood events—and subsequently remobilized by rising flows. This approach allowed us to mimic bottom-originated transport trajectories leading into the vegetated area.

Table 2. 2 - Plastic particles characteristic. * Equivalent sphere diameter considered for the irregular shaped particles

Shape	Thickness [mm]	Diameter (ϕ) [mm]	Density (ρ_P) [g/cm ³]
Disk	0.15	6	1.04
Disk	0.15	4.5	1.04
Irregular	1	9*	1.04
Disk	0.08	6	1.1
Disk	0.08	4.5	1.1
Disk	0.02	6	1.2
Disk	0.08	6	1.4

A total of 100 particles were released per run, a number chosen to ensure sufficient representation of transport and retention dynamics while maintaining practical feasibility for manual tracking and post-run recovery. Similar sample sizes have been adopted in previous flume studies on particle retention (Ijaz et al., 2023; Jalonen et al., 2013).

For each type of particle, the experiment was repeated at least seven times to ensure reproducibility. The coefficient of variation ($CV = \sigma/\mu$) was computed across the seven repetitions per particle type and configuration. A threshold of 21% was adopted to evaluate the consistency of the results, following criteria commonly used in experimental flume studies. In some cases, this threshold was not met, which may indicate higher variability in the retention process or the need for additional repetitions to improve statistical robustness.

The experimental protocol was designed considering three phases. The first one started after the experimental hydraulic condition was reached, the plastic particles were released and the test was run for fifteen minutes. A set of pilot tests was performed prior to the main experimental runs to determine a suitable time window for evaluating stable retention. In these trials, particles were monitored at 5, 10, 15 and 20 minutes after release. Untrapped particles consistently exited the flume within a few seconds, while retained particles remained stationary after 10 minutes, with no visible displacement up to 20 minutes. These observations supported the adoption of 15 minutes as a reliable duration to assess effective retention under steady flow conditions. Keeping these conditions, the channel is visually checked, and the particles trapped are detected with the aid of a UV lamp.

Fluorescent microplastic particles were used in all experiments to ensure high visibility under UV illumination. Pilot tests showed that visual detection without UV light led to substantial undercounting, especially on vegetated surfaces. The UV lamp significantly improved detection reliability, although no formal quantification of accuracy was performed. This method is

suitable for controlled laboratory conditions but not directly transferable to natural environments where MP fluorescence cannot always be ensured. The second phase involved a thorough visual inspection of the flume and individual plants to identify and count the microplastic particles trapped within the vegetation or retained in the channel (Supplementary Video S1). The number of particles trapped was signed and compared with the amount released calculating K associated with the simulation. The third phase consisted of draining the channel and systematically collecting all remaining particles, both from within the flume and from the net positioned at the downstream end.

The TKE values used in this study were calculated by Artini (2025) using flow velocity data measured. In particular:

$$TKE = \frac{1}{2}(u'^2 + v'^2 + w'^2)$$

where u' , v' and w' are the instantaneous velocities in the x, y, z directions, respectively. Velocities were collected using a Nortek Vectrino Plus ADV at a sampling rate of 100 Hz along the water column with a vertical spacing of 1 cm from the bottom to surface. These measurements were collected in the same flume, under identical hydraulic and morphological conditions, including the same flow rate, dune-bed configuration, and artificial vegetation layout, and included a side-wall correction following (Vanoni & Brooks, 1957), to minimize the influence of wall proximity on turbulence estimates. In total, 27 vertical profiles were measured along the channel, with a longitudinal spacing of 30 cm. The measurement section was considered 2.4 m long, corresponding to approximately 4 wave lengths. Given the steady flow conditions and the high spatial and temporal resolution of the measurements, data quality is considered high.

This ensures that the turbulence structures captured by those measurements accurately represent the hydrodynamic context in which our retention experiments were conducted. Given the strong control of TKE on particle transport and deposition, these data provide a robust basis for interpreting the observed retention patterns. These data provide a reliable and representative basis for interpreting the observed retention dynamics.

2.2.4 Statistical analysis

To assess the differences in particle trapping across foliage configurations, plant positions, and particle characteristics (shape, size, density), a non-parametric statistical approach was adopted due to the non-normal distribution of data and the relatively small sample sizes per group ($n = 7$ replicates per configuration). The Kruskal–Wallis H test was applied to compare median retention values across more than two groups, providing a rank-based alternative to one-way ANOVA. The test statistic is reported as H, with corresponding p-values.

When significant differences were found ($p < 0.05$), Dunn's post-hoc tests were used for pairwise comparisons between specific groups. A Bonferroni correction was applied to adjust the significance threshold for multiple testing. The adjusted p-values are reported directly in the text, and denote comparisons between particle types or vertical trapping positions.

This approach was used throughout the analysis of the retention coefficient (K), vertical distribution, and the effects of particle properties. All tests were performed using GraphPad Prism (v9) or R (v4.3) statistical packages.

2.3 Results

2.3.1 MPs retention coefficient - K

The observed retention coefficient against the particle density for the considered plastic particles are shown in Figure 2.2. This result was statistically significant, as shown by the Kruskal-Wallis test ($H = 161.3$, $p < 2.5 \times 10^{-32}$), and confirmed by Dunn's post-hoc test, which indicated that 1.4 – 6 mm particles had significantly higher retention than all other groups (e.g., $p \approx 6.6 \times 10^{-12}$ vs. 1.04 – 6 mm; $p \approx 4.2 \times 10^{-9}$ vs. 1.1 – 6 mm; $p < 0.001$ vs. all others). The highest trapping efficiency (K reaching the value of 93%) was observed for the densest particles. This finding highlights the influence of particle density on the trapping process, with denser particles being more effectively retained.

Among particles with the same shape and density, a clear relationship between particle diameter and trapping efficiency was observed. Specifically, as the particle diameter decreased, the trapped particles also decreased. Particles with a diameter of 6 mm and density of 1.1 g/cm³ achieved a trapping efficiency of 11.6%, which is higher than the 7.3% efficiency observed for particles with a smaller diameter of 4.5 mm and same density. This difference was statistically significant ($p \approx 0.0006$), supporting the hypothesis that larger particles are more prone to retention when shape and density are held constant. Similar trend could be observed for the particles with density 1.04 g/cm³, reaching a trapping efficiency of 9.9 % for particles with 6 mm diameter, and 7% for particles with 4.5 mm diameter, although the difference was only marginal ($p \approx 0.0501$). This finding indicates that, when density and shape are held constant, larger particles are more susceptible to be trapped than smaller ones.

Irregular particles, with a nominal diameter of 9 mm and density of 1.04 g/cm³, demonstrated a significantly higher trapping efficiency, reaching 20% of the total particles released. Retention was significantly higher than that of 1.04 – 6 mm ($p \approx 5 \times 10^{-8}$), 1.1 – 6 mm ($p \approx 1.5 \times 10^{-5}$), and both regular 4.5 mm particle groups ($p < 1 \times 10^{-13}$). This result confirms that particle shape plays a major role in enhancing retention efficiency, even when diameter and density are comparable.

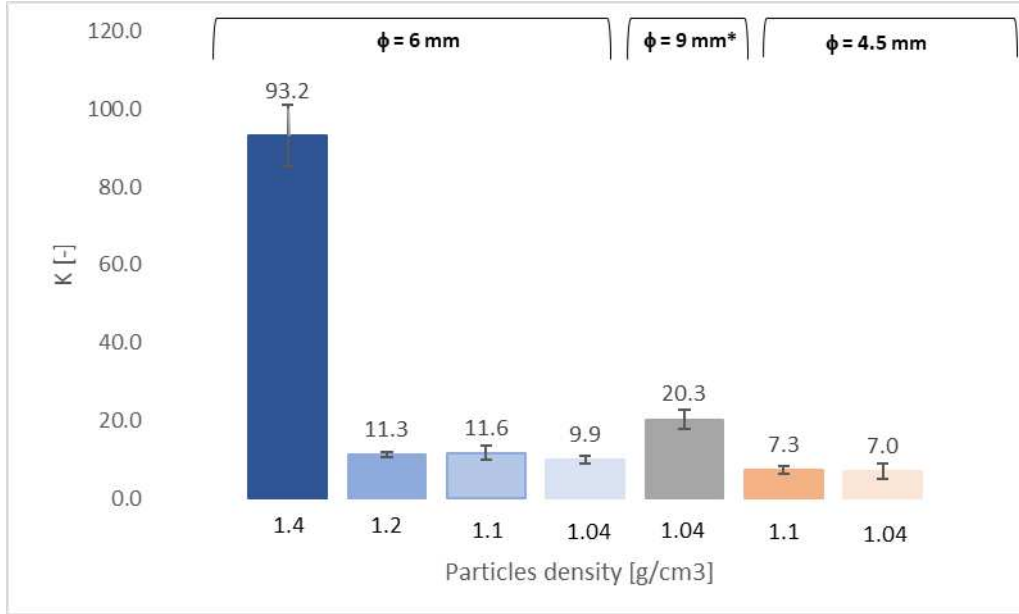


Figure 2. 2 - Percentage of trapped particles in the channel considering density and diameter values. *This value is an equivalent diameter considered for the irregular shaped particles. The equivalent diameter was calculated as $\phi = \sqrt{(4 \cdot (L \cdot W) / \pi)}$, where L is the maximum length and W is the maximum width of the particle, assuming an equivalent area to an ideal circular disk.

2.3.2 Vertical distribution of retained plastic particles

2.3.3 Density influences

To evaluate the vertical distribution of trapping efficiency, we define the position retention efficiency K_{pos} which represents the percentage of particles trapped at specific positions along the plant (Bed, A, B, C, D, E) of the total trapped in the hydraulic flume. This parameter allows a more detailed analysis of the spatial distribution of trapped particles. In particular position retention efficiency K_{pos} was calculated as:

$$K_{pos} = \frac{MP_{trapped,i}}{MP_{trapped}} \cdot 100$$

Where $MP_{trapped,i}$ is the number of particles trapped at a specific vertical position (e.g., A, B, C, etc.), and $MP_{trapped}$ is the total number of particles trapped in the channel.

As shown by Fig. 2.3A, the two lowest positions on the plant (i.e., A and B) are the places where the particles are mostly trapped at all. A general decrease in the number of trapped particles was observed moving upward toward the free surface flow. This trend was confirmed by statistical analysis: Kruskal-Wallis tests showed significant differences among vertical positions for all particle types ($p < 0.001$ in all cases), with Dunn's test identifying position A as significantly different from most other heights for particles with density 1.1 g/cm^3 ($p < 0.001$ for A vs. D and E), and especially for those with 1.2 g/cm^3 ($p < 0.001$ for A vs. B–E). In particular, for the particles with a density of 1.2 g/cm^3 , the trapped particles were observed as decreasing from 46% of their total number at position A to 23%, 9%, 6% and 10% at positions B, C, D, Bed, and E, respectively, showing a peak in position A (e.g., A vs. D and E: $p < 0.001$). Similarly, for the particles with a density of 1.1 g/cm^3 the vertical distribution of the trapped particles was 13%, 39%, 25%, 12%, 5% and 6% of total trapped particles at positions Bed, A, B, C, D and E, respectively, showing a peak in the position A, with statistically significant differences compared to D and E ($p < 0.001$).

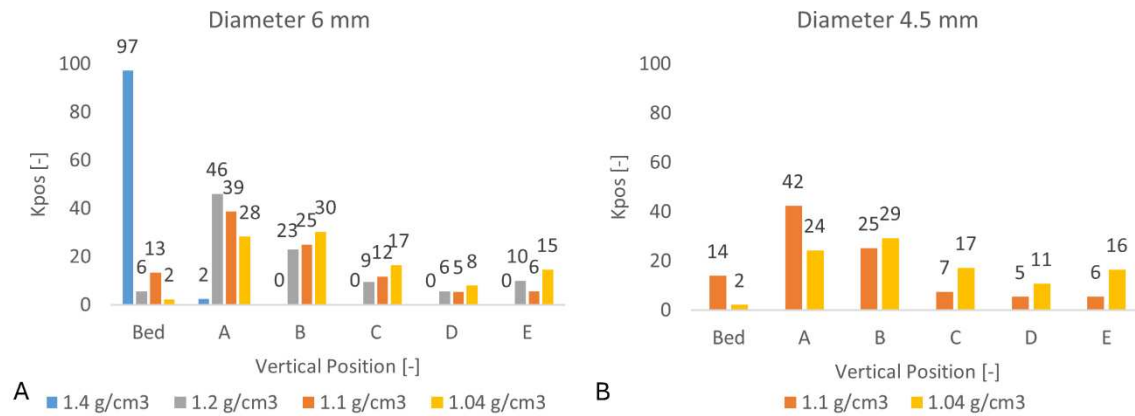


Figure 2.3 - Position trapping efficiency K_{pos} for round-shaped plastic particles with diameters of (a) 6 mm and (b) 4.5 mm. K_{pos} represents the percentage of particles retained at each vertical segment of the plant (Bed, A–E), relative to the total number of trapped particles.

In contrast, considering the densest particles, 97% of the total trapped particles were trapped at the Bed position, and 2% at position A; no particles were trapped at positions B, C, D, and E. The statistical test confirmed this pronounced accumulation at bed level, with position F significantly different from all others ($p < 0.001$).

Also, for particles with a density of 1.04 g/cm^3 , there is a tendency to be trapped in lower positions A and B, with a less evident peak in position A. For these particles, the K_{pos} values were 2%, 17%, 8%, and 15% at positions B, C, D, and E, respectively. Although position A showed a slightly higher trapping percentage, differences with B and C were only marginal ($p \approx 0.012$ – 0.03), suggesting a less marked vertical selectivity for these particles.

Similar patterns were found in Fig. 2.3B for particles with smaller diameters (4.5 mm), particularly for those with density 1.1 g/cm^3 . The Kruskal-Wallis test confirmed significant differences ($p < 0.001$), with Dunn's test indicating a peak at position A ($p < 0.001$ for A vs. C–E), although overall vertical stratification was slightly less pronounced than in larger particles.

2.3.4 Leaf Area Index (LAI) influence

Beyond plastic density, the vertical distribution of particles was influenced by additional factors, including leaf configurations and particle characteristics. Fig. 2.4 reports the variation of the percentage of particles trapped at a given plant position considering various leaf configurations for the particles lighter than 1.4 g/cm^3 . In this section, to better analyze their individual effects on the total trapped particles, the results are reported considering the configurations separately, allowing for a more detailed assessment of their contribution to particle trapping.

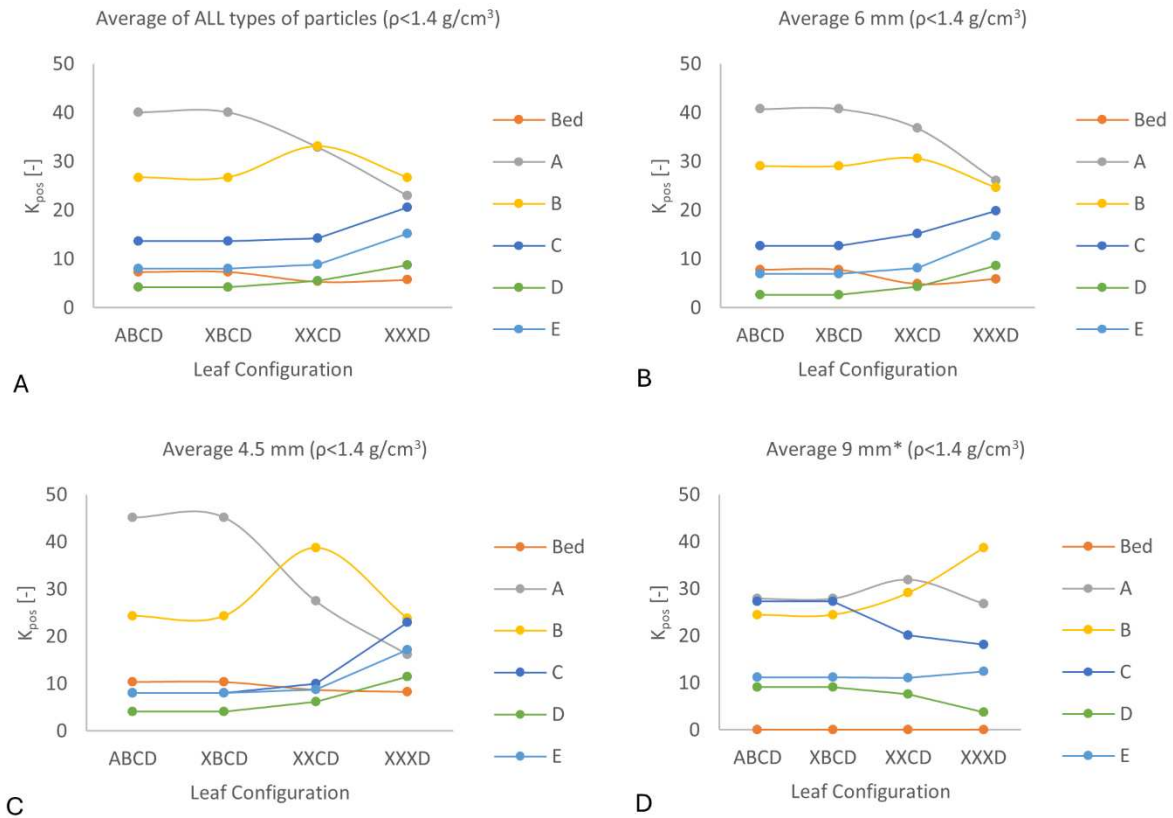


Figure 2. 4 - Position trapping efficiency K_{pos} for the four leaves configurations (ABCD, XBCD, XXCD, XXXD). (a) Average of all types of particles with density below 1.4 g/cm^3 , (b) average 6 mm, (c) average 4.5 mm, (d) average 9 mm*(*this value is an equivalent diameter considered for the irregular shaped particles).

Considering the average results (Fig. 2.4A), the data indicate that the lower position on the plant (A) consistently exhibits the highest trapping efficiency in all the leaf configurations, but, at the same time, displays a slight decline in trapping efficiency as lower branches are removed, moving from the 40% in the configuration ABCD, to 23% in the configuration XXXD ($p < 0.001$). In contrast, positions B and C exhibited moderate increases in trapping efficiency under the XXCD and XXXD configurations ($p < 0.01$ and $p \approx 0.008$, respectively). No significant variations were found for positions D and E, suggesting a stable trapping pattern across configurations. For the Bed position, the trapped particles were almost unaffected by the leaf configuration, maintaining an almost constant value with a subtle reduction in the XXCD and XXXD configurations ($p > 0.85$).

The second graph (Fig. 2.4B) focuses on particles with a diameter of 6 mm. Similar to the overall trend observed in Fig. 2.4A, position A retains the highest trapping efficiency, with values slightly reducing (from 41% to 26%) along with the leaves removal. This reduction was statistically significant when comparing configuration ABCD with both XXCD ($p = 0.002$) and XXXD ($p < 0.001$), confirming the role of lower branches in enhancing capture efficiency at this position. A similar trend could be noted for position B, with values remaining relatively constant across the different vegetation configurations, mildly reducing only in the last configuration XXXD, up to 25% ($p > 0.05$). Meanwhile, position C exhibited a significant increase in trapping efficiency in the XXCD and XXXD configurations compared to ABCD ($p < 0.008$), indicating that the removal of lower branches redirected particles toward intermediate levels of the structure. Positions D and E also did not show significant changes among configurations ($p > 0.05$),

suggesting a relatively stable behavior. Similarly to the Fig. 2.4A, in this graph, the entrapment values for the Bed position remain constant across the four leaf configurations and are among the lowest recorded.

The third graph (Fig. 2.4C) shows the behavior of smaller particles with a diameter of 4.5 mm. In this case, position A shows the same behavior as the other graphs, gradually reducing the retention rate from 45% in the first configuration ABCD, to 16% in the last configuration XXXD ($p < 0.005$). Conversely, position B did not exhibit statistically significant changes across configurations ($p = 0.20$), although a non-significant peak (40%) was observed in the XXCD configuration. These results indicate that smaller particles are more influenced by intermediate branches, particularly when lower branches are absent, further emphasizing the complex interplay between particle size and vegetation architecture. Once again, the entrapment values for the Bed position remain constant across the four leaf configurations, with no significant differences detected ($p = 0.84$).

Finally, the fourth graph (Fig. 2.4D) examines the trapping efficiency for larger and irregular particles (9 mm). Here, the position (B) demonstrates a pronounced increase in trapping efficiency under the XXXD configuration (around 39%), although the statistical test revealed only a marginally significant difference ($p \approx 0.03$) between configurations ABCD and XBCD. Conversely, position A experiences a less evident reduction, almost maintaining the same value along the four-leave configuration. In this case, position C starts with a retention rate value in configuration ABCD comparable to positions A and B (27%), reducing the value to 18% in the configurations with less leaves, but without statistically significant variation. Positions D and E show a minor trapping rate (around 10%) starting from the initial leave configuration, with moderate changes in their values along with the change of the configurations. This highlights the role of vegetation morphology and particle size in influencing particle distribution. Lower branches play a key role in particle retention, while their removal shifts trapping to higher vegetation levels, with the effect varying depending on particle size. For this type of particles, no entrapment was observed in the bed position across any of the leaf configurations.

2.3.5 Turbulence Kinetic Energy

Fig. 2.5 shows a comparison between the normalized *TKE* values at different heights and the number of trapped particles for the ABCD and XBCD leaf-configurations.

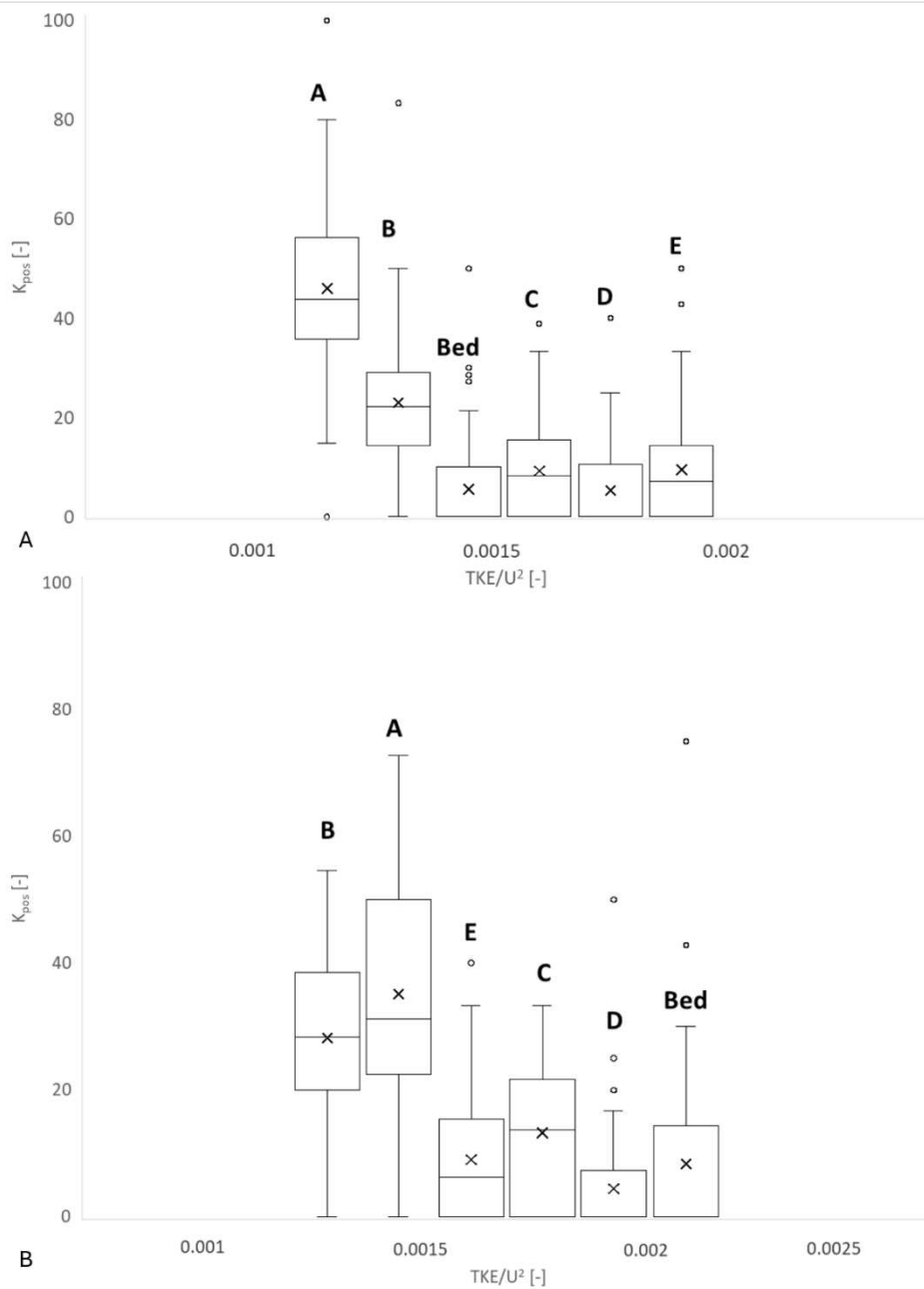


Figure 2. 5 - Influence of normalized turbulence values on the number of trapped particles in configuration (a) ABCD with all leaves and (b) XBCD without the lowest leaves level. The K_{pos} values are average of 9, 6 and 4.5-mm particles with density 1.2, 1.1 and 1.04 g/cm³. The tags A, B, C, D, E and bed indicate the position for the corresponding TKE/U^2 value.

Fig. 2.5A presents the position trapping efficiency (K_{pos}) of plastic particles in relation to the normalized turbulent kinetic energy (TKE/U^2) measured at each trapping position. The data indicate a non-linear relationship between TKE/U^2 and K_{pos} , suggesting that local turbulence conditions significantly influence the retention of plastic particles. The statistical test confirmed these observations (Kruskal-Wallis, $p < 0.0001$), with significant pairwise differences particularly between the lowest turbulence value (0.00113) and all others ($p < 0.001$). Lower turbulence values (e.g., around 0.001) correspond to relatively higher retention rates at certain positions, suggesting that weaker turbulence may facilitate particle trapping at these sites. Conversely, as TKE/U^2 increases toward 0.0015, the position trapping efficiency appears to stabilize, potentially indicating a threshold where higher turbulence disrupts particle retention. Each value of TKE/U^2 represents a specific trapping position (A, B, C, D, E and Bed), although

the sequence along the horizontal axis does not follow the natural order of the positions. This variability underscores the heterogeneity in turbulence conditions and their localized effects on particle retention efficiency. The second graph (Fig. 2.5B) reports the data for the leaf configuration XBCD including a wider range of TKE/U^2 values, extending from 0.001 to 0.0025. The Kruskal-Wallis test confirmed a significant difference across turbulence classes ($p < 0.001$), and post hoc comparisons revealed that the trapping efficiency (K_{pos}) dropped significantly between the lowest turbulence class (0.001166) and most of the higher ones (0.002133, $p < 0.001$). Similar to the first graph, the K_{pos} demonstrates variability across the different trapping positions. However, as turbulence increases, the retention rates start to decline. This trend implies that stronger turbulence reduces the ability of vegetation or other structures to retain plastic particles, likely due to increased kinetic energy disrupting the settling or adhesion processes.

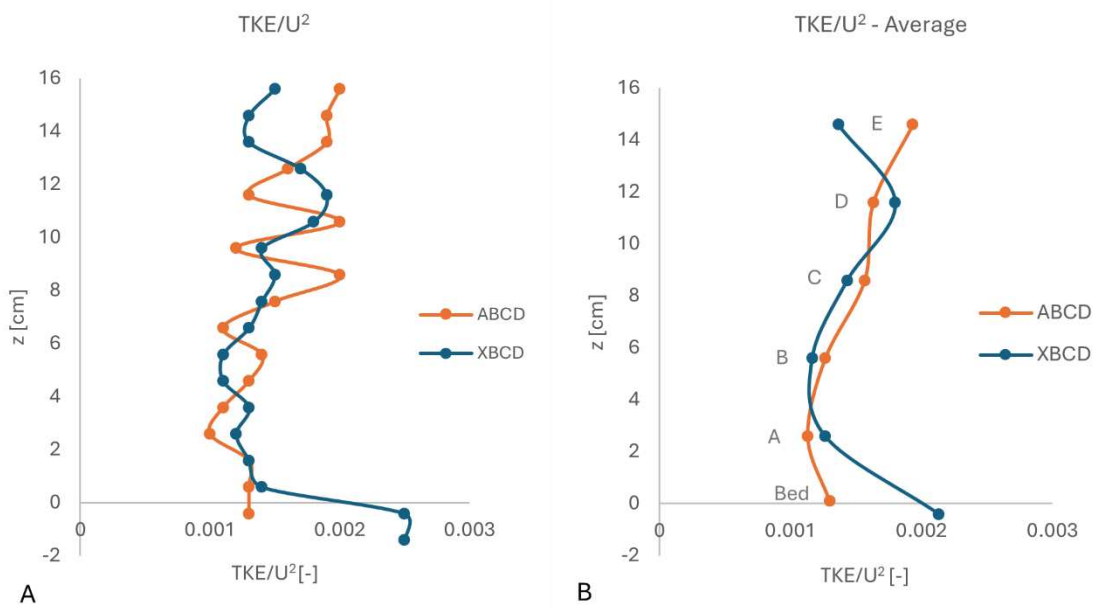


Figure 2. 6 - (a) Dimensionless double-averaged TKE/U^2 data from (Artini et al., 2025) (b) TKE/U^2 values vertically averaged for the entrapment positions

The leaf configuration, and consequently the Leaf Area Index (LAI), showed a greater influence on the vertical distribution of trapped particles (Fig. 2.4). Changes in the leaf configuration tend to affect the pattern of turbulence along the water column. The distribution of TKE values along the vertical axis for the two configurations ABCD and XBCD is shown in the Figure 2.6, illustrating how the removal of lower branches modifies turbulence patterns. It was therefore possible to define a relationship between the number of trapped particles and the values of Turbulence Kinetic Energy (TKE). Specifically, the lowest TKE values are associated with a higher number of trapped particles.

2.4 Discussion

2.4.1.1 Density and LAI vs Retention

The densest particles (1.4 g/cm^3) exhibited a tendency to settle within dune troughs, thus, their transport dynamic was largely driven by interactions with the bed and by recirculating flow

patterns (Supplementary Video S2). Dune-bed influence the local hydrodynamics, particularly by creating recirculation zones and lower-velocity regions near the bed troughs, which may facilitate particle settling and enhance retention at bed position. This morphological influence aligns with findings from sediment transport studies (Venditti et al., 2005) showing preferential deposition in dune troughs. Moreover, this behavior aligns with previous study by Russell et al. (2023), which observed how high-density particles predominantly behave as bed load, rolling or bouncing along the riverbed. In contrast, particles with densities lower than 1.04 g/cm^3 were more influenced by the local turbulence induced by the vegetation, particularly by vertical fluxes. These particles showed a greater propensity for retention at higher vegetative positions, underscoring the interplay between particle buoyancy and vegetative structure. Similar behaviors were reported by Lenaker et al. (2019) for natural river systems in which lighter polymers were distributed at higher levels in the water column, corroborating the role of density in vertical particle transport.

These results highlight a threshold density beyond which trapping mechanisms shift from being vegetation-dominated to bedform-dominated. This distinction highlights the importance of considering both particle characteristics and channel morphology when evaluating microplastic retention dynamics.

Considering the influence of *LAI*, we found that the difference in MPs retention between configurations was almost negligible. One of the reasons was that the MPs were mainly retained by the main stem of the plant, that was always present in all the leaf configurations. The particles were also retained by the branches and only a few by the leaves. This could be explained by the fact that the leaves were the most movable elements of the plant, constantly oscillating because of the flow and turbulence. This result is in line with what found by (de Smit et al., 2021) that showed similar results analyzing the influence of different types of vegetation, and concluding that the plant to retain higher number of microplastics were the one with a complex and rigid architecture, and rough surface, such as corals.

2.4.1.2 Density vs Vertical position

Numerical values of K_{pos} , defined as the percentage of particles retained at each vertical level, are provided and discussed below for all particle types and vegetation configurations. The results suggest that density also influences the vertical position. The trend is not so evident, but we can see that denser particles tend to be trapped in lower positions compared to less denser particles with same dimension (see Fig. 2.3). A similar behavior has been observed by (Lenaker et al., 2019). Considering the results, it is possible to note a higher concentration of denser polymers in the lower layers of the water column.

This behavior could be compared with the theoretical vertical concentration profile of transported MPs. (Born et al., 2023b) observed that the vertical distribution of MPs with a density close to the water density (i.e, $0.91 - 1.13 \text{ g/cm}^3$) could be related to the vertical distribution of sediments and well depicted by the well-known Rouse formula. This vertical concentration profile has been already observed in the field by (Cowger et al., 2021; Valero et al., 2022). Anyway, the formula was not defined for plastic particles, and it was valid for spherical particles, so it has several limitations when applied to plastic litter and in vegetated channel.

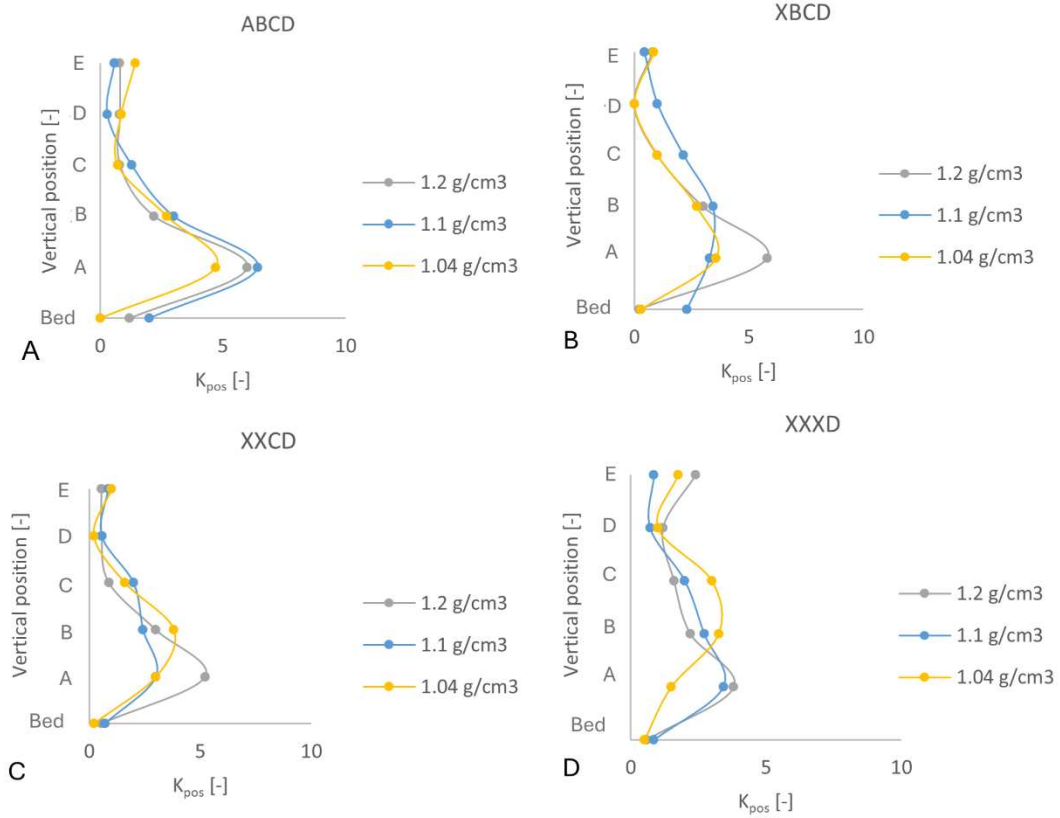


Figure 2.7 - Position trapping efficiency K_{pos} for particles with density 1.04, 1.1 and 1.2 g/cm³, across four different foliage configurations: (a) ABCD – full foliage; (b) XBCD – lower branch removed; (c) XXCD – two lower branches removed; and (d) XXXD – only the top branch retained. K_{pos} quantifies the percentage of particles retained at each vertical segment of the plant (Bed, A–E), relative to the total number of trapped particles

Fig. 2.7A shows the percentage of trapped particles along the vertical axis of plants considering the four leaf configurations for the MPs with a density lower than 1.4 g/cm³. Statistical analyses confirm that the differences among vertical positions are significant for all tested configurations ($p < 0.01$ in all Kruskal-Wallis tests). In the ABCD configuration, Dunn's test highlights significantly higher trapping in position A compared to D and E ($p < 0.003$), confirming the preferential accumulation near the bed. Similarly, in the XBCD and XXCD setups, position A retains significantly more particles than C, D, and E ($p < 0.003$), while for the lowest density configuration (XXXD), the trapping shifts upward, with significant increases in position B compared to A and C ($p < 0.003$). These results align with theoretical concentration profiles (G. Wang & Ni, 1990), suggesting a preferential retention of denser particles at lower positions. A detailed visualization of this trend is provided in supplementary data (Fig. S2.4).

The results also indicate that most particles, regardless of density, tend to accumulate at the lower positions of the plants, particularly in Position A (0–5 cm), where trapping is consistently highest across all scenarios (see Fig. 2.7, all panels). This trend is more pronounced for denser particles ($\rho = 1.2$ g/cm³), which exhibit near-exclusive retention at the bed due to their rapid settling behavior. However, even particles with lower density ($\rho = 1.04$ – 1.1 g/cm³) show a substantial proportion of trapping in lower positions (A and B), especially under the ABCD configuration where foliage is distributed along the entire plant height.

This preferential near-bed retention of low-density particles may appear counterintuitive, yet it can be explained by local hydrodynamic conditions. As shown by Born et al. (2023a) particles

with densities near that of water can follow sediment-like vertical concentration profiles, with increased accumulation near the bed under moderate turbulence. In our flume, the combination of bedforms and dense vegetation likely generates low-velocity zones and recirculation cells that promote near-bed deposition. Likewise, Choy et al. (2019) reported vertical displacement and deep accumulation of buoyant particles driven by flow and biological interactions in estuarine systems.

Notably, changes in foliage configuration affected the vertical distribution of trapped particles, particularly for lowest foliage density configuration. As shown in Fig. 2.7D, when lower branches were removed (XXXD), the peak retention shifted from Position A to higher regions (e.g., B or C), with statistically significant differences ($p < 0.003$), suggesting that the absence of near-bed obstruction allowed particles to rise further before being intercepted. This effect was especially evident for the lighter particles ($\rho = 1.04 \text{ g/cm}^3$), which showed increased trapping in upper positions as foliage mass was progressively shifted upward. These results underscore the importance of both particle density and vegetation architecture in determining vertical retention patterns (Lofty et al., 2024).

Based on our results, the lighter particles are more easily mobilized and transported to upper layers by vertical turbulent fluxes. The reduced obstruction at the base allows the formation of larger vortices, potentially increasing the vertical component of turbulence and enhancing upward transport. Similar effects have been described in canopy flows, where densely distributed structural elements constrain eddy scale and suppress vertical turbulent transport (Nepf, 1999). In our case, removing lower branches may relax these constraints, enabling vertical displacement of lighter MPs and facilitating their entrapment at higher elevations within the canopy structure.

It is also worth noting that the fixed dune-bed morphology used throughout all experiments may have contributed to the preferential accumulation of particles at lower positions (e.g., Position A), by generating low-velocity recirculation zones in the dune troughs that favor settling.

2.4.1.3 TKE vs trapping efficiency

The transport of microplastics in turbulent flow depends on several factors like the characteristics of particles, the flow regime and the hydrodynamic conditions. Based on these factors, the particles could travel for long distances or settle down, either be trapped by vegetation or other elements present in the channel (Cesarini & Scalici, 2022b; Gallitelli et al., 2020, 2023).

The present study points out the relationship between the number of trapped particles and the normalized *TKE* value. As shown by Fig. 2.5, increasing the value of the TKE/U^2 there is a drastic reduction in the number of trapped particles. The behavior could be related to an increase in the bed shear velocity related to the increase of TKE/U^2 , that keep the particles through the vegetation in suspension.

As found by (Wingenroth et al., 2021) canopy-induced turbulence has two primary effects: on one side fewer particles are delivered to the stems due to the turbulence disrupting the flow paths, on the other side it increases resuspension, so the particles that are already attached to the stems are more likely to be resuspended into the fluid due to the increased shear stress. This combined effect leads to fewer particles trapped in vegetation, decreasing the retention

factor. In conclusion they state that *TKE* drives to the resuspension of deposited particles. The effect diminishes with the increase of stem density as eddy scale becomes limited by the stem spacing. So, inside the canopy, because of the turbulence, we have a lower threshold for particle motion initiation. On the other hand, due to the lower flow intensity caused by the presence of vegetation the particle transport load is diminished and deposition may occur. So based on our results, the lighter particles are more easily mobilized and transported in the upper layer by the vertical fluxes generated by turbulence. This could be related to the tendency of turbulence to drag the particles along the channel, in the direction of the flow, but also in the other two directions, vertically and transversally. In this way the particles are more prone to be trapped in the upper positions, in the intricate vegetation structures, by the primary drivers of physical blockage and entanglement (Cesarini & Scalici, 2022b; Gallitelli et al., 2023).

The vertical profiles of the streamwise (*u*), spanwise (*v*), and vertical (*w*) components of turbulence in the two vegetation configurations: ABCD (all branches present) and XBCD (lower branch removed) indicate distinct behaviors (see Fig. S2.5). Above the dune crest ($z > 0$ cm), the streamwise and spanwise components remain relatively constant, whereas below the crest ($z < 0$ cm), turbulence drops sharply due to flow separation and reduced energy in the dune trough's recirculation zone.

The vertical component (*w*) demonstrates more pronounced variability along the vertical axis. In the XBCD configuration, (*w*) reaches higher values than in the ABCD configuration. This suggests that the absence of lower branches enhances vertical turbulence. Thus, it is possible to assume that the vertical contribution of the *TKE* pushed the particles in upper positions where they are trapped in the vegetation, even if in reduced percentage compared to the lower positions. The Reynolds stress vertical component (*w*) could be considered as the cause of the vertical transfer of the particles, pushed by the vertical flux (Hendriks et al., 2010).

As with all flume-based experiments, the simplified and idealized nature of the setup introduces inherent limitations. The use of a straight, rectangular flume with rigid walls and fixed vegetation excludes the morphological and hydraulic complexity typical of natural river systems, such as channel curvature, lateral variability, and bank irregularities. Additionally, the absence of biofilm and other surface-bound biological material may lead to underestimating potential adhesion mechanisms that could enhance particle retention in natural environments. Although flow conditions were kept quasi-steady, unsteady and transient flow events—common in natural rivers—could also affect transport and retention dynamics. These factors should be considered when interpreting the results and extrapolating them to field-scale conditions.

2.5 Conclusions

This study provides new insights into the role of flexible riparian vegetation as a natural barrier for microplastic retention,. Through controlled hydraulic flume experiments, we systematically investigated how microplastic properties, vegetation characteristics, and hydrodynamics interact to influence the retention of plastic debris.

Experimental results reveal the role played by the flow hydrodynamics in microplastic trapping efficiency, demonstrating that *TKE* plays a crucial role in modulating retention patterns. We found that lower turbulence levels favor particle retention, while higher *TKE* values can increase resuspension, reducing the effectiveness of vegetation as a retention barrier. Additionally, while the overall percentage of MPs trapped within the vegetation patch remained consistent

across different leaves configurations, variations in the vertical distribution of retained particles were observed, with a shift towards higher canopy positions when lower branches were removed.

The spatial distribution of trapped particles within the plant canopy is quantified by the parameter K_{pos} which is defined as the percentage of particles trapped at specific vertical position along the plant. This parameter provides a standardized framework for evaluating microplastic retention efficiency in riparian vegetation with different foliage structures (LAI).

Future research should expand upon these findings by investigating the long-term retention of microplastics and prioritize a detailed classification of MPs by shape and material properties, and test their interaction with vegetation using standardized retention metrics. Additionally, given the limited influence of foliage in flexible configurations, further work should investigate whether more rigid or structured vegetation types—such as reeds or woody shrubs. Incorporating variable flow regimes and testing under partial or full unsteadiness could also help clarify the temporal stability of retention under dynamic river conditions.

Overall, these findings have important implications for the design of nature-based solutions to mitigate plastic pollution in freshwater systems. By quantifying the retention efficiency of different vegetation structures and identifying the hydrodynamic conditions that enhance plastic trapping, this study provides a scientific basis for optimizing vegetated buffer zones as natural filters for microplastic pollution. To enhance microplastic retention, riparian buffers should prioritize dense vegetation with rigid stems or branches positioned close to the ground (0–10 cm), where retention was consistently highest. In engineering applications, this suggests favoring shrubby or juvenile woody species, or simulate their structural traits, particularly in zones prone to deposition, such as near banks or in low-flow areas shaped by river morphology.

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2.8 Supporting Material

Supporting Information for:

Riparian vegetation as a natural barrier: experimental analysis of plastic particle retention in a vegetated reach

Daniele Martuscelli^{1*}, Simona Francalanci¹, Lorenzo Innocenti¹, Luca Solari¹

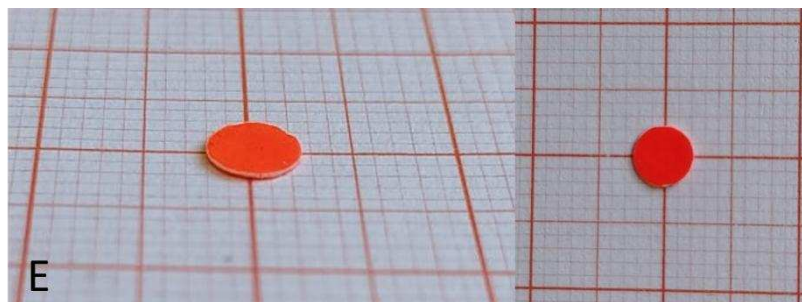
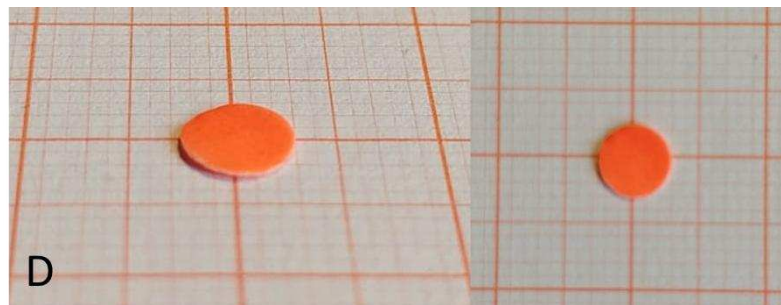
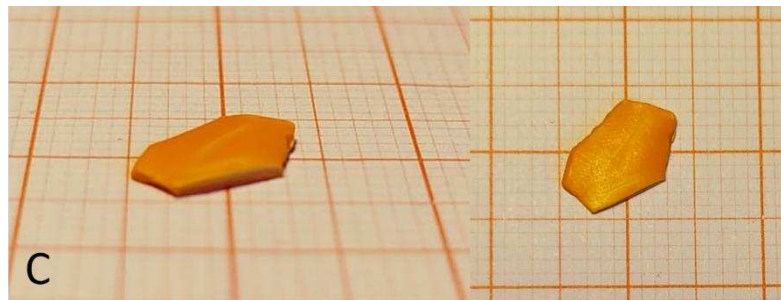
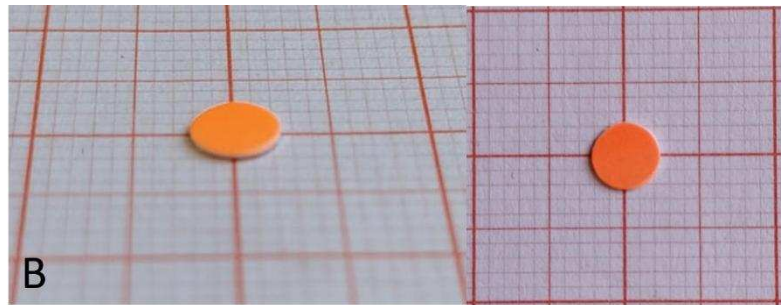
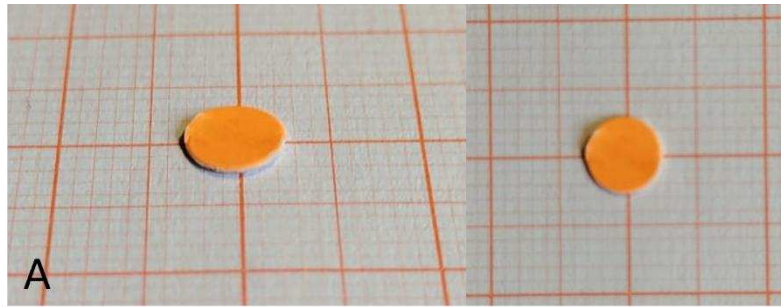
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Figure S2. 1 - Artificial poplar at different stages of foliation (from left to right: stem with blossom, stem with branches carrying one leaf, stem with branches carrying two leaves, and stem with branches and three leaves, respectively. (Schoneboom & Aberle, 2009)



Figure S2. 2 - Views of the experimental flume setup with artificial riparian vegetation. (a) Photograph of the central portion of the hydraulic flume showing artificial *Populus alba* plants positioned over the fixed dune bed. In this configuration (XBCD), the lowest foliage layer (A) has been removed, while branches B, C, and D are present. (b) Top view of the 10.5 m recirculating flume during a test run, showing the vegetated section installed over the fixed dune bed. Flow direction is from bottom to upper part; upstream and downstream sections are stabilized with gravel layers. (c) Lateral view of the vegetated section under quasi-steady flow conditions (mean depth ≈ 23 cm), showing artificial *Populus alba* plants interacting with the current. The dune-shaped bed is made of painted polystyrene and covered with sediment.



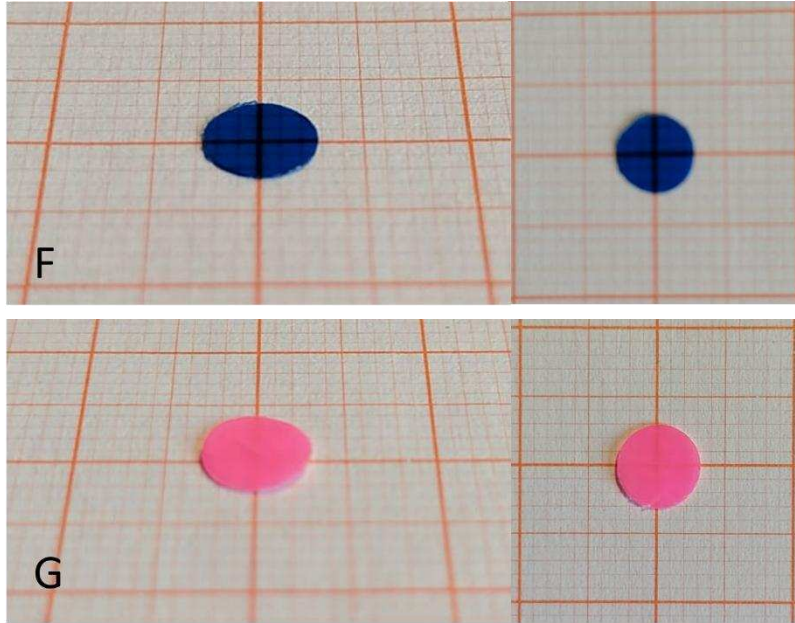


Figure S2. 3 - Visual representation of the plastic particles used in the experiments, categorized by type. (a) height 0.15 mm, diameter 6 mm, density 1.04 g/cm³; (b) height 0.15 mm, diameter 4.5 mm, density 1.04 g/cm³; (c) height 1 mm, diameter 9 mm, density 1.04 g/cm³; (d) height 0.08 mm, diameter 6 mm, density 1.1 g/cm³; (e) height 0.08 mm, diameter 4.5 mm, density 1.1 g/cm³; (f) height 0.02 mm, diameter 6 mm, density 1.2 g/cm³; (g) height 0.08 mm, diameter 6 mm, density 1.4 g/cm³

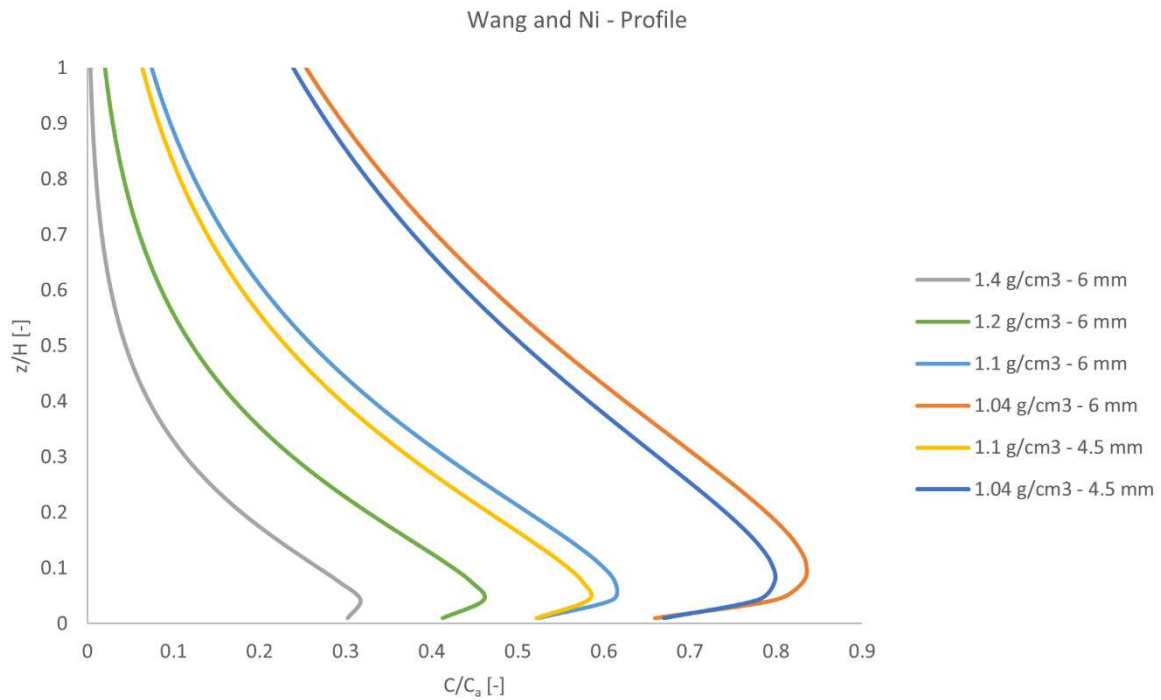


Figure S2. 4 - Wang and Ni (1990) water column concentration profiles. C_a represents the reference concentration of particles at a specified height calculated using Van Rijn's (1984) formula

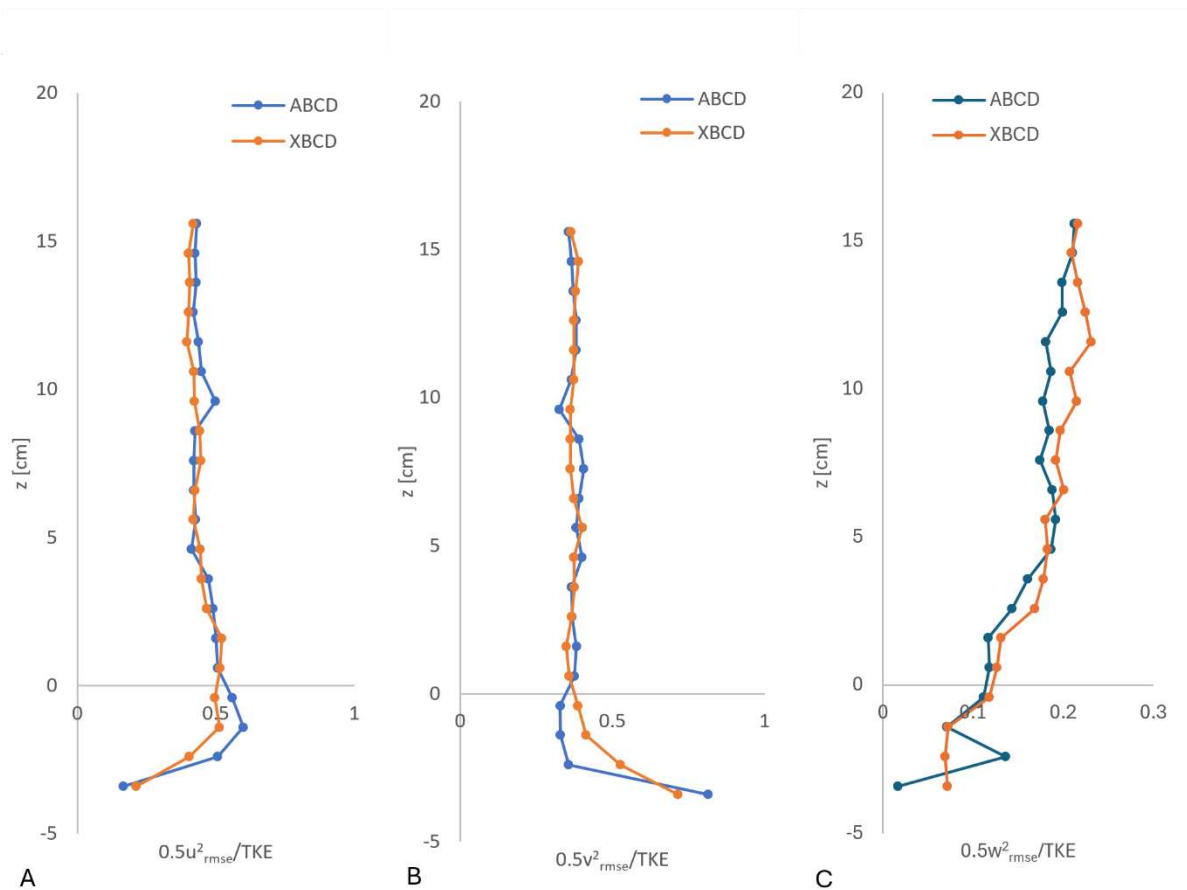


Figure S2. 5 - (a) Streamwise, (b) spanwise, and (c) vertical fractions of the turbulent kinetic energy for the two configurations ABCD and XBCD

Video_S1

The video_S1 illustrates the second phase of the experimental protocol, where the flume and individual plants are visually inspected to identify and count trapped microplastic particles. A UV lamp is used to enhance the detection of retained particles, making them more visible against the background. The footage captures the careful examination of the vegetation and the channel bed, highlighting the locations where microplastics accumulate within the system.

<https://www.sciencedirect.com/science/article/pii/S2666765725000377#ecom0002>

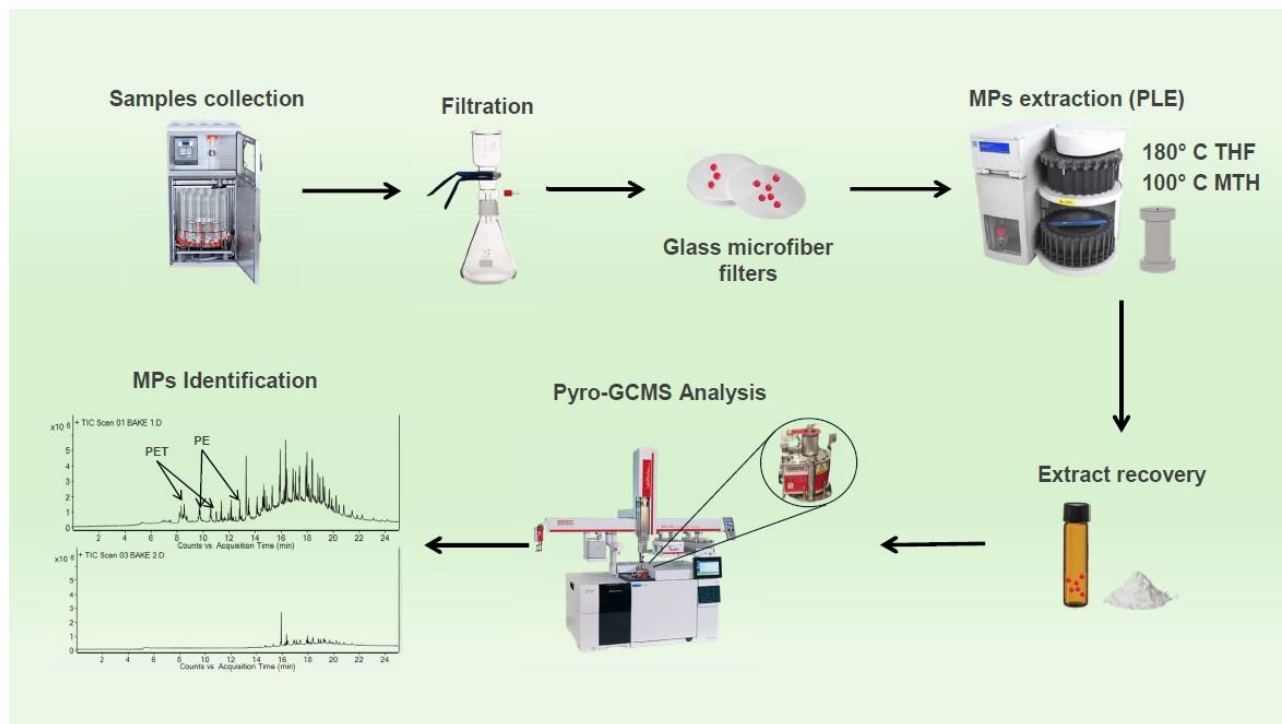
Video_S2

This video illustrates the settling behavior of the densest microplastic particles (1.4 g/cm^3) within dune troughs. As observed, particles tend to accumulate downstream of the dunes, likely due to the formation of localized recirculating flow patterns that enhance retention in these areas. This phenomenon supports the hypothesis that bed morphology plays a key role in microplastic transport and deposition dynamics.

<https://www.sciencedirect.com/science/article/pii/S2666765725000377#ecom0003>

3 Chapter 3: Quantification Challenges in Polymer Analysis in Urban Runoff and Wastewater using Pressurized Liquid Extraction and Double-Shot Pyrolysis-Gas Chromatography-Mass Spectrometry

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Abstract

Microplastics are persistent environmental pollutants with potential risks to ecosystems and human health. This study optimized methods for isolating and quantifying polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), and polystyrene (PS) in environmental water and wastewater samples using Pressurized Liquid Extraction (PLE) combined with Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS).

The optimized two-step PLE protocol, consisting of methanol pre-extraction at 100°C followed by tetrahydrofuran (THF) at 180°C, achieved MP recoveries of 43-58%. Calibration curves were established using both solubilized and solid MP standards, with solubilized calibrations providing higher accuracy for PET and PP. Py-GC/MS conditions were optimized at 625°C for 40 seconds to ensure maximum sensitivity and reproducibility.

PE and PET were identified as the dominant MPs in the wastewater samples, with concentrations of $99.4 \pm 71.8 \mu\text{g/L}$ and $16.2 \pm 13.3 \mu\text{g/L}$ in Avedøre, and significantly higher levels of $749.0 \pm 200.0 \mu\text{g/L}$ and $56.7 \pm 22.6 \mu\text{g/L}$ in Pontedera. PP and PS were detected at lower concentrations, with PP ranging from $8.2 \pm 4.2 \mu\text{g/L}$ to $16.9 \pm 6.5 \mu\text{g/L}$, and PS from $2.2 \pm 1.5 \mu\text{g/L}$ to $8.4 \pm 2.6 \mu\text{g/L}$.

Our results highlight the challenges of MP analysis in water samples emphasizing the impact of key method parameters (e.g., MP isolation, pyrolysis and calibration) on analytical reliability. The proposed method enhances MP detection in different water matrices, offering a reliable approach for routine environmental monitoring. Future efforts should focus on refining protocols to improve accuracy and precision and advancing standardization to ensure consistent and accurate MP quantification.

Keywords: Microplastics, wastewater analysis, Py-GC/MS, pressurized liquid extraction, polymer quantification, environmental water samples

3.1 Introduction

Microplastics (MPs), defined as plastic particles smaller than five millimeters, have become a significant environmental concern due to their widespread presence and persistence in ecosystems. When ingested by animals, MPs can cause physical harm, toxic exposure, and bioaccumulation, ultimately entering the human food chain.(Jiménez-skrzypek et al., 2021; Martín et al., 2022) This has raised increasing concerns about their impact on human health, as studies continue to report their presence in food and water sources.(Eze et al., 2024; Mamun et al., 2023)

Numerous studies have detected MPs across environments, from urban areas to remote regions like the North Atlantic, highlighting the global scale of this issue.(Bui et al., 2020; Halle et al., 2017) While much research has focused on marine environments, the roles of rivers, runoff, wastewater, and overflows in MP pollution remain poorly understood.(R. Hurley et al., 2018) A key challenge is the lack of standardized analytical protocols, leading to inconsistent and difficult-to-compare results across studies. Despite the development of methods for isolating and quantifying MPs, a universal standard is still lacking, with evolving methodologies failing to establish consistent benchmarks.(Fok et al., 2020; Mai et al., 2018)

MP analysis involves two key steps: isolation from environmental matrices and quantification. Traditional isolation techniques, such as density separation (e.g., NaCl or high-density solutions), can be inefficient due to variations in MP density or adhesion to particulate matter, leading to biased quantification.^{10–12} Chemical digestion with oxidative agents (NaClO, H₂O₂), bases (NaOH, KOH), or acids (HCl, HNO₃) is also commonly used to remove organic material, but it can degrade certain polymers and hinder the detection of plastic additives or adsorbed micropollutants.^{13–15}

To improve isolation efficiency, emerging methods such as the Oil Extraction Protocol (OEP), which exploits the oleophilic nature of plastics, have been tested in combination with density separation.¹⁶ Electrostatic and magnetic separation techniques have also been explored to selectively isolate MPs based on their electrical or magnetic properties.^{17–19} These alternatives aim to enhance recovery and reduce biases in MP quantification

Pressurized Liquid Extraction (PLE) has recently shown promising results for isolating MPs. (Fuller & Gautam, 2016; Okoffo et al., 2020) PLE uses solvents at high pressure and temperature, enhancing MP extraction by keeping solvents in a liquid state at elevated temperatures, thereby increasing solubility. This method is particularly effective for isolating MPs from complex matrices like sediments and biological tissues and can detect even low MP concentrations (Dierkes et al., 2019).

MP quantification of solid or solubilized MP samples can be performed with laser scanning or chromatography-based methods. Laser scanning analyzes MP spectra to visualize characteristics like shape, number, polymer type, and color but can struggle with very small particles. In contrast, chromatography-based methods, especially Pyrolysis- gas

chromatography/mass spectrometry (Py-GC/MS), are better for analyzing MPs of all sizes. Py-GC/MS breaks down polymers through pyrolysis and detects thermal degradation products and reliable quantification requires monitoring of selective products at specific mass-to-charge (m/z) ratios. This method also reduces the need for extensive sample preparation as it can be performed on both solid and liquid (solubilized) samples, but sample cleanup is beneficial to avoid quantification biases (Akoueson et al., 2021; Jiménez-skrzypek et al., 2021; Shin et al., 2011) (Stile et al., 2021) (Li et al., 2025)

Py-GC/MS is used not only for identifying and quantifying MPs but also for detecting associated micropollutants, such as plastic additives or environmental pollutants absorbed by MPs (Bart, 2001; F. C. Y. Wang, 2000). Common pyrolyzers include Curie Point, Micro Furnace, and filament types, which, despite varying heating methods, consistently produce reliable results for MP quantification, enabling cross-study comparisons (Fischer & Scholz-Böttcher, 2019). However, quantifying MPs in the low range of 25-385 μg poses challenges in preparing both MP standards and samples. Calibration curves can be created by diluting MPs with inert materials like glass microfibers or silica for even pyrolysis distribution (de Carvalho et al., 2021; Fischer & Scholz-Böttcher, 2019), but this process is prone to errors during preparation.

Alternatively, MP solubilization can be performed prior to analysis using solvents like dichloromethane (DCM) or tetrahydrofuran (THF). It has been demonstrated that (Okoffo et al., 2020) PLE with DCM effectively solubilizes polyethylene (PE) and polypropylene (PP), while another study (Leslie et al., 2022) used THF for polyethylene terephthalate (PET). For polymers such as PE and PP, where solubilization is difficult, MPs are analyzed in solid form (Z. Steinmetz et al., 2020). Selecting appropriate solvents to solubilize various MP mixtures is crucial. One strategy that will be tested in this study involves using Hansen solubility parameters to evaluate the solubilization potential of organic solvents on the different MP polymers treated in this study (Hansen, 1967).

The creation of solid or liquid calibration curves is essential for reliably quantifying MPs. Dierkes et al. (2019) reported limits of quantification (LOQs) between 1.4-1.6 μg for PE, PP, and polystyrene (PS), calculated from the standard deviation of blank samples. Matsueda et al. (2021) found higher LOQs for PET, around 10 μg , reflecting the varying challenges in quantifying different polymer types.

PLE was selected in this study for MP due to its suitability for handling complex matrices rich in organic matter, such as wastewater and combined sewer overflows (CSOs). Unlike conventional density separation and filtration, PLE allows for selective solubilization of MPs while simultaneously removing dissolved organic matter through controlled temperature and solvent polarity. Its high degree of automation reduces contamination risk and improves reproducibility between replicates. Moreover, the dry residues obtained from PLE with trapping material in the collection vials (in this study hydromatrix) are fully compatible with Py-GC/MS, enabling an efficient transition from extraction to quantification without additional filtration steps.

This study aims to refine MP quantification by optimizing PLE for particulate matter extraction and evaluating two calibration approaches for Py-GC/MS analysis of PE, PP, PS, and PET. It focuses on selecting optimal solvents, optimizing pyrolysis conditions, and assessing key PLE parameters (pressure, temperature, and extraction cycles) to enhance MP recovery from complex water matrices. Additionally, Py-GC/MS settings (pyrolysis temperature, split ratio, and flow conditions) will be fine-tuned to improve sensitivity and reproducibility. By refining these methodologies, the study seeks to establish more reliable and standardized protocols for MP isolation and quantification.

Although PLE has been applied for to solid samples, its application to filtered particles from aqueous samples remains limited. In this study, we adapted a dual-solvent PLE protocol, using methanol for matrix cleanup and THF for polymer solubilization, and collection of MPs on a solid material in the PLE collection vial. The solid material is then analysed by a double-shot Py-GC/MS method for MP quantification. Calibration in both solid and liquid phases, enabled detection of PE, PP, PET, and PS in a realistic concentration range of 200 ng to 10 µg.

The method was applied to environmental samples collected from CSOs, a highly relevant but under-investigated source of MP pollution. These episodic discharges, triggered by intense rainfall, can introduce large volumes of particulate matter into receiving waters and are recognized as major contributors to urban MP loads. To date, no quantitative studies employing thermal analysis techniques have targeted these complex matrices. This study provides the first chemically resolved MP concentrations from CSO events, contributing novel insights into the role of urban drainage in plastic pollution.

3.2 Material and Methods

3.2.1 Reference Standards and Calibrations

MP reference standards used for calibration and recovery experiments included PE, PP, PET, and PS in pellet form, provided by CC Plast (Hillerød, Denmark), with declared purities >99%. Pellets were cryo-milled using a TissueLyser II (QIAGEN) after liquid nitrogen immersion and sieved to obtain particles >100 µm, in agreement with environmental survey thresholds.

Calibration was performed using both solubilized and solid-phase MP standards. For solubilized calibrations, MPs were dissolved in solvent mixtures optimized per polymer type (e.g., 20:80 octanol:toluene for PE/PP and 70:30 chloroform:TFA for PET/PS), heated (100–130°C) to assist dissolution, and evaporated at 100°C overnight. Aliquots of the resulting dry film, corresponding to 200 ng to 10 µg of polymer, were transferred into quartz pyrolysis vials (Gerstel) using pre-heated glass syringes.

For solid-phase calibration, the same mass range (200 ng–10 µg) of cryo-milled MPs was manually mixed with 2–3 mg of Hydromatrix and spiked with 0.95 µg of 4-fluorostyrene (Polymer Source), used as internal standard. The standard was solubilized in THF before addition. Mixing was performed manually using a metal spatula for 4–5 minutes to ensure homogeneity prior to loading into pyrolysis vials.

Limits of detection (DL) and quantification (LOQ) were determined from blank sample analyses and are reported in the Supporting Information (SI11)

3.2.2 Pressurized liquid extraction

PLE was used to isolate MPs while minimizing interference. Samples were filtered (GB-140, 7 μm), dried, folded, and placed in 33 mL ASE-200 cells packed with Ottawa sand (1 cm from the top).

A two-step PLE method was optimized: a 100°C methanol pre-extraction to remove micropollutants, followed by THF extraction at 180°C. Key parameters: 1500 psi, 5 min static time, 3 methanol cycles, 2 THF cycles, flush volumes (MeOH 45%, THF 80%), 75 s purge. Extracts were collected in 80 mL vials with preheated Hydromatrix (450°C).

PLE efficiency was assessed via hydrothermal extraction (180°C, 16 h, 1°C/min increase) and by weighing vials before/after solvent evaporation. Two methods were tested: (1) three rinses (50% volume, combined) and (2) one rinse (150% volume). A four-cycle extraction was also evaluated for full polymer recovery

3.2.3 Py-GC/MS Method: Development and Final Protocol

Py-GC/MS method was developed and optimized for MP analysis, following an initial protocol where 5 mg of milled MP sample were placed in pyrovials and analyzed in a Gerstel TDU 2 (thermal desorption unit). The method employed a double-shot approach: thermal desorption at 300°C to remove volatile and semi-volatile compounds, followed by pyrolysis at 625°C for MP degradation and analysis of pyrolyzates.

The initial method(Fuller & Gautam, 2016) applied pyrolysis at 600°C for 20 s, injecting pyrolysis products at a 1:20 split ratio, followed by another 1:20 split (total 1:400 split). A split vent flow controlled the transfer through the TDU, while a second split was applied during column injection. The temperature between the pyrolysis unit and the injection port was maintained at 300°C. Mass spectra were acquired on an Agilent 5975C inert XL MSD, with an interface temperature of 320°C.

To improve sensitivity and prevent MSD saturation, particularly for PS, the split ratio was refined. The final optimized method maintained the double-shot approach, with thermal desorption at 300°C (100 s hold) followed by pyrolysis at 625°C (40 s hold, plus 1 min post-pyrolysis transfer). Helium was supplied at 51.1 mL/min, with a 1:10 split ratio and a 10 mL/min purge flow. The CIS (cooled injection system) trapped analytes at 30°C, then ramped to 300°C at 12°C/s, holding for 3 min before column transfer.

Separation was performed on an Agilent 7890A GC equipped with a Zebron ZB5 column (5% phenyl methyl siloxane, 30 m $\times$ 250 μm $\times$ 0.25 μm). The oven program started at 35°C, ramping at 15°C/min to 320°C, followed by a 6-minute hold. The helium flow was 1.1 mL/min, with detection in SIM mode (six ions per retention time window, 30 ms dwell time, 4.89 scans/s) or

scan mode (m/z 10–400, 3.66 scans/min). The ion source and quadrupole were kept at 230°C and 150°C, respectively.

Through this methodological refinement, the final Py-GC/MS protocol ensured detection of all polymer types, optimizing split ratios to prevent signal saturation while maintaining high analytical sensitivity and reproducibility.

The method was validated using six-point calibration curves (200 ng–10 µg) with replicates ($n = 2$ –3), internal standard correction, blank vials between samples, and pre-analysis cleaning of pyrolysis interfaces. R^2 values, 95% CI, and carryover tests are provided in SI6–SI8.

3.2.4 Peak identification, detection and quantification

For MP polymer identification, specific indicator compounds are needed for each plastic type. In py-GC/MS analysis, PS is typically identified and quantified by styrene, α -methylstyrene, and cumene, while PET is quantified by dimethyl terephthalate and bis(2-hydroxyethyl) terephthalate. PE is recognized by linear alkenes and alkanes such as 1-octadecene and 1,14-tetracosadiene, and PP by 2,4-dimethyl-1-heptene and 2,4-dimethyl-1-decene.

Reference standards were analyzed, and pyrograms were compared with literature data for accurate identification. Styrene (m/z 104) was used for quantifying PS, vinyl benzoate (m/z 105) for PET, 1-decene (m/z 83) for PE, and 2,4-dimethyl-1-heptene (m/z 126) for PP.

Table S3.2 lists the plastic polymers, target pyrolyzates, and corresponding m/z values used as quantified and qualified ions. SIM data files from the py-GC/MS were processed in Masshunter's MS Quantitative Analysis software, with manual adjustments to ensure consistency.

3.3 Result and discussion

3.3.1 Homogeneity of MP

Sample homogeneity is a key challenge in MP analysis (Dierkes et al., 2019). For particles >100 µm, high sample weights are typically required. Thermo-analytical methods use 10–50 mg, but our study was limited to 5–10 mg due to pyro vial capacity. Aggregate formation post-extraction further reduced homogeneity, with polymers sometimes re-polymerizing upon solvent evaporation, forming fragile aggregates atop the Hydromatrix layer, which were disaggregated using a handheld miller.

To enhance homogeneity and reduce statistical uncertainty, we compared samples with and without homogenization (Figure S3.4). Aliquots (5 mg) were analyzed, the maximum workable in 10 mg pyrolysis cups. Initially, direct PY-GC/MS analysis was used. A mixing step with stainless steel spheres in a Reax mixer (overnight) was then tested. The most effective method was manual mixing (4–5 min with a spoon), previously reported but not quantified for effectiveness.

Figure S3.4 shows that manual mixing significantly improved reproducibility, reducing relative standard deviation (RSD) from 0.95% to 0.14% (2 µg/mg sample) and from 0.94% to 0.22% (160 µg/mg sample). Proper grinding/homogenization is essential for reliable MP analysis, ensuring uniform particle distribution and lower RSD in technical replicates.

3.3.2 Polymer solubility and handling challenges

The solubility of MPs varied significantly among polymers, with PS dissolving easily while PE and PP required heating and specific solvent combinations. Further details on polymer solubility and handling challenges are provided in the Supplementary Information (SI8).

3.3.3 PLE for isolation of MPs from sample matrix

The two-step PLE method began with a pre-extraction using methanol at 100°C to remove organic contaminants that could otherwise interfere with subsequent MP analysis. This step was followed by an extraction with THF at 180°C to solubilize MPs effectively. Figure 3.1 presents the peak areas for PS and PE obtained during sequential extractions, highlighting the method's selectivity.

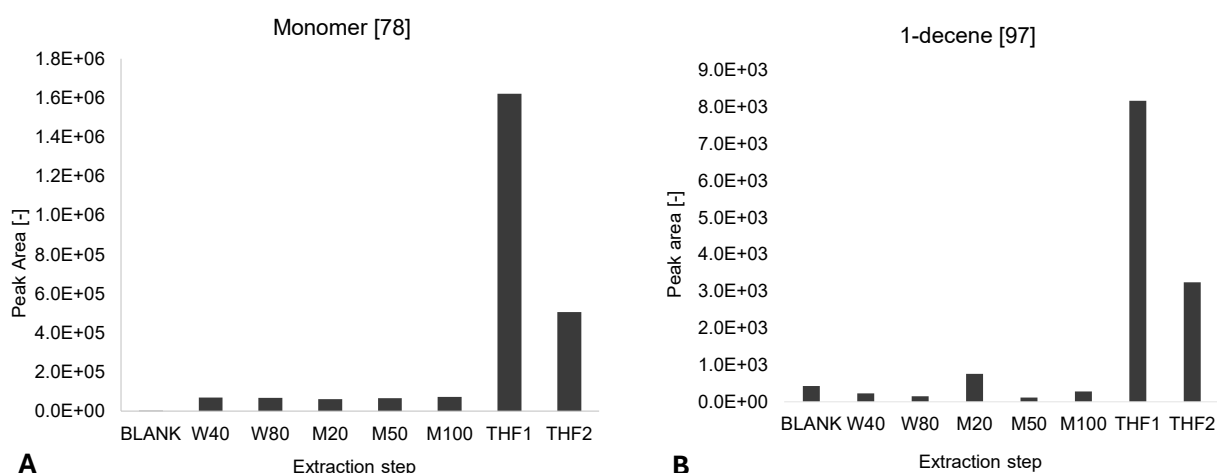


Figure 3. 1 - Peak area of representative compounds for PS (styrene, m/z 78) (A) and PE (*i*-decene, m/z 97) (B) after sequential extraction conditions. The blank represents the extraction of a PLE cell without spiking with MPs using THF at 180°C. W40 and W80 refer to milliQ water extractions at 40°C and 80°C, respectively. M20, M50, and M100 represent extractions with 20%, 50%, and 100% MeOH in MilliQ water at 100°C. THF1 and THF2 denote two consecutive THF extractions at 180°C.

Results demonstrated that water and methanol extractions, regardless of temperature, did not solubilize the polymers, as peak areas remained like blank values, indicating minimal polymer extraction. In contrast, the first THF extraction yielded significantly higher MP concentrations, confirming effective polymer isolation with little need for a second THF extraction where the peak area was around 30% compared to the first extraction peak area for both polymers, PS and PE. This outcome confirms that the methanol pre-extraction step can successfully remove potential organic interferences without compromising MP recovery. However, at the same time it requires full solubilization of the plastic polymers with the extraction solvent (in this case THF) at the extraction temperature 180°C, and this has not been confirmed with PET or PP.

Each extracted fraction was collected and analyzed by Py-GC/MS to quantify MPs. Recovery was determined by comparing the peak areas of PS, PE, PP and PET in the Hydromatrix

collected from PLE with those in the extract obtained by sixteen hours of heating in a metal cell, yielding recoveries between 43% and 58% for the different MPs (43% for PS, 46% for PET, 44% for PE, and 58% for PP).

A second evaluation method involved weighing the Hydromatrix in the collection cells before and after PLE. Results for PS confirmed that no extraction occurred during the MeOH pre-extraction, while the THF extract recovered $87 \pm 1\%$ ($n=2$) of the PS initially added to the PLE cell.

Although previous studies have used multi-step solvent extraction with PLE, the present method introduces key improvements that improve both versatility and environmental relevance. The dual-phase calibration strategy - employing both solubilized polymer films and solid-phase mixtures with Hydromatrix - offers performance across a range of sample types, improving method transferability. More importantly, the method was successfully applied to samples from CSO events, which constitute episodic but intense releases of MPs into the aquatic environment.

3.3.4 Influence of temperature and time on pyrolysis efficiency

Before analyzing real samples, we focused on selecting the pyrolysis temperature to ensure complete polymer degradation and consistent signals responses across samples with identical polymer quantities. Various pyrolysis technologies exists (Al-Rumaihi et al., 2022; Canabarro et al., 2013; Pahnla et al., 2023), and previous studies (Okoffo et al., 2020) have identified effective pyrolysis conditions to minimize inter-laboratory discrepancies.

A preliminary study on PS pyrolysis duration found that extending the time from 20 to 40 seconds resulted in higher peak areas. Based on this, a pyrolysis time of 40 seconds was selected for further temperature optimization without additional refinements.

The effect of pyrolysis temperature on the abundance of target pyrolyzates was evaluated for all four MPs. Six temperatures, ranging from 550°C to 675°C, were tested using a 40-second pyrolysis time, a 1:50 split ratio, and a CIS transfer temperature of 250°C. Each temperature was tested in triplicate across three batches, with batch orders varied (low to high, high to low, and random), and cleaning steps performed between runs.

Results (Figure 3.2) showed that PE pyrolysis was most efficient at 625°C, with C10-C14 α -alkene peaks nearly doubling compared to other temperatures. A similar trend was observed in another study (Okoffo et al., 2020), where selected 600 °C to maximize PE detection in biosolid matrices using Pyr-GC/MS. PET pyrolyzates showed high temperature sensitivity; while vinyl benzoate remained constant, biphenyl and benzophenone increased 8-10 times between 550°C and 675°C, in line with thermal decomposition profiles described in pyrolysis reviews (Jiménez-skrzypek et al., 2021; Mai et al., 2018; Samples, 2021). PP pyrolyzates, particularly the early-eluting trimer “2,4-dimethyl-1-hept-1-ene(126)” and the “2,4,6,8-tetramethyl-1-undecene (111)” peaks, remained stable between 550°C and 625°C but decreased at higher temperatures. For PS, styrene levels remained consistent across temperatures, while the PS-dimer “3-buten-1,3-diyl dibenzene (91)” and 2,5-diphenyl-1,5-hexadiene (234) showed slight decreases at higher temperatures.

Systematic comparison of six pyrolysis temperatures between 550 and 675 °C showed compound-specific responses, such as a sharp increase in biphenyl and benzophenone from PET, and a decline in PP trimers above 625 °C. The selected 40 s pyrolysis time was validated through preliminary tests on PS and is consistent with results from another study(Okoffo et al., 2020) who reported no signal suppression using 20–40 s in their double-shot setup.

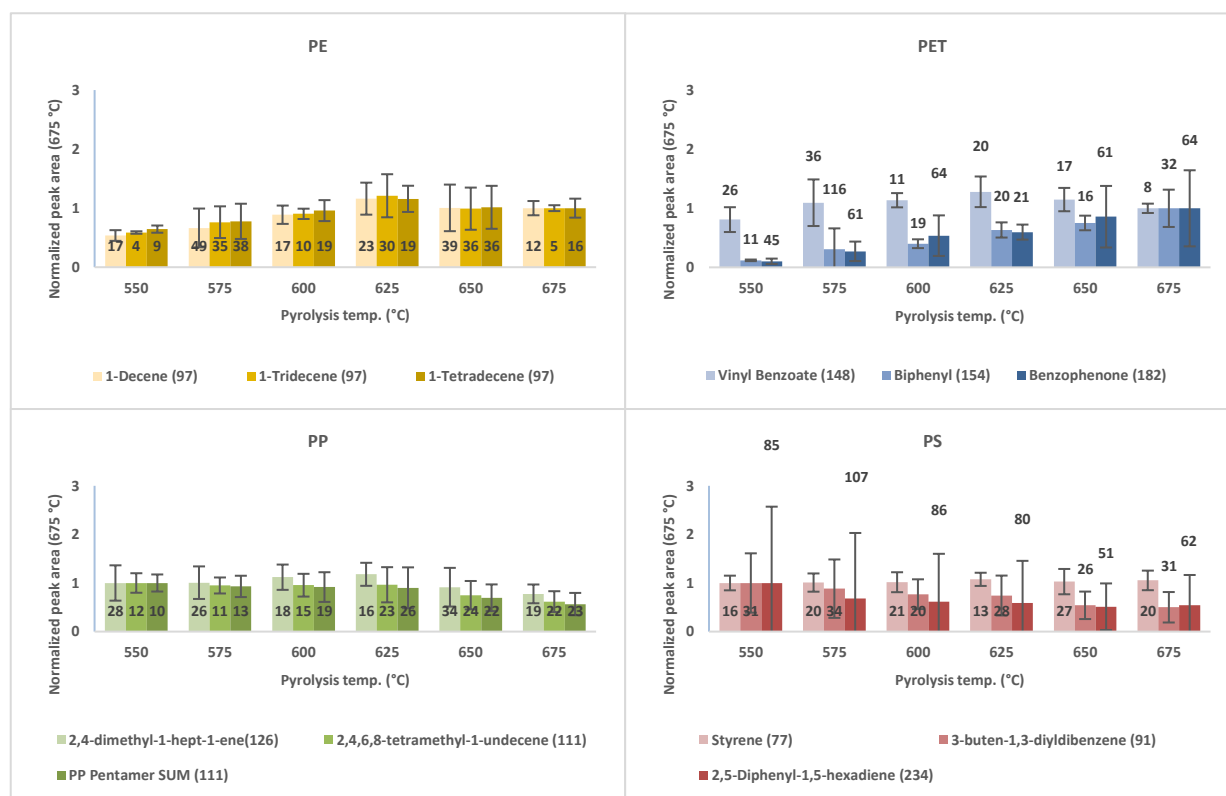


Figure 3. 2 - Normalized peak areas of selected pyrolyzates for PE, PET, PP, and PS at different pyrolysis temperatures. The x-axis represents the pyrolysis temperature (°C), while the y-axis shows the normalized peak areas relative to 675°C. Pyrolyzate names and their corresponding extracted ion (m/z) values are indicated in the legends below each chart. Error bars represent the 95% confidence interval ($n=3$), with relative span percentages displayed above or within the bars for each temperature condition.

3.3.5 Influence of inorganic matrix in pyrolysis-vials

Under standard pyrolysis conditions without a matrix, PS pyrolysis primarily produces styrene (monomer), 3-butene-1,3-diylidibenzene (dimer), and 5-hexene-1,3,5-triyltribenzene (trimer), with the highest signals observed for the monomer, trimer, and dimer. Additional byproducts include α -methylstyrene, benzene, and xylene (Maafa, 2021; Onwudili et al., 2009).

The presence of an inorganic matrix significantly alters the yield of these characteristic PS pyrolysis products, even with identical PS amounts. For PET, the addition of silica gel caused a near-complete suppression of diagnostic peaks, suggesting that silica gel interacts with analytes or decomposes key markers such as benzoates and terephthalates (Lauschke et al., 2023).

Comparative tests using three inert matrices and PS showed varying effects on styrene peak areas. Silica gel caused the most deviation, glass beads produced values closer to pure PS, and

Hydromatrix provided the most stable pyrolysatogram (Figure S3.6), making it the preferred inorganic matrix in our study. These results are comparable with those obtained in a similar study (Lauschke et al., 2023), who observed a reduced signal intensity of PS marker compounds when pyrolyzed with silica. However, our study extends this comparison by simultaneously evaluating three matrices under controlled conditions and by monitoring multiple PS markers (monomer, dimer, trimer). Notably, Hydromatrix provided the best reproducibility, measured as relative standard deviation (RSD), with values as low as 0.7% for styrene, compared to 31.9% with silica gel, 9.2% with glass beads, and 7.5% with no matrix. Similarly, lower RSDs were observed for the PS dimer (4.9%) and trimer (14.0%) with Hydromatrix, confirming its superior consistency. These results contrast with the greater variability reported in a previous study (Lauschke et al., 2021) for both alumina and silica (see Figure 3.3).

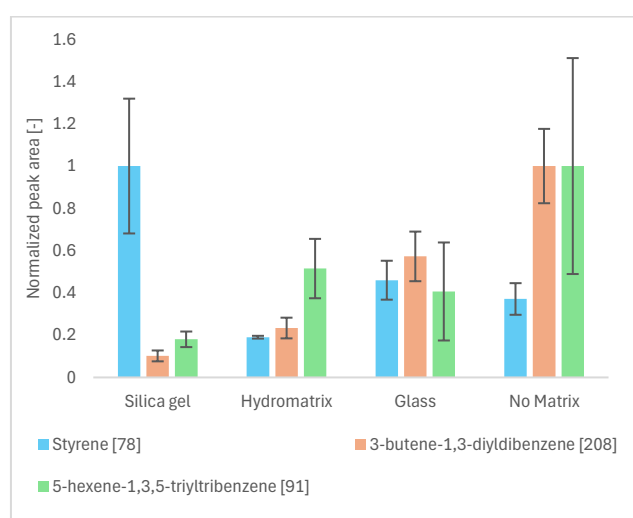


Figure 3. 3 - Influence of inorganic matrices on PS pyrolysis products. All values are normalized to the highest detected signal. Peak areas of styrene monomer (m/z 78), 3-butene-1,3-diyltribenzene (m/z 208) and 5-hexene-1,3,5-triyltribenzene (m/z 91) for PS pyrolysis with different inorganic matrices: silica gel, Hydromatrix, glass beads, and no matrix. Equal sample amounts were analyzed, with error bars representing relative standard deviations and mean peak areas shown. Silica gel yielded the highest peak area for monomer and dimer, while Hydromatrix ensured consistent results.

3.3.6 Effects of split ratios and flows and temperatures in the TDU

A test was conducted to evaluate the impact of split ratios in the py-GC/MS method on selected pyrolyzate peak areas. Split ratios of 1:50, 1:25, 1:10, and splitless were tested with single replicates, and the MS operated in scan mode. Results (Table S3.3) show that for PE, peak areas did not scale proportionally with decreasing split ratios, remaining under a 2-fold increase for most pyrolyzates, except 1-tetradecene in splitless mode. PET pyrolyzates followed a similar pattern from 1:50 to 1:10, but in splitless mode, peak areas of vinyl benzoate and biphenyl increased 6-7 times, while divinyl terephthalate and benzophenone rose 25-fold. PP pyrolyzates, including the trimer and pentamer peaks, showed minimal change across split ratios. The PS styrene monomer remained stable, while the PS dimer, 2,5-diphenyl-1,5-hexadiene, and trimer increased similarly to PET's divinyl terephthalate and benzophenone.

These results are consistent with other studies (Fischer & Scholz-Böttcher, 2019; Ishimura et al., 2021) who observed that Py-GC/MS peak areas often increase with lower split ratios,

though the response is not always linear due to saturation effects or secondary reactions among pyrolyzates. Moreover has been noted(Ishimura et al., 2021) unexpectedly high responses of certain PET and PC markers under high load or mixed-polymer conditions, an effect also evident in our data, particularly for PET in splitless mode. Similarly, has been demonstrated(C. Steinmetz et al., 2020) stable responses for PP trimer and PS monomers under varied preparation conditions, supporting the stability observed in our study for these markers

Comparable conclusions were reported in another study(Kwon et al., 2025), showing that high calibration curve linearity ($R^2 > 0.995$) in MP quantification required split ratio adjustment, specifically 100:1 for high-load and 10:1 for low-load samples, as a single calibration curve failed to maintain linearity for all polymers at a large mass range.

Compared to these studies, our results provide a more detailed polymer-specific analysis under varying split conditions, particularly highlighting the disproportionate signal increase of selected PET and PS pyrolyzates in splitless mode. This suggests that splitless injection can lead to overestimation of specific markers, especially in complex matrices or at high analyte loads, and should therefore be carefully validated for quantitative applications.

3.3.7 Reduction of carry-over

Following sample pyrolysis, the TDU hold time can be adjusted to stay warm for a set duration. This study tested 1 min (n=2) and 5 min (n=2) hold time to assess their impact on carryover. Pyro-vials containing a mixture of four MPs were analyzed using the double-shot method, followed by blanks to evaluate carryover. A 1:50 split ratio was applied with the MS in scan mode.

TDU hold time influenced both signal intensity and carryover for certain analytes. A longer hold time caused earlier elution of smaller analytes compared to the 1 min hold. For example, the PP trimer, the earliest eluting analyte, shifted by 1.26 min with a 5 min hold. Analytes eluting at 9 min or later were unaffected. Additional details are in Table S3.4 (left side).

Carryover signals of PE pyrolyzates in blanks showed minimal differences between hold times (Table S3.4, right side), with both resulting in 1-3% carryover, slightly lower with the 5 min hold. This trend was consistent for PP pyrolyzates and other early-eluting analytes. However, later-eluting analytes—PS dimer, trimer, and 2,5-diphenyl-1,5-hexadiene— showed reduced carryover with the longer hold time.

The calibration curve for solubilized MP polymers was analyzed in three batches, progressing from lowest to highest concentration, with blank pyro-vials between samples to monitor and minimize carryover. Figure 3.4 shows carryover signals for selected pyrolyzates, used in calibration curves described in the next section. For PE, 1-decene showed the lowest carryover, while 1-dodecene and 1-tetradecene had higher levels. Carryover remained stable from 200 ng to 2 μ g, but blanks following the 6 and 10 μ g showed increased peak areas across all three pyrolyzates.

These observations are consistent with general recommendations from instrument manufacturers such as Gerstel, which advice extended hold times at high temperature in the TDU to reduce memory effects between injections, particularly for high-boiling analytes. However, such guidelines are qualitative and lack numerical quantification. In contrast, this study provides a systematic, quantitative evaluation of carryover reduction as a function of hold time for a range of MP pyrolyzates, offering a novel and practical contribution to method optimization.

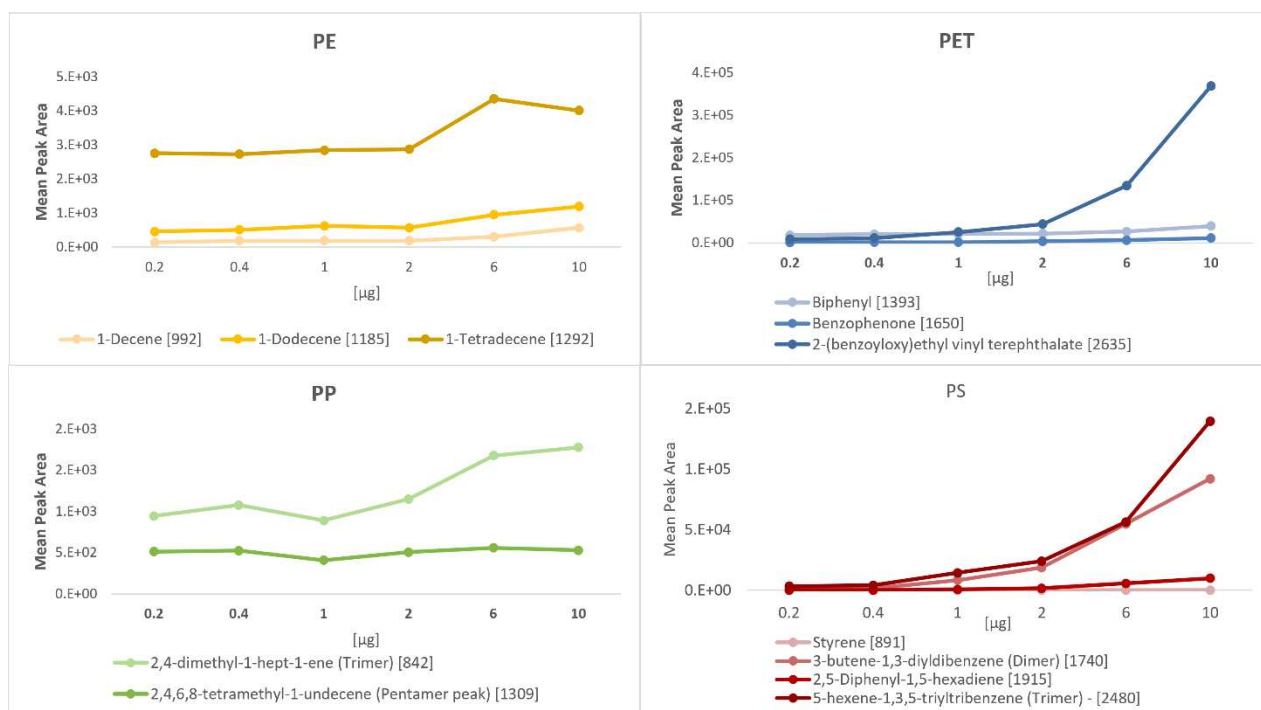


Figure 3. 4 - Mean peak area ($n=3$) of blank samples analyzed between consecutive calibration curve points, with samples run in ascending concentration order.

The PP trimer followed this trend, while the first PP pentamer peak showed minimal carryover change. The biphenyl peak from PET had the highest initial value, doubling from 17,700 to 39,500 between the 200 ng and 10 μg samples, with benzophenone showing a similar pattern at lower levels. The larger PET pyrolyzate, 2-(benzoyloxy)ethyl vinyl terephthalate, showed an 80-fold carryover increase from the lowest to highest concentrations. PS dimer and trimer followed a similar trend, while 2,4-diphenyl-1,5-hexadiene showed distinct peaks in blanks starting at 1 μg injections. The styrene monomer produced minimal carryover, appearing as integrated noise at 3.7 min.

In addition to carry-over effects, the influence of sample loading volume on response signal was tested, showing that smaller incremental loads resulted in a higher detector signal due to concentrated deposition at the vial bottom. Further details are provided in the Supplementary Information (SI9).

3.3.8 Comparison of solid and liquid calibration curve methods

Calibration curves for the four MP polymers (PE, PET, PP, and PS) were prepared from both solubilized polymers and solid MP particles diluted in Hydromatrix (Table 3.1 and SI6-SI8). PE calibration curves showed minimal differences between the two forms. For PET, the solid calibration curve had steeper slopes for biphenyl and benzophenone, with a similar but less pronounced trend observed for PP. In contrast, the solubilized PS curve had a steeper slope than its curve based on solid MP particles diluted in Hydromatrix. Regression parameters for calibration curves, using fluoro-styrene as an internal standard, are presented in Table 3.1.

Table 3. 1 - Comparison of second order regression parameters for calibration curves of solubilized and solid MP polymers. The left side contains parameters for solubilized MP polymers, while the right side represents solid MP particles diluted in Hydromatrix.

Polymer	Solution							Solid						
	a1	a2	R2	LOQ (µg)	Ratio	±	CI(95)	a1	a2	R2	LOQ (µg)	Ratio	±	CI(95)
PE	12760	28.84	0.94	0.13	5.14	±	0.37	17530	-251	0.97	0.09	5.29	±	0.56
PET	27350	-847	0.95	0.45	0.19	±	0.03	119500	-6968	0.54	0.10	1.08	±	0.97
PP	47290	276	0.95	0.13	2.67	±	0.06	65040	93	0.97	0.09	2.82	±	0.09
PS	3711000	-187000	0.95	0.00	230.6	±	55.23	2650000	-98670	0.89	0.00	291.9	±	129.7

Table 3.1 compares the regression parameters of solubilized (Solution) and solid-phase (Solids) MP calibration for each polymer, highlighting differences due to sample preparation and MP distribution.

For PE, both methods produced comparable results, with the solid calibration yielding a slightly higher a_1 value (17530 compared to 12760 in solubilized calibration) and a marginally improved model fit ($R^2 = 0.97$ vs. 0.94). The ratio values were consistent, indicating that both approaches provide reliable quantification.

For PET, significant discrepancies were observed. The solubilized method exhibited a much lower a_1 value (27350, whereas the solid calibration reached 119500) and a better model fit ($R^2 = 0.95$ compared to 0.54), suggesting that the solid calibration introduces higher variability, potentially due to matrix effects or polymer degradation. Additionally, the solubilized approach showed lower and more stable ratio values (0.19 ± 0.03 , in contrast to 1.08 ± 0.97 in the solid method).

For PP, moderate differences were found, with the solid calibration yielding a higher a_1 value (65040 relative to 47290 in solubilized calibration). However, both methods produced similar determination coefficients ($R^2 = 0.97$ and 0.95 , respectively), and ratio values remained close (2.82 ± 0.09 for solid calibration and 2.67 ± 0.06 for solubilized calibration), indicating consistent quantification across approaches.

For PS, the solubilized calibration resulted in a higher a_1 value (3711000, while the solid calibration reached 2650000) and a better model fit ($R^2 = 0.95$ compared to 0.89). However, higher response variability in the solid method (291.9 ± 129.7 vs. 230.6 ± 55.23 for solubilized

calibration) suggests potential differences in pyrolysis behavior between calibration approaches.

Overall, while solid calibration was effective for PE and PP, solubilized calibration provided superior consistency for PET and PS, with higher determination coefficients and more stable ratio values. Given these findings, solubilized calibration was preferred for MP quantification in environmental samples.

3.3.9 Validation tests on environmental samples: Utterslev Mose samples

The quantification of MPs in filtered water samples from Utterslev Mose (SI1 and Fig. S3.2), using calibration curves from solubilized PE, PET, PP, and PS, highlight the variability in concentrations in polymer concentrations (Table 3.2). PS concentrations were near the detection limits (DL). PP concentrations were below LOQ in all samples. Notably, PP levels in tap water were comparable to those in environmental samples A, B, and C, which ranged from 0.7 to 1.2 µg/L.

Table 3. 2 - MP concentrations in water samples from Utterslev Mose and blanks. Concentrations of PE, PET, PP, and PS in water samples A, B, and C (n=4) from Utterslev Mose, as well as MilliQ and tap water blanks (n=6), were quantified using calibration curves from solubilized MPs without an internal standard. Mean concentration and absolute 95% confidence interval are presented in µg/L. Superscripts indicate: (a) below DL, (b) below LOQ, and (c) above the highest calibration curve point.

Polymer	Sample A [µg/L]			Sample B [µg/L]			Sample C [µg/L]			MilliQ [µg/L]			Tap water [µg/L]		
	Mean	±	CI(95)	Mean	±	CI(95)	Mean	±	CI(95)	Mean	±	CI(95)	Mean	±	CI(95)
PE	72.9	±	33.8	148.9	±	62.0	232.0	±	118.0	5.3	±	2.1	4.4	±	3.2
PET	63.7	±	0.5	99.6	±	19.4	135.1	±	66.9	0.4	±	0.5	0.2	±	0.1
PP	1.0	±	0.7	0.7	±	0.4	1.2	±	1.4	1.2	±	1.0	0.5	±	0.2
PS	1.2	±	0.7	3.1	±	1.3	5.1	±	2.3	0.0	±	0.0	0.0	±	0.0

These findings are consistent with recent studies reporting low detectability of PP and PS in environmental water samples. Research suggests that micro-sized PS particles are prone to degradation and dispersion in natural waters, which can affect their quantification reliability (Dierkes et al., 2019). Moreover, the low PP levels align with its limited solubility and high buoyancy, which affect its environmental presence and detectability (Alimi et al., 2018).

PET and PE concentrations were considerably higher compared to other MPs, with PET levels of 63.7, 99.6, and 135.1 µg/L in samples A, B, and C, respectively. The elevated PET concentrations correspond to findings in other studies, attributing its accumulation in aquatic environments to its high density and environmental persistence (Horton, Walton, et al., 2017b). Benzophenone has been shown to be a reliable marker for PET detection with consistent quantification results even in complex environmental matrices (Lauschke et al., 2023).

PE showed the highest concentration among the tested MPs, with values of 72.9 ± 33.8 µg/L (sample A), 148.9 ± 62.0 µg/L (sample B), and 232.0 ± 118.0 µg/L (sample C). These findings reflect PE's extensive use in consumer products and its strong environmental persistence

(Geyer et al., 2017). Its lower density compared to PET contributes to its widespread distribution in surface waters, where it persists despite natural degradation processes (Hartmann et al., 2017).

Overall, these results demonstrate the diverse environmental behaviors of MPs depending on polymer type, with PET and PE showing higher concentrations in filtered water samples, consistent with literature. The use of specific quantifier ions such as benzophenone for PET, enhances detection accuracy and underlines the importance of rigorous analytical methods to accurately capture concentration variability across polymer types.

3.3.10 Wastewater samples

MP analysis in wastewater samples from Avedøre (Denmark) and Pontedera (Italy) (SI1 and Fig. S3.1) revealed significant MP concentration differences, reflecting site-specific variations commonly reported in wastewater studies (Table 3.3). The quantification of MPs in samples from both Avedøre and Pontedera, was made using calibration curves from solubilized PE, PET, PP, and PS, considered more reliable compared to the solid calibration curve (Table 3.1). In Avedøre, the mean PE concentration was 99.4 µg/L, while Pontedera exhibited substantially higher levels at 749.0 µg/L. These elevated concentrations in Pontedera are likely to result from higher wastewater volumes and industrial discharges, which are known to contribute to increased MP loads in WWTP influents.

Table 3. 3 - MP concentrations in WWTP samples from Avedøre and Pontedera. PE, PET, PP, and PS concentration in wastewater samples (n=5) from Avedøre and Pontedera were quantified using calibration curves based on solid MP particles. Values represent mean concentrations and 95% confidence interval (µg/L). Superscript: (a) below DL, (b) below LOQ.

Polymer	Avedøre [µg/L]			Pontedera [µg/L]		
	Mean	±	CI(95)	Mean	±	CI(95)
PE	99.4	±	71.8	749.0	±	200.0
PET	16.2	±	13.3	56.7	±	22.6
PP	8.2	±	4.2	16.9	±	6.5
PS	2.2	±	1.5	8.4	±	2.6

PET concentrations varied between sites, with Avedøre at 16.2 ± 13.3 µg/L and Pontedera at 56.7 ± 22.6 µg/L. The higher PET levels in Pontedera may be attributed to its persistence and accumulation in wastewater systems.

PP concentrations were 8.2 ± 4.2 µg/L in Avedøre and 16.9 ± 6.5 µg/L in Pontedera. The buoyancy of PP likely contributes to its retention in wastewater, resulting in moderate but consistent effluent levels.

PS concentrations were below the LOQ at both sites, a common finding in wastewater studies showing PS degradation nor removal during preliminary treatment stages. Its rapid breakdown and lower stability in wastewater systems often result in concentrations below DL.

In comparison to other results (Okoffo et al., 2020), influent PE concentrations in other WWTPs were reported at 2340, 1481, and 607 µg/L — 5 to 20 times higher than Avedøre samples but comparable to Pontedera. PP levels in Avedøre were below Okoffo's LOQ of 8 µg/L.

These findings align with current wastewater research, demonstrating the influence of site-specific factors, influent wastewater composition, and operational differences in WWTPs on MP concentrations. The observed variability highlights the need for customized MP quantification protocols to accurately track MP distribution and behavior in wastewater systems.

Moreover, a detailed comparison between the quantified MP ratios and their expected confidence intervals is provided in the Supporting Information (Table S3.10). While most samples exhibited ratios consistent with expected ranges, PET and PP presented notable deviations, particularly in lake samples (A, B, and C). These discrepancies may be attributed to polymer degradation (Rostampour et al., 2024), environmental accumulation patterns (Colombini et al., 2024; Xu et al., 2022), or matrix interferences affecting quantification (Lauschke et al., 2023). Given the inherent variability in MP composition and the influence of additives and polymer aging, further refinement of calibration strategies and extraction protocols could enhance quantification accuracy. However, the overall consistency in PE and PS detection, along with the observed trends in wastewater and surface water samples, supports the robustness of the analytical approach applied in this study.

3.4 Conclusions

This study established a refined approach for isolating MPs—PE, PET, PP, and PS—in environmental and wastewater samples using double-shot pyrolysis-gas chromatography/mass spectrometry, with or without PLE. The method effectively addressed matrix interferences, by either thermal desorption to remove volatile and semi-volatile compounds before pyrolysis and/or by pre-extraction with methanol using PLE.

Key findings provide valuable insights into critical parameters influencing MP quantification. The selected extraction strategy, whether using PLE with methanol pre-extraction followed by tetrahydrofuran or direct analysis via Py-GC/MS will reduce quantification biases and improve MP recovery. The final pyrolysis conditions, set at 625°C for 40 seconds, delivered consistent sensitivity and reproducibility. The study also evaluated different quantification strategies, showing that solid MP calibration curves improved accuracy for PET and PP, while solubilized calibrations provided better linearity for PE and PS.

Tests of surface water from a lake in Copenhagen and wastewater from two sites in Italy and Denmark showed that PET and PE were the most abundant MPs, likely due to their stability and density, whereas PP and PS were detected at lower levels, influenced by their buoyancy and degradation potential. Variations observed between WWTPs showed the impact of influent composition and operational factors on MP concentrations.

Further efforts should focus on refining Py-GC/MS conditions and evaluating the necessity of PLE for different sample types to enhance detection limits and overall performance. Developing consistent calibration protocols to ensure comparability across laboratories and sample types – the study clearly showed the challenge with obtaining reliable calibration solution either solid or liquid.

This study presents a analytical strategy for MP monitoring, offering flexible approaches for effective extraction, pyrolysis, and quantification to support environmental assessments and regulatory initiatives.

Supporting Information

The Supporting Information is available free of charge at the [ACS website](#)

General experimental methods; Materials and reagents, Synthesis; Characterizations; Calibration data, Additional figures and spectra

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3.7 Supporting Material

Supporting Information for:

Quantification Challenges in Polymer Analysis in Urban Runoff and Wastewater using Pressurized Liquid Extraction and Double-Shot Pyrolysis-Gas Chromatography-Mass Spectrometry

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SI1 - Sampling sites and preparation

The PLE-Py/GC-MS method was applied to wastewater samples from two WWTPs: Pontedera (Italy), where the municipal spillway receives residential, road runoff, and industrial wastewater (Figure SI1), and Avedøre (Denmark), where effluent is discharged after treatment. During heavy rainfall, the Pontedera spillway activates per Italian regulations. Samples were filtered to isolate MPs.

Additionally, surface water was collected from three sites (A, B, C) in Utterslev Mose (Denmark), with site C located under a bridge with traffic (Figure SI2). At each site, 2.5 L of water was sampled in glass bottles and refrigerated until analysis.

Samples from Pontedera and Avedøre WWTPs were vacuum-filtered (0.7 μm), requiring multiple rounds due to clogging. The retained material was analyzed for MPs. Before PLE-Py/GC-MS, samples were extracted using PLE, with dried filters folded, placed in 33 mL ASE-200 cells, and packed with Ottawa sand (1 cm from the top), following the Section 2.8 protocol.

Utterslev Mose samples were sequentially filtered (1.6 μm and 0.7 μm , Whatman GF/A) to match control spike conditions. MilliQ and tap water controls were processed alongside. Filters were dried (105 $^{\circ}\text{C}$, 4 h) and cryo-milled, with both filter sizes combined before milling.

SI2 - Materials

The plastic polymers used were: “Flexirene” linear low-density polypropylene (LLDPE or PE for simplicity, Versalis, pellets), “PP1013H1” polypropylene homopolymer (PP, Exxonmobile, pellets), “Eastar copolyester MN058” polyethylene terephthalate (PET, Eastman, pellets), “Styron 485” polystyrene (PS, Trinseo, pellets), Poly(4-fluorostyrene) (fluoro-PS, sample #: P43494-4FS, Polymer Source). The MP materials were provided by CC Plast in Hillerød, Denmark. The MP materials were in pellet form, each a few millimeters in diameter.

Quartz glass pyrolysis vials, with no slit (length 17 mm, part No. 018133-100-00, Gerstel), were used as sample containers during pyrolysis. Tools and containers made of plastic materials were avoided during the handling and preparation of plastic polymer solutions. Glass- and metalware was used instead. The only plastic material in contact with the reagents during sample preparation and analysis was PTFE-vial caps, used to close the 8 mL glass vials for polymer solubilization.

SI3 - Chemicals

The solvents used in this study were chloroform (ACS grade, $\geq 99.8\%$, Sigma-Aldrich), toluene (glass distilled grade, Rathburn), dichloromethane (glass distilled grade, Rathburn), 1-octanol (Reidel-de Haën), trifluoroacetic acid (TFA, $\geq 99\%$, Sigma-Aldrich), 1,2,4-trichlorobenzene (TCB, $\geq 99\%$, Sigma-Aldrich), and tetrahydrofuran (THF, ACS reagent, with BHT inhibitor, $\geq 99\%$, Sigma-Aldrich).

These chemicals are volatile and toxic; all handling was performed in a fume hood with appropriate PPE.

SI4 - Preparation of MP materials

MP sample preparation involved cryo-milling the pellets (few mm size) into fine powder. 1–2 g of polymer were placed in stainless-steel grinding jars with a 20 mm steel ball, submerged in liquid nitrogen (10 min, twice), and milled using a TissueLyser II (30 Hz, 3–10 min, QIAGEN). For tougher polymers (e.g., PE), milling cycles were repeated with additional cooling.

The powder was sieved (100, 250, 500 μm), repeating the process as needed to collect 100–200 μm particles. Only >100 μm particles were used, aligning with environmental survey thresholds (Cutroneo et al., 2020; Lindeque et al., 2020; Nihan, 2020).

SI5 - Polymer solutions in pyrovials for preparation of calibration curves

Solubilized polymer solutions were prepared by dissolving cryo-milled MPs in 8 mL glass vials with selected solvents (20:80 octanol for PE/PP; 30:70 TFA for PET/PS). Solutions were heated to dissolve MPs (40°C for PET/PS, 130°C for PE/PP), with volatile solvents added first and evaporated at 100°C overnight (≥ 8 h).

MP solutions were loaded into pyro-vials using climatized syringes (50 μL for MPs, 10 μL for fluoro-PS), starting with lower MP concentrations to avoid contamination. Syringes were equilibrated by flushing three times before use and cleaned in heated octanol (130°C) using a two-step rinse process.

SI6 - Solubilized MP Calibration Curves

Stock solutions of PE/PP (octanol 20:80) and PET/PS (TFA 30:70) were prepared at 0.9 $\mu\text{g}/\mu\text{L}$ (I), 0.3 $\mu\text{g}/\mu\text{L}$ (II), and diluted to 35 $\text{ng}/\mu\text{L}$ (III). A six-point calibration curve (200 ng –10 μg , $n = 3$) was constructed.

Samples were analyzed in three batches, with pyrolysis vial adaptors pre-cleaned at 750°C (40 s). Cleaning steps included TDU at 350°C (30 min) and CIS at 300°C (25 min). To reduce carryover, samples were run low to high concentration, with blank vials in between.

SI7 - Solid Particle MP Calibration Curves

A six point calibration curve was created using solid MP particles (PE, PET, PP and PS) mixed with Hydromatrix. The MP particles were cryo-milled, sieved to >100 μm , and combined with Hydromatrix to achieve pyro-vial loadings of 200 ng , 400 ng , 1 μg , 2 μg , 6 μg , and 10 μg , with two replicates per point ($n = 2$). Each pyro-vial was spiked with 0.95 μg fluoro-PS (solubilized in THF), dried, and filled with 2–3 mg of the Hydromatrix-MP mix.

SI8 - Polymer solubility and handling challenges

PS was the easiest polymer to dissolve due to its large interaction radius (R_o), dissolving in most tested solvents except TFA and octanol (high δH values, Table 3.1), consistent with studies using DCM:THF (50:50) (Matsueda et al., 2021) and TCB at 120°C (Z. Steinmetz et al., 2020). PET dissolved in 70:30 Chloroform:TFA ($RED = 0.92$) at room temperature, while DCM ($RED = 0.99$) required high temperature and pressure (Okoffo et al., 2020). PE and PP were harder to dissolve; 20:80 octanol:toluene was most effective for PE, but both required heating (100°C for PE, 130°C for PP), suggesting HSP spheres do not fully predict solubility at high temperatures (Howell et

al., 2017). Attempts with TCB(Z. Steinmetz et al., 2020) failed due to density differences, causing polymers to float and precipitate upon cooling, in line with Matsueda et al. (2021). PE and PP also presented handling issues, solidifying on vial surfaces upon repeated heating, leading to concentration loss. Stock solutions were prepared in larger volumes to minimize surface exposure, and heated syringes (50 µL, larger inner diameter) were used to prevent clogging. In contrast, PET and PS in chloroform allowed precise aliquoting with smaller syringes.

SI9 - Effect of loading volume on pyro-vial response signal

The impact of loading volume on response signal was evaluated by loading a 20 µL PP solution using three methods: a single 20 µL load, two sequential 10 µL loads with evaporation in between, and four sequential 5 µL loads with evaporation. A 50 µL syringe was used, with a 1:100 split ratio and a 180 mL/min TDU flow during pyrolysis (MS scan mode: 10-500 m/z).

Incremental loading of smaller volumes resulted in a slightly higher detector signal for the PP trimer, likely due to more concentrated deposition at the vial bottom. A similar trend was observed for the two larger PP pentamer peaks. Visual inspection confirmed that smaller volumes concentrated PP at the vial base, while larger volumes caused distribution further up the pyro-vial. When adding the volume as 4 × 5 µL method, a thin PP layer formed along the inner vial walls, likely due to surface tension between the syringe needle and vial wall, creating a “curtain” effect (Figure SI5).

SI10 - Pressurized liquid extraction

PLE was used to isolate MPs while minimizing interference. Samples were filtered (GB-140, 7 µm), dried, folded, and placed in 33 mL ASE-200 cells packed with Ottawa sand (1 cm from the top).

A two-step PLE method was optimized: a 100°C methanol pre-extraction to remove micropollutants, followed by THF extraction at 180°C. Key parameters: 1500 psi, 5 min static time, 3 methanol cycles, 2 THF cycles, flush volumes (MeOH 45%, THF 80%), 75 s purge. Extracts were collected in 80 mL vials with preheated Hydromatrix (450°C).

PLE efficiency was assessed via hydrothermal extraction (180°C, 16 h, 1°C/min increase) and by weighing vials before/after solvent evaporation. Two methods were tested: (1) three rinses (50% volume, combined) and (2) one rinse (150% volume). A four-cycle extraction was also evaluated for full polymer recovery

SI11 - DL and LOQ determinations

DL and LOQ were determined by calculating the standard deviation of 23 blank samples, adding the mean blank signal for each pyrolyzate (Table S3.2), and dividing by the slope of the calibration curve, as shown in equations 1 and 2:

$$DL = \frac{(3\sigma + \text{Mean of blanks})}{m} \quad (1)$$

$$LOQ = \frac{(10\sigma + \text{Mean of blanks})}{m} \quad (2)$$

Where σ represents the standard deviation of the blank samples (n=23), and m is the slope of the calibration curve.

Table S3. 1 - Dispersive (δ_D), polar (δ_P), and hydrogen-bonding (δ_H) parameters for microplastics (MPs) and selected solvents, along with the interaction radius (R_o) for each MP.

	δ_D	δ_P	δ_H	R_o
Polymers				
PE	16.0	0.8	2.8	3.2
PET	19.1	6.3	9.1	4.8
PP	18.0	0.0	1.0	6.0
PS	22.3	5.8	4.3	12.7
Solvents				
TFA	15.6	9.7	11.4	
DCM	17.0	7.3	7.1	
THF	16.8	5.7	8.0	
TCB	20.2	4.2	3.2	
Toluene	18.0	1.4	2.0	
Chloroform	17.8	3.1	5.7	
Octanol	16.0	6.0	11.2	

Table S3. 2 - Plastic polymers, target pyrolyzates, and corresponding m/z ions for peak integration with quantifier and qualifier ions used for peak integration are listed, where bolded m/z values represent ions used for quantification.

Polymer	Indicator Compound(s)	Indicator Fragments (m/z)	
		Qualifier ions	Quantifying ion
PS	<u>Styrene</u>	<u>51, 104</u>	78
	3-buten-1,3-diyl dibenzene (styrene dimer)	91, 130	208
	5-hexen-1,3,5-triyltribenzene (styrene trimer)	117, 194, 207, 312	91
PET	<u>Benzophenone</u>	<u>77, 105, 152</u>	182
	Dimethyl terephthalate	76, 104	175
	Vinyl benzoate	51, 77	105
	Biphenyl	76, 152	154
PP	<u>2,4-dimethyl-1-hept-1-ene</u>	<u>43, 55, 70</u>	126
	2,4,6,8-tetramethyl-1-undecene	43, 69	111
PE	<u>1-Decene (C₁₀)</u>	<u>83, 140</u>	97
	1-Undecene (C ₁₁)	82, 96, 112	154
	1-Tridecene (C ₁₃)	96, 112, 154, 182	196
	1-Tetradecene (C ₁₄)	69, 97	83

Table S3. 3 - Comparison of selected ion peak areas, normalized to the 1:50 split ratio, where Pyrolyzate names are listed under each MP with extracted ion m/z values in brackets.

Pyrolyzate	Split ratio:	Normalized peak area to 1:50			
		1:50	1:25	1:10	Splitless
<u>PE</u>					
	RI				
1-Decene (97)	992	1	1.3	1.9	1.5
1-Undecene (97)	1092	1	1.2	1.5	1.7
1-Dodecene (97)	1185	1	1.1	1.7	1.7
1-Tridecene (97)	1292	1	1.2	1.6	1.2
1-Tetradecene (97)	1393	1	1.2	1.4	4.1
<u>PET</u>					
Vinyl Benzoate (148)	1137	1	0.9	0.8	6.4
Biphenyl (154)	1393	1	0.8	1.0	7.1
Divinyl terephthalate (104)	1577	1	1.9	3.1	24.5
Benzophenone (182)	1650	1	1.0	1.8	26.8
<u>PP</u>					
PP - Trimer (126)	842	1	1.4	1.5	1.3
PP - Pentamer 1 (111)	1309	1	1.4	1.5	1.6
PP - Pentamer SUM (111)		1	1.3	1.4	1.6
<u>PS</u>					
Styrene (77)	891	1	1.3	1.4	1.3
PS - Dimer (91)	1740	1	2.2	4.1	22.4
2,5-Diphenyl-1,5-hexadiene (234)	1915	1	3.3	11.8	33.3
PS - Trimer (91)	2480	1	4.5	19.1	25.8

Table S3. 4 - Effect of TDU hold time on carryover showing retention time changes and carryover percentages for 1-min and 5-min holds after pyrolysis

TDU hold:	Retention time [min]			Carryover %			
	1 min	5 min	Δ Rt	1 min	5 min	1 min	5 min
<u>PE</u>							
1-Decene	4.968	4.681	0.29	2.9	1.1	2.3	2.6
1-Undecene	6.110	5.996	0.11	2.9	0.9	2.2	2.2
1-Dodecene	7.151	7.106	0.04	3.1	1.0	2.3	1.7
1-Tridecene	8.125	8.107	0.02	2.7	0.9	1.8	1.5
1-Tetradecene	9.030	9.025	0.00	2.5	0.9	2.4	1.3
<u>PET</u>							
Vinyl Benzoate	6.624	6.537	0.09	0.9	0.6	1.0	1.0
Biphenyl	9.034	9.021	0.01	8.8	6.4	9.2	6.5
Divinyl terephthalate	10.540	10.540	0.00	5.5	0.9	6.4	1.8
Benzophenone	11.113	11.113	0.00	13.7	8.3	17.9	8.5
<u>PP</u>							
PP - Trimer	3.030	1.770	1.26	1.7	0.4	1.1	1.2
PP - Pentamers							
Peak 1	8.279	8.261	0.02	1.2	0.3	0.8	0.6
Peak 2	8.352	8.339	0.01	2.3	1.1	1.4	1.2
Peak 3	8.434	8.420	0.01	1.0	0.3	0.7	0.5
<u>PS</u>							
Styrene	3.731	2.929	0.80	0.3	0.1	0.2	0.2
PS - Dimer	11.786	11.786	0.00	9.0	1.9	11.4	3.3
2,5-Diphenyl-1,5-hexadiene	13.010	13.010	0.00	10.4	2.7	18.8	4.2
PS - Trimer	16.344	16.344	0.00	69.0	7.3	123.5	10.0

Table S3. 5 - Linear regression parameters for calibration curves of PE, PET, PP, and PS using 4-fluorostyrene ($m/z=96$) as an internal standard, with y as the analyte-to-internal standard peak area ratio and x as the analyte-to-internal standard concentration ratio

PE	Solution - IS		Solids - IS	
	Intercept	Slope	Intercept	Slope
1-Decene (97)	-4.1E-04	5.3E-03	4.1E-04	6.7E-03
1-Undecene (97)	-7.8E-04	4.0E-03	-3.3E-05	4.4E-03
1-Undecene (154)	-7.5E-05	4.7E-04	2.4E-05	4.9E-04
1-Dodecene (97)	-2.9E-04	4.8E-03	9.5E-04	4.6E-03
1-Tridecene (97)	-5.6E-04	4.4E-03	3.0E-04	5.3E-03
1-Tetradecene (97)	6.7E-05	5.5E-03	2.3E-03	6.9E-03
PET				
Biphenyl (182)	6.5E-03	3.4E-02	1.2E-02	9.9E-02
Benzophenone (182)	-9.5E-05	8.2E-03	-9.0E-04	2.7E-02
PP				
Trimer (126)	-3.7E-03	2.0E-02	-1.6E-03	2.8E-02
Pentamer 1 (111)	-1.3E-03	7.6E-03	-8.1E-04	1.0E-02
Pentamer SUM (111)	-2.4E-03	1.4E-02	-1.7E-03	1.9E-02
PS				
Styrene (78)	2.1E-01	8.6E-01	-8.5E-03	7.6E-01
Styrene SUM (77+78)	3.2E-01	1.3E+00	1.6E-02	1.1E+00
2,5-Diphenyl-1,5-hexadiene (234)	-1.6E-03	8.0E-03	-1.2E-03	4.0E-03

Table S3. 6 - Quantifier-to-Qualifier ratios for PE, PET, PP, and PS in environmental samples representing the ratio of the quantifier peak to the qualifier peak for each polymer

Sample	Ratio			
	PE	PET	PP	PS
Alba	3,35	0,44	2,05	181,38
Ponte	3,50	0,45	2,36	550,96
Sample A	3,53	3,91	0,42	274,44
Sample B	3,00	1,84	0,93	247,04
Sample C	3,19	1,10	0,00	530,24
MilliQ	5,22	0,01	2,66	0,58
Tap Water	3,45	0,02	2,31	0,99

Table S3. 7 - Overview of scan windows used, including target microplastic polymer, pyrolytic target, scan start time (min), and ion masses with high resolution, six unique ions per window (30 ms dwell time), and adjustments excluding vinyl benzoate while including PP pentamer peaks.

Target MP	Name	Time (min)	m/z
PP	2,4-Dimethyl-1-heptene	0	126
PS	Styrene		104, 77, 78
IS-PS	Fluoro- Styrene		122, 96
PS	α -Methylstyrene	4.4	118, 103
PE - C10	1-Decene		140, 97
PE - C10	1,9-Decadiene		110, 81
PET	Acetophenone	5.4	120, 105
PE - C11	1-Undecene		154, 97
PE - C11	1,10-Undecadiene		124, 81
PET	Vinyl Benzoate	6.5	105, 148
PE - C12	1-Dodecene	7.5	168, 97
PE - C12	1,11-Dodecadiene		81
PE - C13	1-Tridecene		182, 97
PE - C13	1,12-Tridecadiene		81
PP	Pentamer		210, 111
PET	Biphenyl	8.7	154, 76
PE - C14	1-Tetradecene C14		196, 97
PE - C14	1,13-Tetradecadiene		194, 81
PS	Bibenzyl	9.6	182, 91
PET	Divinyl terephthalate		175, 154, 105
PET	Benzophenone		182, 105
PS	Dimer	11.3	208, 130, 91
IS-PS	Dimer - fluoro		229, 133
PS	2,5-Diphenyl-1,5-hexadiene		234, 130
PS	Trimer	13.4	312, 207, 91
PET	2-(benzoyloxy)ethyl vinyl terephthalate		297, 149, 105

Table S3. 8 - Example of a calibration curve batch design with samples from 200 ng to 10 µg, including exact MP polymer weights per vial, cleaning steps, blanks, internal standard (fluoro-PS), and calibration samples with notes on MP solutions and volumes used.

			Solvent	Octanol:Toluene (20:80)	TFA:Chloroform (30:70)	Octanol:Toluene (20:80)	TFA:Chloroform (30:70)	THF					
			Stocks 1	0.90 $\mu\text{g}/\mu\text{L}$		0.86 $\mu\text{g}/\mu\text{L}$		0.90 $\mu\text{g}/\mu\text{L}$		0.87 $\mu\text{g}/\mu\text{L}$		0.32 $\mu\text{g}/\mu\text{L}$	
			Stocks 2	0.30 $\mu\text{g}/\mu\text{L}$		0.31 $\mu\text{g}/\mu\text{L}$		0.34 $\mu\text{g}/\mu\text{L}$		0.28 $\mu\text{g}/\mu\text{L}$			
			Dilutions 3	34.12 $\text{ng}/\mu\text{L}$		35.10 $\text{ng}/\mu\text{L}$		39.02 $\text{ng}/\mu\text{L}$		32.49 $\text{ng}/\mu\text{L}$			
Place #	Run #	Name	PE		PET		PP		PS		Fluoro-PS		Note
			μL		μL		μL		μL		μL	μg	
1	1	Bake 1											
1	2	Blank 1											
2	3	Syringe cleaning	10										
1	4	Blank 2											
3	5	SIM IS-PS Only									3	0.95	
1	6	Blank 3											
4	7	SIM 200ng 1	6	205 ng	6	211 ng	6	234 ng	6	195 ng	3	0.95	Dilutions 3
1	8	Blank 4											
5	9	SIM 400ng 1	12	409 ng	12	421 ng	11	429 ng	12	390 ng	3	0.95	Dilutions 3
1	10	Blank 5											
6	11	SIM 1ug 1	4	1.2 μg	4	1.2 μg	4	1.4 μg	4	1.1 μg	3	0.95	Stocks 2
1	12	Blank 6											
7	13	SIM 2ug 1	7	2.1 μg	7	2.2 μg	7	2.4 μg	7	2.0 μg	3	0.95	Stocks 2
1	14	Blank 7											
8	15	SIM 6ug 1	7	6.3 μg	7	6.0 μg	7	6.3 μg	7	6.1 μg	3	0.95	Stocks 1
1	16	Blank 8											
9	17	SIM 10ug 1	12	10.8 μg	12	10.3 μg	12	10.8 μg	12	10.4 μg	3	0.95	Stocks 1
1	18	Blank 9											
1	19	Bake 2											
1	20	Blank 10											

Table S3. 9 - PE, PET, PP, and PS concentrations in water samples A, B, and C from Utterslev Mose, MilliQ, and tap water blanks, quantified using solubilized MP calibration with fluorostyrene ($m/z=96$) as an internal standard, showing mean concentration and 95% confidence interval in $\mu\text{g/L}$ with superscripts indicating values below DL, LOQ, or above the calibration range.

Pyrolyzate (m/z)	Sample A [$\mu\text{g/L}$]	Sample B [$\mu\text{g/L}$]	Sample C [$\mu\text{g/L}$]	MilliQ [$\mu\text{g/L}$]	Tap water [$\mu\text{g/L}$]
	Mean $\pm$ CI(95)	Mean $\pm$ CI(95)	Mean $\pm$ CI(95)	Mean $\pm$ CI(95)	Mean $\pm$ CI(95)
PE					
1-Decene (97)	81.7 $\pm$ 32.1	158.4 $\pm$ 68.6	268.1 $\pm$ 143.9	10.5 $\pm$ 2.2	9.3 $\pm$ 3.7
1-Undecene (97)	111.4 $\pm$ 79.8	322.3 $\pm$ 108.9	2816.7 ^c $\pm$ 1297.6	13.3 $\pm$ 1.0	13.1 $\pm$ 4.3
1-Undecene (154)	101.8 $\pm$ 41.9	237.6 $\pm$ 108.2	398.4 $\pm$ 182.3	12.5 ^a $\pm$ 1.6	14.6 ^a $\pm$ 4.7
1-Dodecene (97)	90.8 $\pm$ 27.6	187.4 $\pm$ 159.0	630.4 ^c $\pm$ 81.2	11.2 ^b $\pm$ 2.3	11.2 ^b $\pm$ 4.7
1-Tridecene (97)	134.3 $\pm$ 29.2	363.1 $\pm$ 126.4	291.6 $\pm$ 111.4	12.0 ^b $\pm$ 1.6	11.8 ^b $\pm$ 3.7
1-Tetradecene (97)	190.0 $\pm$ 47.3	195.1 $\pm$ 41.9	363.2 $\pm$ 155.5	18.5 ^a $\pm$ 4.3	16.3 ^a $\pm$ 6.7
PET					
Biphenyl (182)	-1.9 ^a $\pm$ 1.0	9.6 ^a $\pm$ 18.7	33.2 ^b $\pm$ 62.5	5.6 ^a $\pm$ 8.8	-2.8 ^a $\pm$ 0.7
Benzophenone (182)	91.6 $\pm$ 18.6	133.5 $\pm$ 27.1	195.0 $\pm$ 94.1	1.2 ^a $\pm$ 0.9	0.8 ^a $\pm$ 0.2
PP					
PP - Trimer (126)	8.3 $\pm$ 0.7	8.2 $\pm$ 0.4	10.6 $\pm$ 1.6	8.6331 $\pm$ 1.1	7.8 $\pm$ 0.4
PP - Pentamer peak 1 (111)	14.2 $\pm$ 5.9	9.4 $\pm$ 1.5	1219.4 ^c $\pm$ 397.8	8.4631 $\pm$ 1.0	7.8 $\pm$ 0.5
PP - Pentamer SUM (111)	13.0 $\pm$ 3.3	11.1 $\pm$ 1.2	1348.8 ^c $\pm$ 440.0	8.4237 $\pm$ 0.9	7.7 $\pm$ 0.5
PS					
Styrene (78)	-7.3 ^a $\pm$ 1.3	-4.2 ^a $\pm$ 2.5	-2.1 ^a $\pm$ 5.0	-9.4 ^a $\pm$ 0.0	-9.4 ^a $\pm$ 0.0
Styrene SUM (77+78)	-7.8 ^a $\pm$ 1.4	-4.5 ^a $\pm$ 2.7	-2.4 ^a $\pm$ 5.3	-9.9 ^a $\pm$ 0.0	-9.9 ^a $\pm$ 0.0
2,5-Diphenyl-1,5-hexadiene (234)	8.8 ^b $\pm$ 0.3	10.6 ^b $\pm$ 1.5	12.2 ^b $\pm$ 1.5	8.2 ^b $\pm$ 0.9	7.8 $\pm$ 0.1

Table S3. 10 - Ratios of the peak area of the qualifier compound to the peak area of the quantifier compound for the quantification of microplastics in water samples (*values outside of the confidence interval).

Ratio qualifier					
Pyrolyzate (m/z)	Sample A	Sample B	Sample C	MilliQ	Tap water
PE					
1-Decene (97)	1.0	1.0	1.0	1.0	1.0
1-Undecene (154)	0.8	0.7	0.7	1.1	0.7
1-Tridecene (97)	0.6	0.4	0.9	1.1	0.9
1-Tetradecene (97)	0.5	0.9	0.8	0.5	0.4
PET					
Benzophenone (182)	1.0	8.1	4.8	0.1	0.1
Biphenyl (182)	16.8	1.0	1.0	1.0	1.0
PP					
2,4-dimethyl -1-hept-1-ene (126)	1.0	1.0	1.0	1.0	1.0
2,4,6,8-tetramethyl-1-undecene (111)	0.2	0.2	0.0	1.0	0.8
PS					
Styrene (78)	1.0	1.0	1.0	1.0	1.0
2,5-Diphenyl-1,5-hexadiene (234)	1.6	1.4	3.1	0.0	0.0

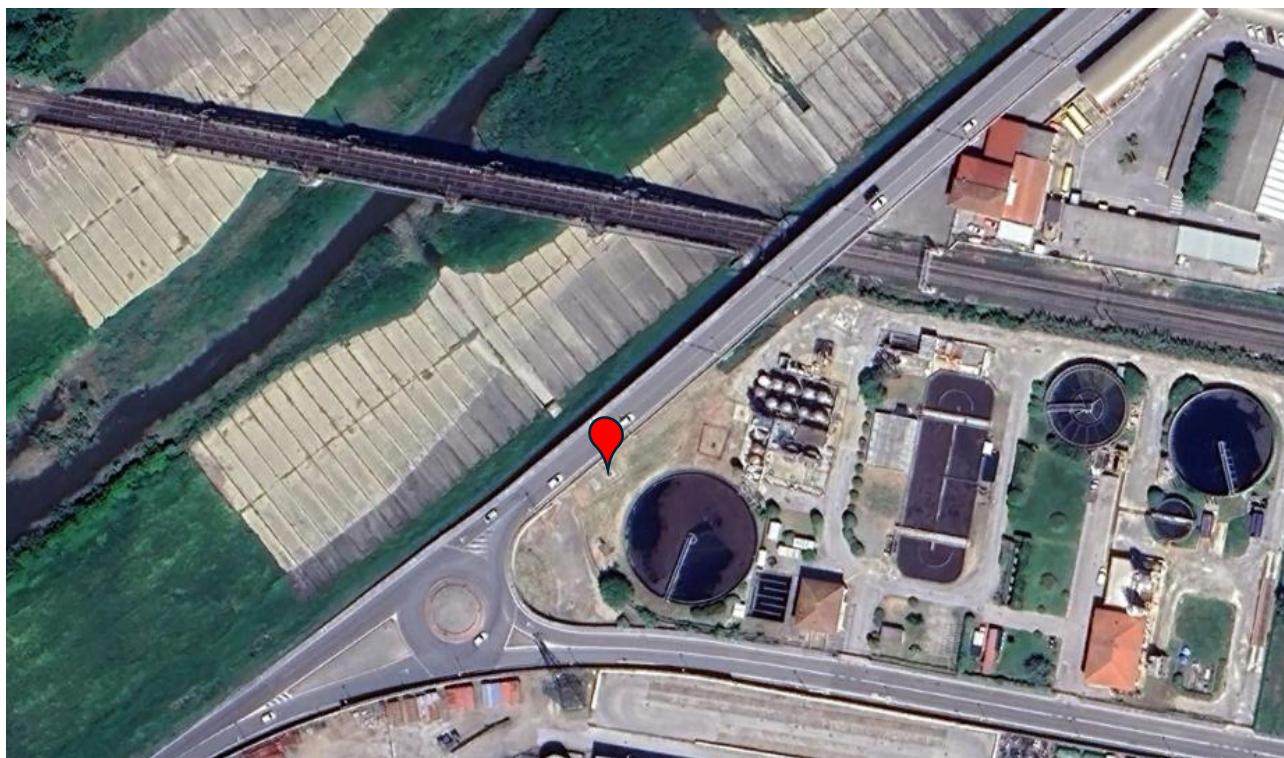


Figure S3. 1 - Location of WWTP water sample ($43^{\circ}39'48.8''\text{N}$ $10^{\circ}36'53.4''\text{E}$) at Pontedera (PI) Italy



Figure S3. 2 - Location of water sample A ($55^{\circ}42'56.9''\text{N}$ $12^{\circ}30'09.3''\text{E}$) and water samples B and C ($55^{\circ}43'07.8''\text{N}$ $12^{\circ}30'46.8''\text{E}$) at Utterslev Mose.

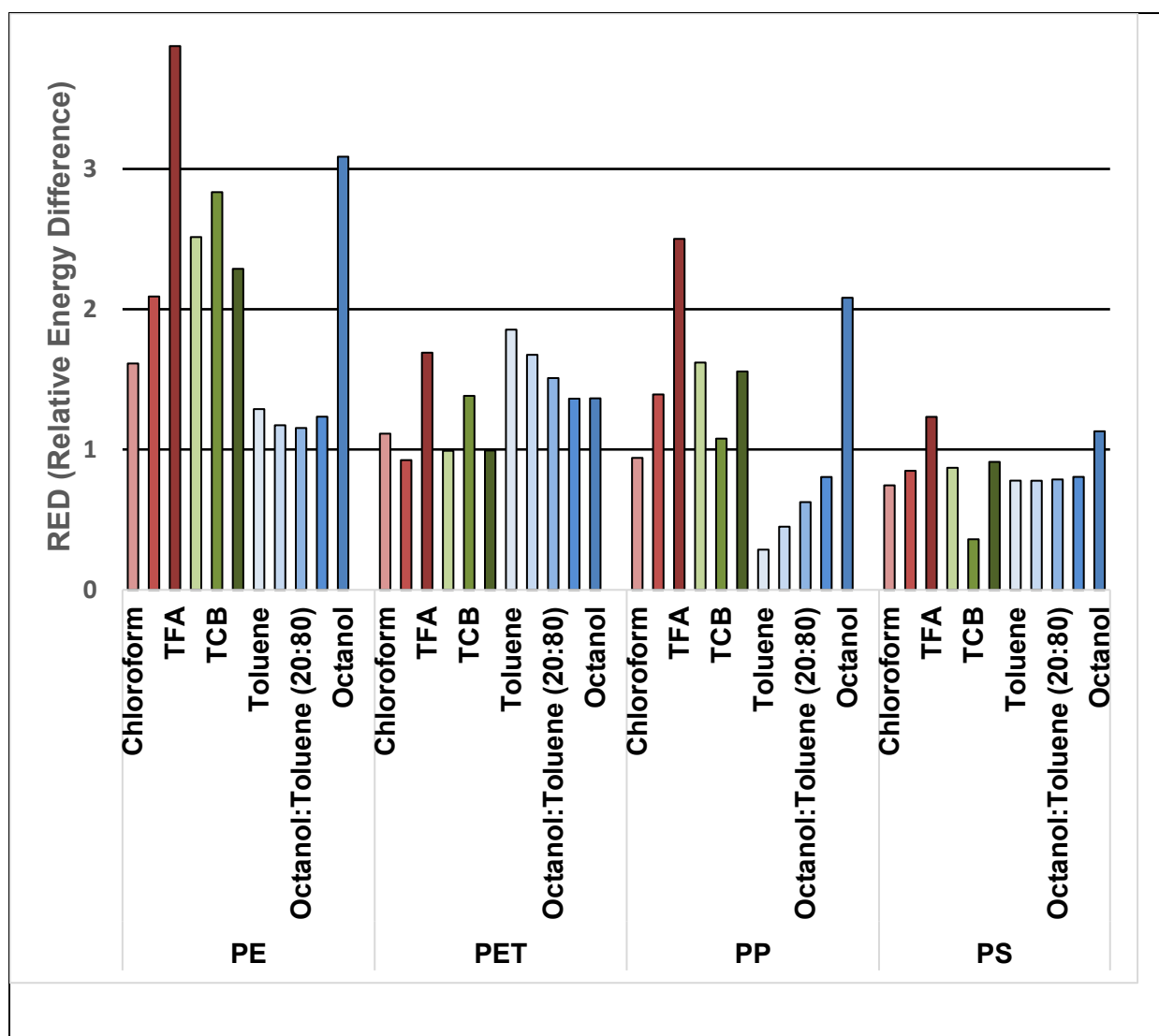


Figure S3. 3 - RED values (y-axis) for seven solvents (chloroform, TFA, DCM, TCB, THF, toluene, and octanol) and four solvent mixtures (one chloroform and three octanol-based) evaluated for the four polymers: PE, PET, PP, and PS (x-axis).

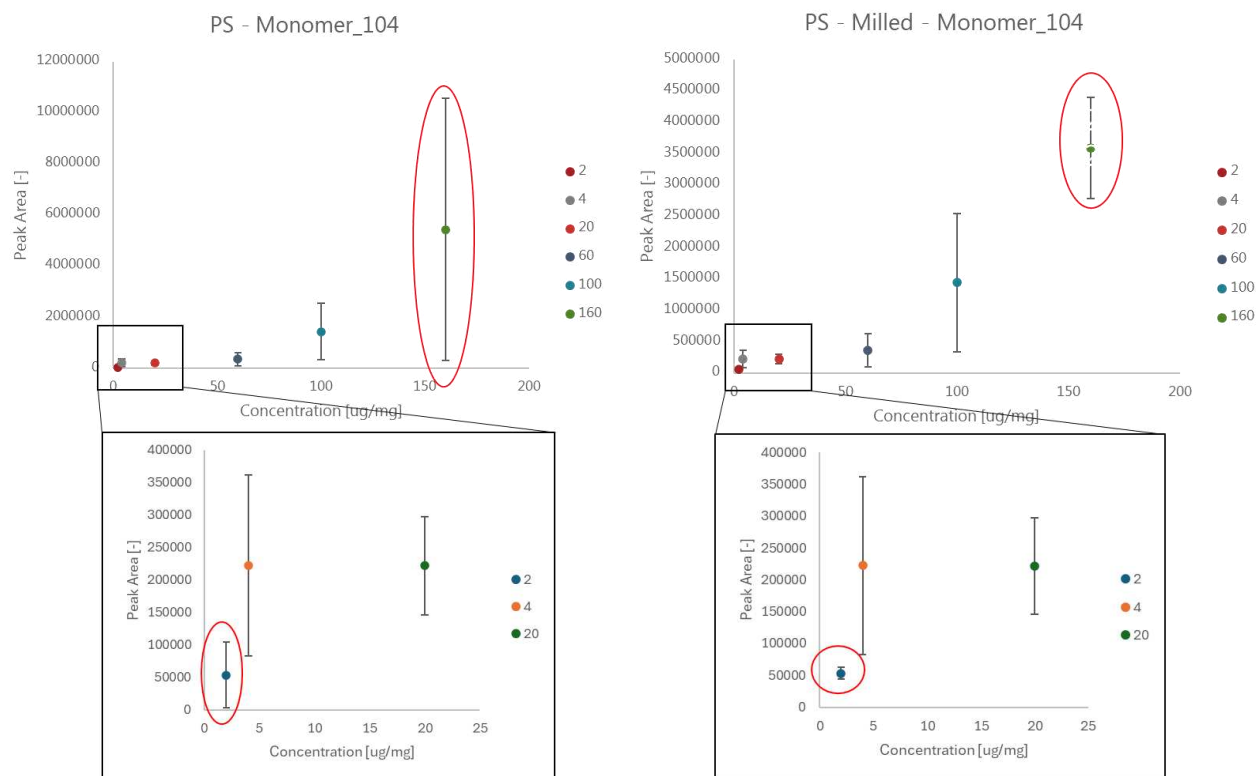


Figure S3. 4 - Comparison of the quantification of polymer PS present in the samples with and without homogenization phase

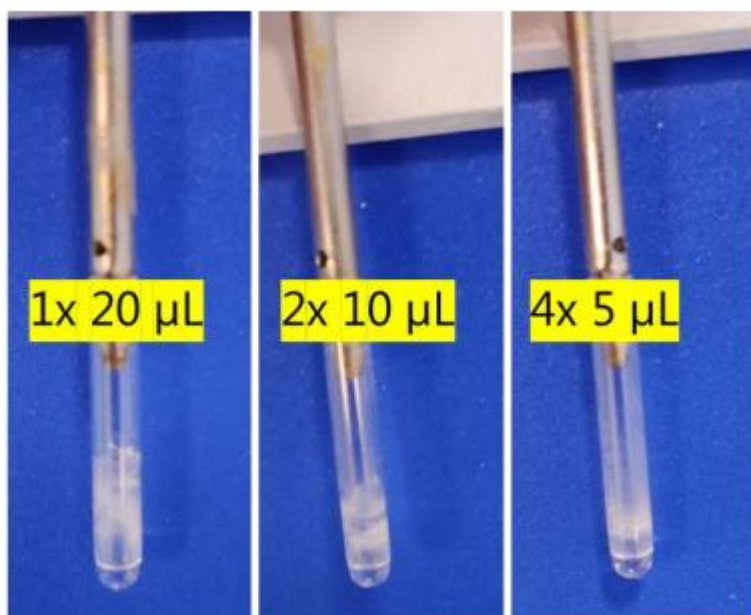
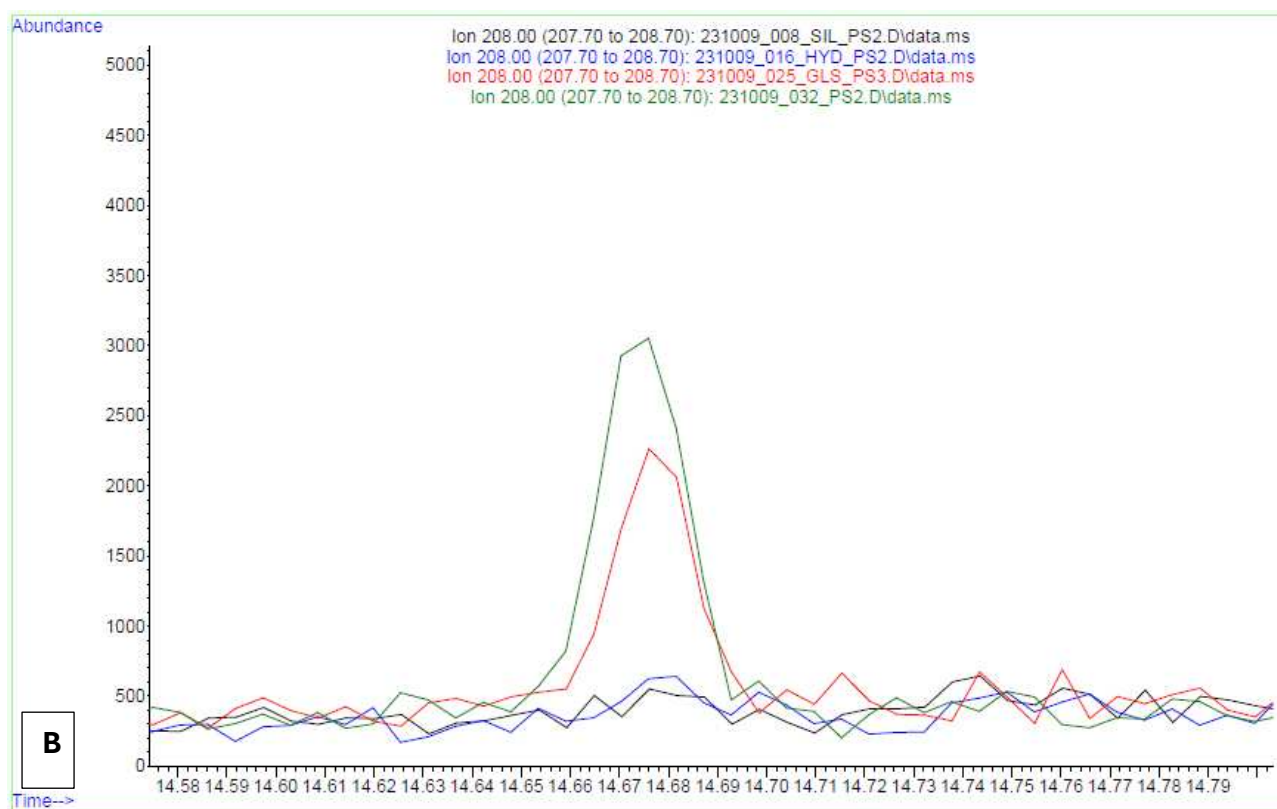
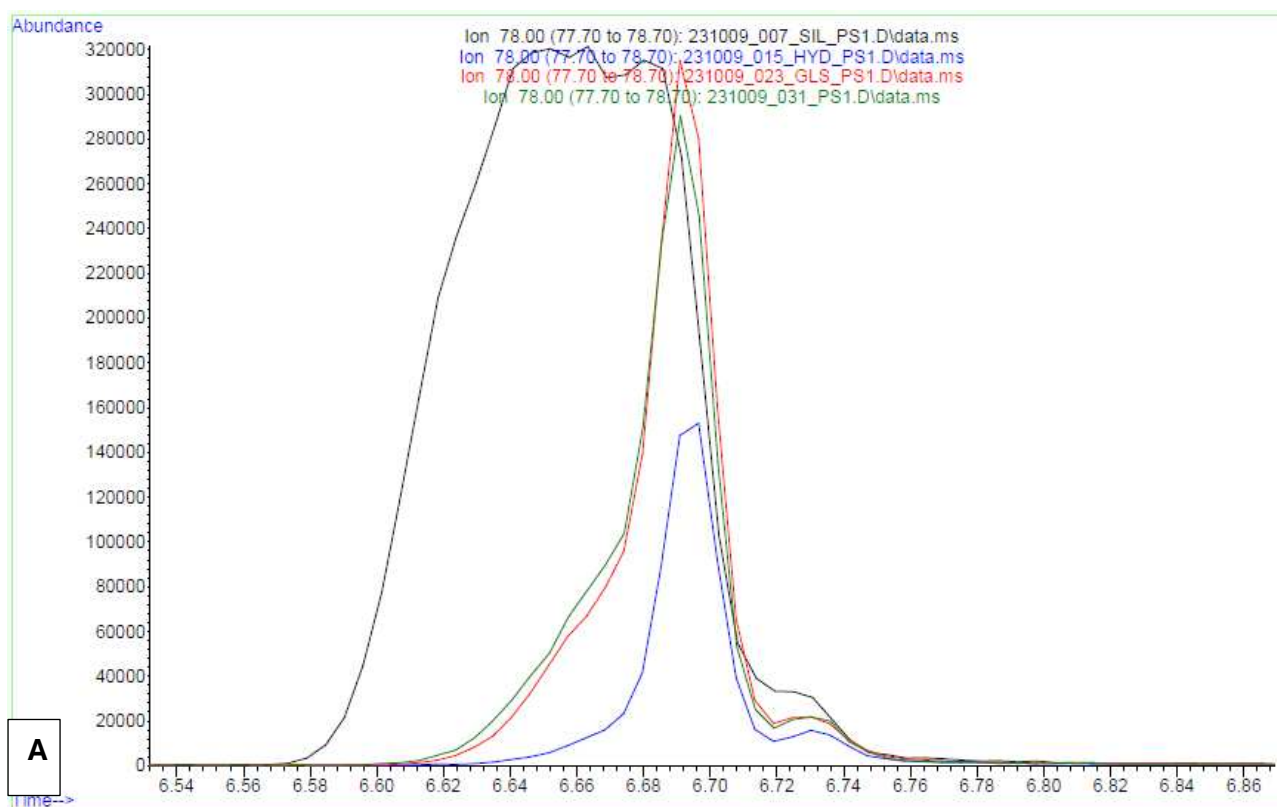


Figure S3. 5 - Images of dried pyro-vials loaded with 20 µL of PP solution in 1, 2, or 4 injections.



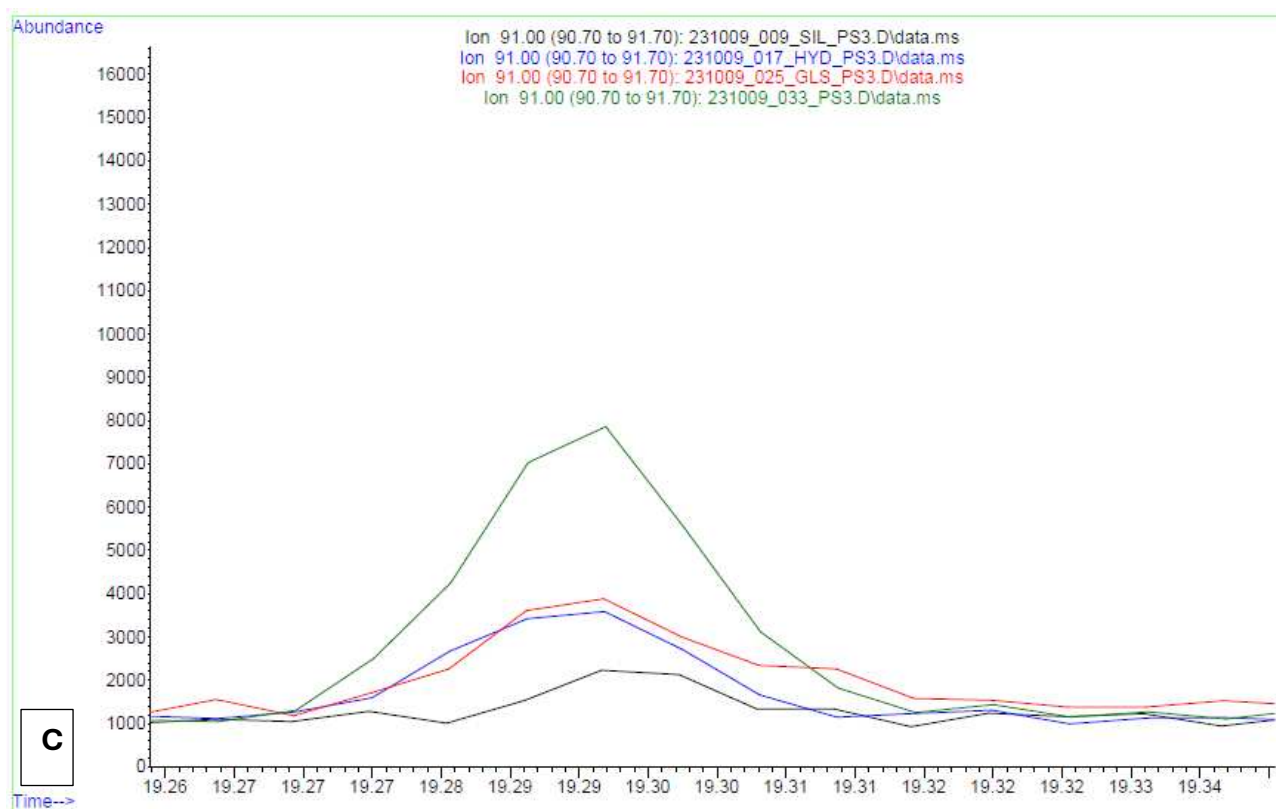


Figure S3. 6 - Overlaid chromatogram peak of monomer (m/z 78) (A), 3-butene-1,3-diyl dibenzene (m/z 208) (B) and 5-hexene-1,3,5-triyltribenzene (m/z 91) (C) for PS pyrolysis with different inorganic matrices: silica gel, Hydromatrix, glass beads, and no matrix

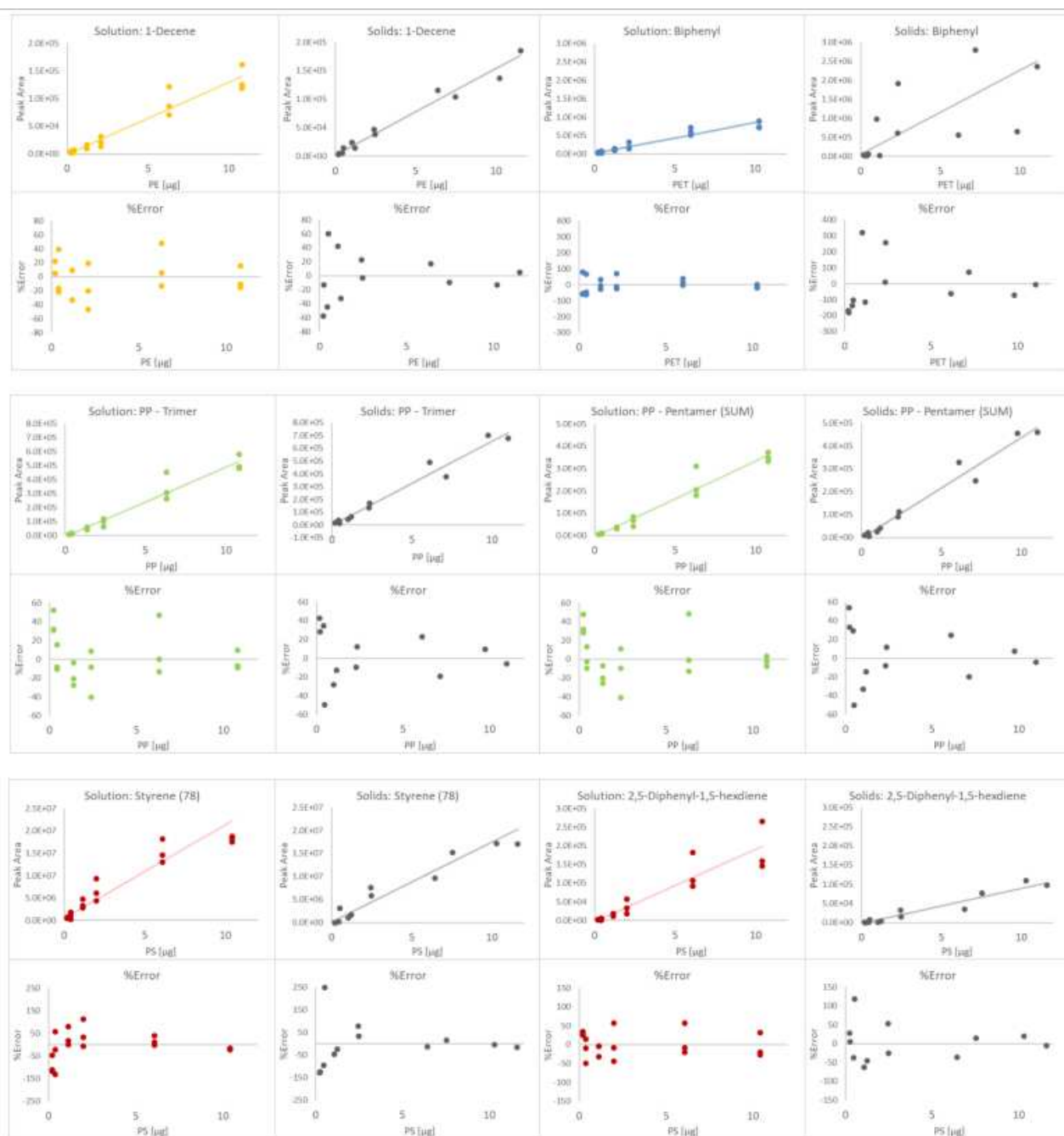


Figure S3. 7 - Linear calibration curves, data points, and % error plots for 1-decene, biphenyl, PP trimer, PP pentamer (sum of three peaks), styrene, and 2,5-diphenyl-1,5-hexadiene, comparing solubilized and solid MP calibrations.

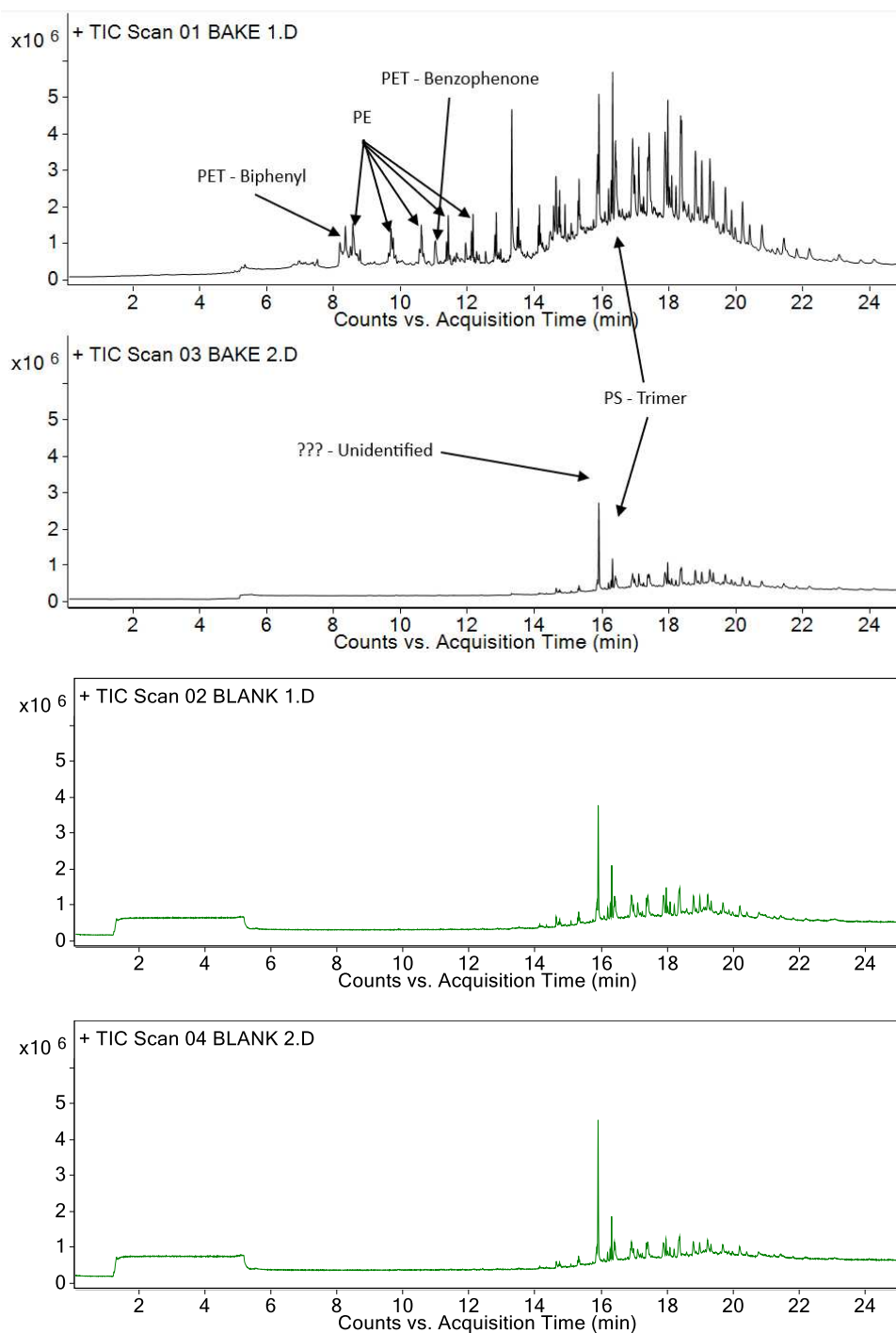


Figure S3. 8 - TICs from TDU cleaning process before sample analysis, with TDU heated to 350°C for 30 min followed by blank analysis, performed twice ("TIC Scan 01 BAKE 1" and "TIC Scan 02 BLANK") in MS scan mode, resulting in four pyrograms locked to the same y-axis scale to assess residual signals and cleaning efficiency.

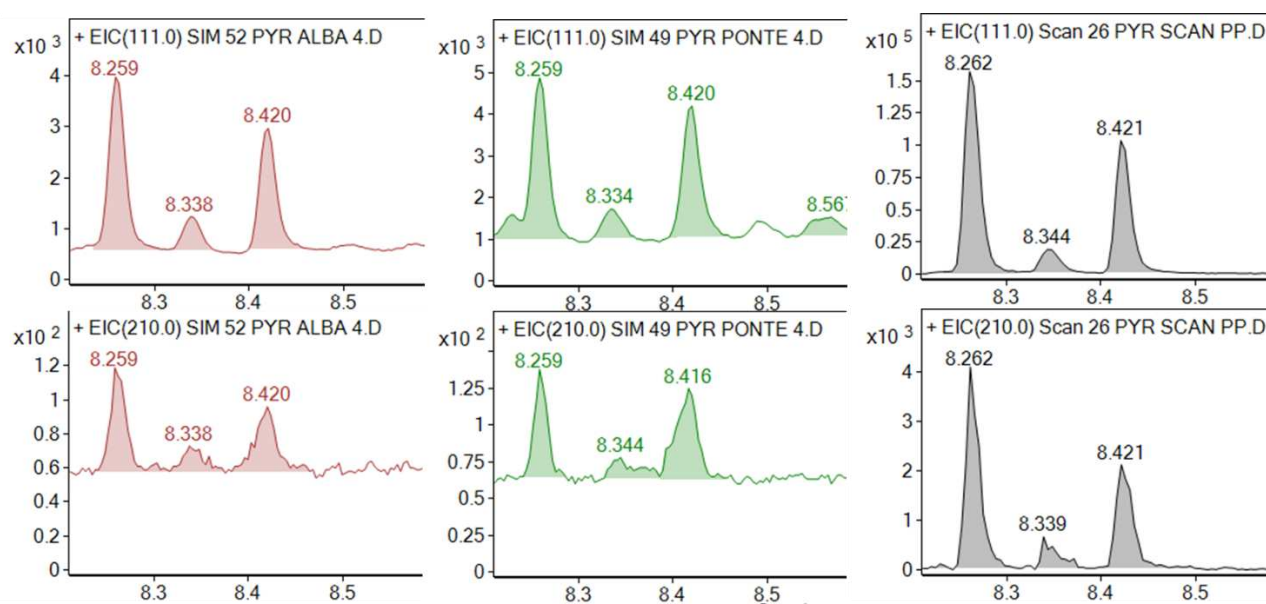


Figure S3. 9 - EICs of $m/z=111$ (quantifier ion for PP pentamer, top row) and $m/z=210$ (qualifier ion, bottom row) for Alba, Ponte, and pure PP samples, showing peak alignment and presence of three expected peaks for the PP pentamer.

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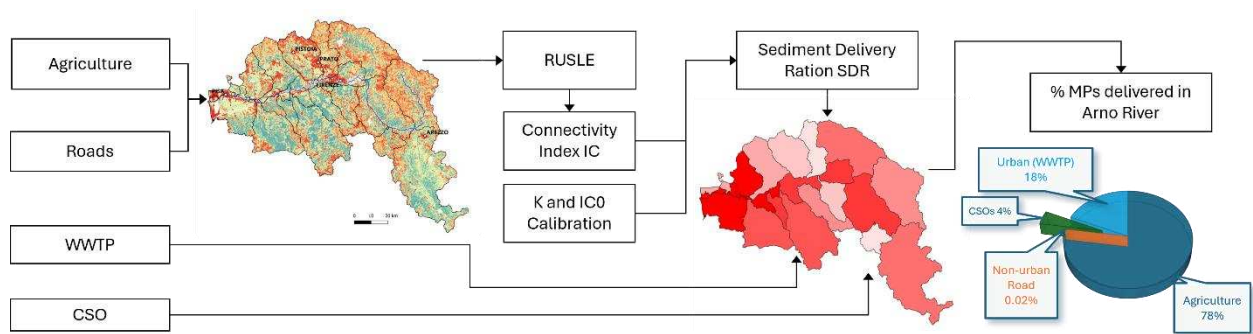
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4 Chapter 4: Microplastic emissions in river catchments using a distributed erosion-connectivity model

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Abstract

Microplastic (MP) pollution from terrestrial sources is increasingly recognized as a major environmental issue, yet integrated spatial models quantifying land-to-river fluxes remain limited. Here, we present a high-resolution, process-based framework to estimate MP emissions from multiple sources across the Arno River catchment (Italy), combining fluvial erosion dynamics, sediment connectivity, and hydrological routing.

The model is designed for estimating the yearly mass of MP delivered in the river network from agriculture, non-urban roads, wastewater treatment plants (WWTPs), and combined sewer overflows (CSOs). It is structured in three main sections: MP production, mobilization of MP from source locations, and MP delivery to rivers; to do that, it integrates emission factors, varying with the characteristics of the source, with spatial data on land use, topography, and drainage. Moreover, the MP mobilization through soil erosion is estimated by coupling the Revised Universal Soil Loss Equation with a sediment delivery ratio derived from the Index of Connectivity. Finally, it reveals the land-to-river flow pathways, highlighting the presence of MPs accumulation locations all along the selected study area.

The model is firstly applied to the Arno River catchment (Italy), results reveal that the main contributors to MP loads delivered into the Arno River are agricultural land (1253 t/year), followed by WWTP effluents (187 t/year), CSOs (43 t/year), and non-urban roads (0.2 t/year). Spatial patterns identify sub-catchments with high erosion potential and strong connectivity, such as Era, Elsa, and Chiana, as critical zones for downstream delivery.

This approach offers a scalable and data-efficient tool to trace MPs from land to river, supporting source attribution, scenario testing, and catchment-scale management. By resolving multiple emission pathways in a unified framework, it advances our understanding of diffuse and point-source microplastic transport across heterogeneous landscapes.

Keywords: microplastics, IC-SDR model, Land-to-river transport, Combined sewer overflows (CSOs); Catchment modeling

Highlights:

- A spatial model quantifies MP emissions across 16 Arno River sub-catchments.
- Agricultural MP sources exceed 3100 t/year in the most impacted catchment.
- CSOs cause sharp peaks during floods while WWTPs show steady emissions.
- MP delivery mainly depends on source location, connectivity index and infrastructure.

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4.1 Introduction

Plastic particles are increasingly recognized as pervasive pollutants in both aquatic and terrestrial environments. While much attention has focused on marine systems, growing evidence highlights the key role of river corridors as conduits for these elements originating from land-based sources. Rivers act as pathways from sources to oceans and open waters particularly for microplastics (MPs) (Van Emmerick et al., 2020), i.e., small pieces of plastics, usually smaller than 5 mm (Frias and Nash, 2019). Yet a comprehensive understanding of the terrestrial pathways that transport MPs from land-based sources to rivers remains limited.

Recent studies have identified multiple land-based sources for MPs, including agricultural activities (Cusworth et al., 2024; Seo et al., 2025), roads (Monira et al., 2022; Vercauteren et al., 2023), wastewater treatment plants (WWTP) (Becucci et al., 2022; Magni et al., 2019; Okoffo et al., 2023; Pagliaccia et al., 2025), and combined sewer overflows (CSOs) (Di Nunno et al., 2021; Sarti et al., 2024). Although these sources have very different characteristics, they all share the potential for episodic or chronic input of MPs to surface waters, often exacerbated by rainfall and hydrological connectivity. For example, MPs in agricultural soils can persist for decades and be mobilized through erosion (Quilliam et al., 2023), while urban infrastructures such as CSOs and side weirs release pulses of particles during storm events (Hurley et al., 2018). Yet, a quantitative comparison of these sources at catchment scale remains rare, limiting our understanding of their cumulative impacts.

Many studies were conducted in last decades to quantify land-based sources of MPs. These efforts typically rely on production rates estimated from field observations or laboratory experiments (Periyasamy and Tehrani-Bagha, 2022; Soon et al., 2024), which are then applied to spatial proxies such as land cover, traffic intensity, or population density, to quantify the MPs entering the environments. While this approach provides valuable first-order estimates, it often lacks spatial resolution (e.g. $> 1\text{km}^2$ grid cell or aggregated by administrative units) and does not account for heterogeneity in land use practices or infrastructure. In addition, more than two source types are rarely considered in the same study, thus, comparison on their relative importance at a large scale, e.g. at a river catchment scale (Hurley et al., 2024; Long et al., 2021; Murphy et al., 2016). A further limitation in available literature is that MP production estimations are usually treated as static inputs, without integrating temporal variability or links to downstream transport processes. This underscores the need for spatially explicit frameworks capable not only of quantifying MPs production across multiple source types, but also of linking these inputs to environmental transport mechanisms.

Unice et al. (2019a) have recently attempted in integrating MPs in erosion models with the aim of applying these tools to a river catchment scale domain, however, these remain limited to coarse spatial scales or rely on global datasets not suited for process-based assessments at regional or catchment level. Additionally, while conceptual models outlining possible pathways of MP movement exist (Besseling et al., 2017; Horton et al., 2017; Mennekes and Nowack, 2023; Unice et al., 2019b), there is still a need for spatially explicit tools capable of estimating the

relative contributions of different land use categories and infrastructure types (e.g., agriculture, roads, impervious areas).

Recent studies have addressed the identification of agricultural sources of MP pollution at the landscape scale, with some studies focusing on modelling cumulative MP emissions into soils (e.g., Brandes et al., 2021, Rehm and Fiener, 2024). Brandes et al. (2021) developed a spatially explicit model for Germany that quantifies MP inputs from three main agricultural pathways: sewage sludge, compost, and plasticulture. Their study emphasizes the heterogeneity of MP emissions and highlights areas with potentially high contamination. While the approach represents an important step toward understanding terrestrial MP inputs, it does not account for subsequent mobilization processes such as runoff or erosion, nor does it model transport toward water bodies. This reinforces the need for integrated models capable of linking terrestrial emissions to fluvial transport. Rehm and Fiener, (2024) developed a spatially distributed model to estimate MP transport from arable soils to stream networks considering erosion. The approach combines long-term soil erosion simulations with historical MP input estimates from diffuse agricultural sources, including sewage sludge, compost, atmospheric deposition, and tire wear. While the study provides one of the first large-scale assessments of erosion-driven MP delivery, it focused exclusively on agricultural sources. Moreover, geomorphological factors such as lateral connectivity and floodplain retention are not explicitly considered, limiting the ability to capture the full complexity of MP routing in real catchments. These limitations underline the need for more comprehensive and integrative frameworks able to incorporate multiple source types and catchment-specific transport dynamics.

From the available literature, a key knowledge gap remains regarding the development of spatially explicit models capable of estimating MP emissions from multiple land-based sources through an integrated, high-resolution framework that captures both diffuse and point-source delivery to rivers. Moreover, most existing approaches do not integrate agricultural inputs, CSO-related discharges, and urban sources within a unified framework.

To address this gap, we developed a spatially explicit modelling framework that integrates MP emission factors with established soil erosion and connectivity models. The proposed model allows to estimate the yearly produced MP mass from land-based sources, and then assesses the yearly mass of MPs that is transported to rivers. This modelling framework advances previous efforts (e.g., Brandes et al., 2021; Rehm and Fiener, 2024) by linking land-based MP sources to fluvial delivery using spatially explicit erosion and hydrological routing, enabling catchment-scale source comparison and prioritization.

With the aim of testing the proposed model, the Arno River catchment (Italy) was selected as a case study due to the presence of important cities and industrial districts (particularly agricultural districts) in the area and the importance of the river in the region through which it flows.

With regards to the modelling framework, the model encompasses several specific aims: (i) to quantify the spatial distribution and magnitude of MPs produced from agriculture, non-urban

roads, WWTPs, and CSOs across the Arno River catchment (Italy); (ii) to simulate the mobilization and delivery of MPs from surface-bound sources through erosion and hydrological connectivity; and (iii) to assess how land use, topography, and connectivity influence transport efficiency and downstream accumulation. By integrating source-specific emission estimates with erosion and connectivity modeling, the framework supports the identification of key contributing areas and provides a flexible tool for scenario analysis and mitigation planning.

The manuscript is structured in six sections. The Introduction is followed by the presentation of the study area (section 2) that reports the main characteristics of the Arno River catchments, particularly in terms of land covering and topography. The model is later presented in detail in section 3. Then results are presented and discussed in sections 4 and 5, respectively. Lastly, conclusions and outcomes are reported in section 6.

4.2 Study area

The Arno River catchment, located in the central Italy (Figure 1), was selected as the study area due to its representativeness of Mediterranean features, characterized by marked seasonal rainfall and diverse land use. The catchment spanning approximately 9116 km² in total (8228 km² of hydrological catchment), includes heterogeneous landscape that embrace urban centers, agricultural areas, forested hills, and industrial zones.

Major cities such as Florence and Pisa contribute to the presence of significant anthropogenic pressures, including multiple WWTPs, dense road network, and combined sewer system (CSOs), whose role in pollutant release during rainfall events has been documented in other European cities (Jalbert et al., 2024; Montoya-Coronado et al., 2024; Quaranta et al., 2022; Rodriguez et al., 2023).

The Arno River catchment also presents extensive agricultural areas, such as Chiana, Era and Bientina, where the intense use of plastic in field is strongly contributing to MPs production. This combination of diverse and interconnected sources of MPs makes the catchment an ideal case study for developing and testing a spatially model that integrates source-specific MPs emissions and sediment-based transport mechanism.

Additionally, the catchment is well documented and monitored, with extensive hydrological and environmental datasets available, including long-term sediment yield measurements at hydrometric stations (e.g., Subbiano, Nave di Rosano, San Giovanni alla Vena), high-resolution land cover maps, and a 10 m-resolution digital elevation model (DEM). This wealth of geospatial and environmental information enables relatively robust implementation and validation of the proposed modeling approach, such as the calibration of sediment delivery parameters and the spatial allocation of emission sources based on land use, topography, and hydrological characteristics.

In the Arno catchment (Table 1) forests cover about 46 % of the area, primarily in the upper and hilly regions, agricultural lands constitute approximately 41 %, predominantly in the plains and valleys, while urban and built-up areas account for about 13 %. Soil types in the catchment are

equally varied, reflecting the complex geological history of the region. In the Florence area, for instance soils are primarily composed of Early Pleistocene palustrine and alluvial deposits, as well as Holocene fluvial and alluvial materials. The heterogeneous land use and soil characteristics affect both erosion rates and the transport of particulates (Billi and Rinaldi, 1997; Caporali et al., 2005; Coli et al., 2015; Diodato et al., 2021a, 2021b).

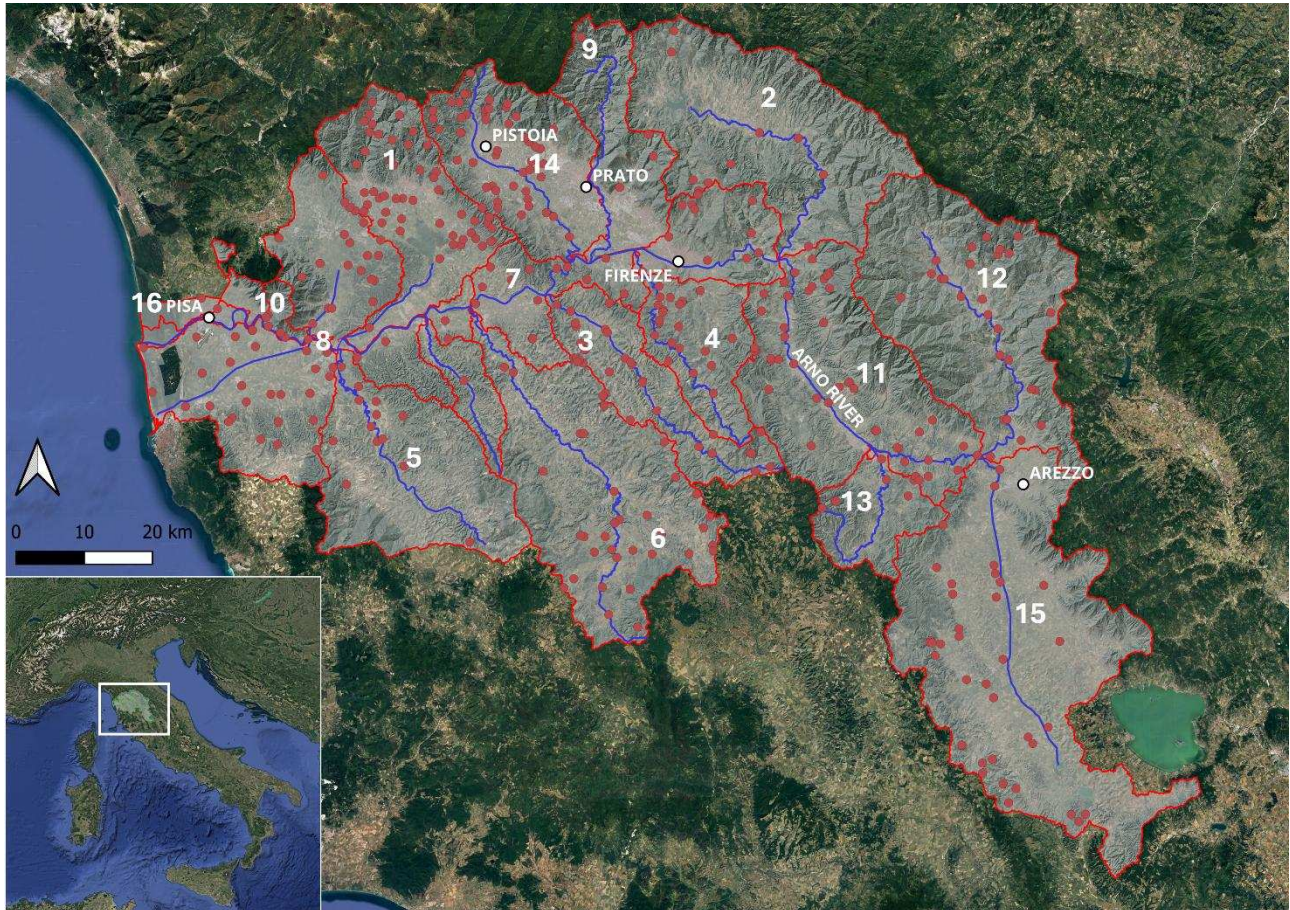


Figure 4. 1 - Overview of the Arno River catchment (Central Italy), including major tributaries (blue lines), WWTPs positions (red dots) and sub-catchment boundaries (red lines) considered in this study. Each sub-catchment is labelled with an identification number (ID), which corresponds to the values reported in Table1. The location of main cities (Pisa, Firenze, Prato, Pistoia, Arezzo) is also indicated for reference. The inset map shows the geographical location of the catchment within the Italian territory. Background image is the Google Satellite map.

Table 4. 1 - Percentage distribution of major land cover types in each sub-catchment of the Arno River catchment. Land cover information was obtained from regional datasets consistent with the Corine Land Cover standard. The table reports the relative proportions of urbanized areas, agricultural land, and forested or semi-natural cover (woods), based on the total area of each sub-catchment.

Sub-catchment ID	Sub-catchment - name	Urban Area [%]	Agriculture Area [%]	Woods Area [%]
1	Usciana	17	43	40
2	Sieve	7	25	68
3	Pesa	8	48	44
4	Greve	12	41	47
5	Era	6	56	38
6	Elsa	9	53	38
7	Valdarno Med.	20	47	33
8	Bientina	17	56	28
9	Bisenzio	19	18	63
10	Valdarno Inf.	17	43	39
11	Valdarno Sup.	10	35	55
12	Casentino	5	21	74
13	Ambra	5	28	67
14	Ombrone	23	40	38
15	Chiana	11	62	27
16	Outlet	19	40	41

4.3 Methods

4.3.1 Model structure overview

Figure 2 shows the conceptual structure of the proposed modelling framework. The model is structured into three main sections: Production, Mobilization, and Delivery; representing the sources of microplastics, their transport mechanisms, and their eventual input into the river network. The model combines both diffuse and localized sources, each associated with different pathways and modeling approaches.

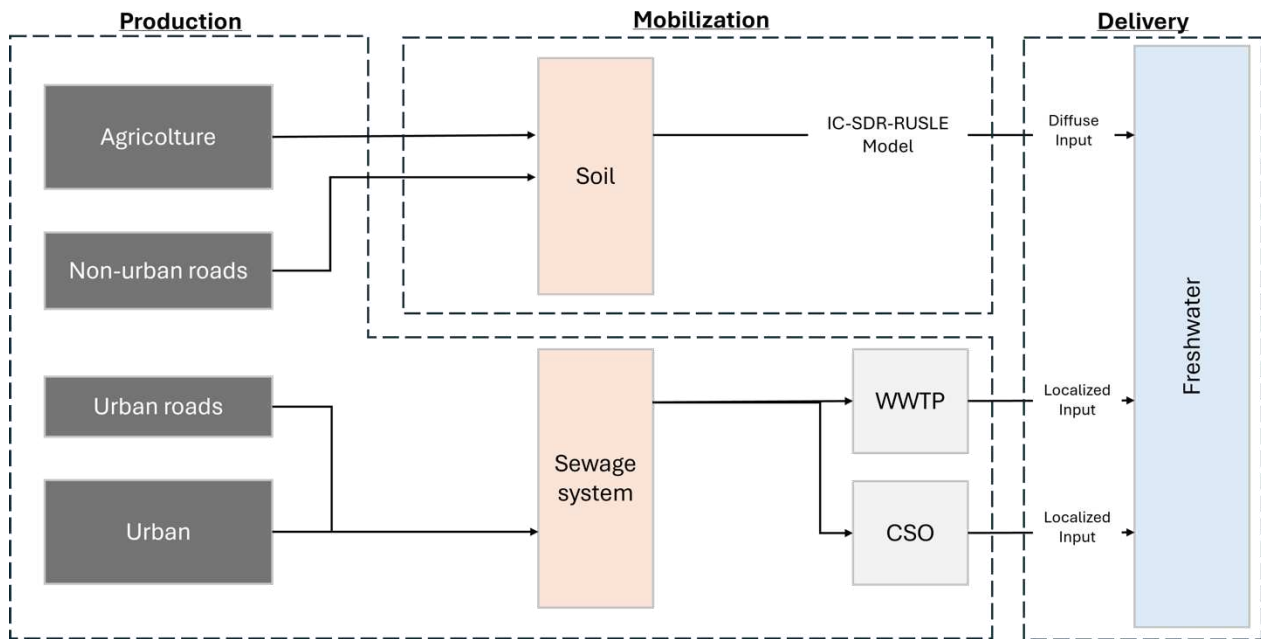


Figure 4. 2 - Conceptual structure of MP input pathways and modeling structure. Agriculture and non-urban road MPs are routed through the soil compartment and mobilized via erosion-based modeling (IC-SDR-RUSLE), producing a diffuse input. MPs from urban sources enter the sewage network and are delivered to surface waters through direct localized inputs from WWTPs and CSOs. The diagram highlights the separation between landscape-mediated and infrastructure-based contributions.

MPs generated from agricultural activities and non-urban roads (defined here as road segments not connected to sewer systems, where runoff flows directly into adjacent soil, ditches, or drainage channels, potentially reaching the river network) are assumed to enter the environment without connection to any sewage infrastructure. These sources are considered diffuse and are routed through the soil compartment, where mobilization occurs via soil erosion. Their delivery to surface waters is estimated through the Connectivity Index (IC) and Sediment Delivery Ratio (SDR) model (Crema and Cavalli, 2018; Vigiak et al., 2012), coupled with RUSLE (see Supplementary Material - S2 for details on the IC-SDR approach and its implementation in the transport module), which incorporates slope, land cover, and hydrological connectivity. The resulting contribution is spatially distributed and represents the progressive accumulation of MPs mobilized from the landscape.

On the contrary, MPs produced from urban sources (i.e., cities and urban roads) follow a different pathway through the sewer network. They are considered as point sources along the

river network, placed at WWTP outflows. From sewage systems the model also considers point sources for CSOs, these sources are estimated considering the urban imperviousness and placed to the same points as WWTP outflows. This assumption reflects the common hydraulic configuration of combined sewer systems, for which CSO structures are typically located nearby WWTPs in the study area, making these locations a reasonable proxy for riverine input in the absence of detailed spatial data. The framework integrates these two parallel routes, diffuse erosion-based inputs and localized infrastructural discharges, into a single spatially explicit simulation. This structure enables the comparison of both emission intensity and delivery efficiency across source types and locations within the catchment.

4.3.2 Estimation of microplastic production from terrestrial sources

The modeling framework developed in this study aims to estimate MP emissions from multiple terrestrial sources and assess their potential transfer to the river network through surface processes. Four major source categories were considered: (i) agriculture, (ii) roads, (iii) WWTPs, and (iv) CSOs. These sources represent both diffuse (agriculture, roads) and point (WWTPs and CSOs) inputs, and were treated accordingly within the model.

The annual production of MPs in the Arno River catchment was estimated using a spatially distributed approach over a 1x1 km grid. For each cell, the total MPs produced was computed based on the type of land use and the presence of specific sources. Diffuse sources (agriculture, roads) were assigned production values directly at the pixel scale, while point-source emissions from WWTPs and CSOs was quantified separately and later introduced at specific discharge locations along the river network.

Thus, the MP production map ($massMPs_{ij}$) for each cell (i,j) is defined as:

$$massMPs_{i,j} = \begin{cases} massMPs_{AGR,i,j} + massMPs_{URB,i,j} + massMPs_{CSO,i,j} & \text{when } massMPs_{URB,i,j} \neq 0 \\ massMPs_{AGR,i,j} + massMPs_{ROAD,i,j} & \text{when } massMPs_{URB,i,j} = 0 \end{cases} \quad (1)$$

where:

$massMPs_{i,j}$ is the total mass of microplastics (in t/year) produced in the cell located at row i and column j of the raster grid;

$massMPs_{AGR,i,j}$ is the mass of MPs generated from agricultural areas in cell (i,j), estimated based on land use and known emission factors (t/year);

$massMPs_{URB,i,j}$ is the mass of MPs generated from urban areas in cell (i,j), estimated by redistributing the total inflow mass of MPs to each WWTP across its connected sewer area, proportionally to the urban surface within the cell (in t/year);

$massMPs_{CSO,i,j}$ is the mass of MPs generated from impervious areas in cell (i,j), estimated by scaling total CSO emissions from a conceptual urban drainage model (Quaranta et al., 2022) according to local impervious surface area;

$massMPs_{ROAD,i,j}$ is the mass of MPs originating from road traffic, primarily tire wear and brake dust, in cell (i,j), assigned based on road presence, type, and traffic intensity.

This distinction reflects the assumption that in urbanized areas served by a sewer network, MPs produced from road surfaces (e.g., tire wear, brake dust) are fully captured by stormwater infrastructure. Conversely, in non-urban settings lacking drainage systems, runoff from roads is assumed to flow directly into adjacent soils, entering the surface environment as a diffuse input.

This equation is applied only to agriculture and non-urban roads, which are treated as diffuse sources distributed across the landscape. Their MPs accumulate on the soil and are mobilized by soil erosion.

4.3.2.1 Agricultural sources (MP_{AGR})

MPs from agriculture were estimated by associating production rates (in kg/ha/year), based on values from FAO (2019), to selected Corine Land Cover classes. The MPs mass produced in each cell was calculated as:

$$massMPs_{AGR,i,j} = \sum (a_{AGR} \cdot r_{AGR})_{i,j} \quad (2)$$

where:

- a_{AGR} is the area (in m^2) of crop type i within the cell;
- r_{AGR} is the MPs production rate.

Further details on the land cover classes, data sources, and calculation method are provided in Supplementary Material – S4.

4.3.2.2 Road traffic sources (MP_{ROAD})

MPs from tire and road wear were estimated using road type, length, and vehicle classes. Annual traveled kilometers for each cell were scaled from COPERT 2019 (European Environment Agency (EEA), 2019) :

$$massMPs_{ROAD,i,j} = \sum_k (m_{ROAD} \cdot nvC \cdot r_{ROAD})_{i,j} \quad (3)$$

Where:

- $k = (Bus, Moped, Motorcycle, Passenger cars, Truck and Lorry, Van);$
- m_{ROAD} is the annual traveled kilometers;
- nvC is the number of the vehicles passing through that cell;
- r_{ROAD} is the MPs production rate.

Further details on the estimation of MPs from road traffic are provided in Supplementary material – S5.

4.3.2.3 Urban sources (MP_{URBAN})

Urban MP production was estimated indirectly by working backwards from measured MP effluent concentrations at wastewater treatment plants (WWTPs). Starting from measured MP outflow values (Becucci et al., 2022), the total inflow mass ($massMPs_k^{inflow}$) for each WWTP was calculated using literature-based MP removal efficiencies (Sun et al., 2019):

$$massMPs_k^{inflow} = \frac{massMPs_k^{outflow}}{rp_k}$$

This inflow mass ($massMPs_k^{inflow}$) was then spatially distributed across the urban territory connected to each WWTP (based on sewer network data and CORINE land cover), assigning to each cell a fraction of the total according to its urbanized surface:

$$massMPs_{URB,i,j} = massMPs_k^{inflow} \cdot \frac{a_{URB,cell,k}}{a_{URB,k}}$$

where $a_{URB,cell,k}$ is the urban area of cell (i,j) connected to plant k, and $a_{URB,k}$ is the total urban area served by K-th WWTP.

Detailed information about the calculation of agricultural MP production are available in the Supplementary material – S4.

4.3.2.4 MPs from CSOs

CSOs represent an important but highly uncertain source of MP emissions in urban environments. Due to the scarcity of site-specific data on CSO locations, activation frequency, and discharge volumes, we relied on a regional-scale modeling approach developed by Quaranta et al., (2022). This conceptual hydrological model, based on a cascade of three linear reservoirs, was designed to estimate annual CSO discharges across European Functional Urban Areas (FUAs), using standardized rainfall inputs and default parameter values derived from engineering literature.

The model simulates stormwater runoff and dry weather flow (DWF) interactions within the urban drainage system, generating overflow events when system capacity is exceeded. Its ability to operate without local calibration makes it suitable for large-scale applications under data-limited conditions.

Building on this framework, Obermaier and Pistocchi, (2022) extended the methodology to estimate MP loads associated with CSO discharges across broader spatial domains. In our study, we adopted their scaling approach, adjusting MP release estimates according to the impervious surface area of the Arno catchment.

$$MP_{CSO,TOT} = MP_{CSO,DWF} + MP_{CSO,TWP} = \left(C_{sewage} * \frac{Q_{DWF,CSO}}{Q_{PE}} \right) + MP_{CSO,TWP} \quad (4)$$

where:

- $MP_{CSO,TOT}$ is the total annual mass of MP released through CSOs kg/year);
- $MP_{CSO,DWF}$ is the estimated MP load related to the portion of dry weather flow that is discharged via CSOs due to system malfunction or limited capacity;
- $MP_{CSO,TWP}$ is the MP load associated with wet weather events (e.g., storm-induced overflows);
- $Q_{DWF,CSO}$ is the volume of sewage released by CSOs under dry weather conditions (m^3 /year);
- Q_{PE} is the average volume of sewage produced per population equivalent, assumed to be 200 L/PE/day (including sanitary flow, industrial wastewater, and infiltration), based on values commonly adopted within EU28 (Abdellatif et al., 2015; David and Matos, 2005; DEFRA, 2008; Launay et al., 2016; Mascher et al., 2017);
- C_{sewage} is the average concentration of MPs in sewage entering WWTPs ($\mu g/L$), assumed from literature-based European average values.

The C_{sewage} value was initially explored across three pollution scenarios (low, medium, and high), as reported in previous studies (Funck et al., 2021; Okoffo et al., 2021; Simon et al., 2018). Among the three, the value reported by Okoffo et al. (2021) was selected for final implementation, given its consistency with measurements conducted in a WWTP located within the Arno catchment (Becucci et al., 2022; Pagliaccia et al., 2025).

For the emissions from tire wear particles (TWP), the contribution is calculated considering that not all the TWP generated from the traffic is transferred into the sewage system but only the 10%, the rest is considered to be removed by street sweeping or retained on urban land (Quaranta et al., 2022).

4.3.3 Monte Carlo Analysis

To assess the uncertainty associated with MP production estimates from terrestrial sources, a Monte Carlo simulation was performed. This approach focused on key input parameters, such as emission rates, vehicle mileage, and WWTP retention efficiency, and aimed to capture the variability in source-specific emissions prior to transport modeling. The analysis involved 10,000 iterations for each 1×1 km cell of the Arno River catchment and accounted for the variability of four key parameters: the production rate from agricultural sources (r_{AGR}), the production rate from road traffic (r_{ROAD}), the vehicle mileage (m_{ROAD}), and the retention efficiency of wastewater treatment plants (r_p). All four parameters were modeled using Gaussian distributions defined over plausible ranges derived from literature (Bryan, 2013; Uusitalo et al., 2015).

For agricultural sources, the values of r_{AGR} were sampled from normal distributions defined for each land cover class using the minimum and maximum values reported by FAO (2019). For road traffic, r_{ROAD} values were differentiated by road type (urban/rural vs. highway) and vehicle

class, with distributions built using COPERT 2019 data (European Environment Agency (EEA), 2019) (a European road transport emission model developed by the European Environment Agency, which supplies standardized estimates of vehicle activity and emissions based on fleet composition and driving conditions). In the case of highways, the Gaussian distribution was defined with a mean equal to the COPERT value and a standard deviation of 0.15. Similarly, m_{ROAD} was varied using a normal distribution centered on the COPERT-reported mean, with a standard deviation of 10%. These standard deviations were selected by analogy with the other Gaussian distributions in the model, where literature-based minimum and maximum values were used to define the plausible range.

For urban sources, uncertainty was introduced in the retention efficiency r_p , modeled via a Gaussian distribution spanning 2% to 98%, as reported by (Sun et al., 2019), reflecting the variation between basic and advanced treatment systems.

This probabilistic approach enabled the propagation of input uncertainties through the spatial model, generating a robust range of possible outcomes for MP emissions across the catchment. Full methodological details and parameter ranges are provided in Supplementary Material (Section S4).

4.3.3.1 Urban MP: production and delivery

As a final note, it is important to distinguish, in the case of MPs from urban sources, between the estimation of their production and the calculation of their input into the river system. While the Monte Carlo analysis described above was applied to all terrestrial sources to assess the uncertainty in MP production (including agriculture, road traffic, and urban areas), in the case of MPs from urban sources, production estimates were obtained by back-calculating inflow masses from measured effluent concentrations, taking into account WWTP retention efficiency. Conversely, riverine inputs were estimated directly from the observed effluent data.

Specifically, effluent MP concentrations measured at a WWTP located within the Arno catchment (Becucci et al., 2022) were used to calculate outflow masses. These values were then scaled to other WWTPs in the catchment based on the size of their connected population. This decoupled approach ensures that modeled production reflects potential variability across the landscape, while riverine MP inputs are based on direct observations and used as a more robust basis for downstream transport modeling.

In other words, modeled MP production from urban areas was used to explore emission uncertainty and spatial patterns, whereas riverine inputs were fixed using field-derived data. This methodological separation supports both exploratory analysis and realistic boundary conditions for land-to-river MP flux estimation.

4.3.4 Mobilization of MPs through soil erosion

MPs originating from agricultural practices were assumed to accumulate primarily within the upper 20 cm of the soil profile, consistent with the depth affected by erosion as estimated by

the RUSLE model (Renard, 1997; Yoon et al., 2024). This assumption is supported by field studies indicating that MPs in soil tend to remain near the surface due to their limited vertical mobility (Rillig et al., 2017a; Zubris and Richards, 2005).

Each raster cell was defined as a 10×10 m unit, corresponding to a surface area of 100 m² and a soil volume of 20 m³ for the upper 20 cm. Assuming an average bulk density of 1.6 g/cm³ (Panagos et al., 2024), this yields a total soil mass of approximately 32,000 kg per cell. When the estimated annual soil loss from RUSLE, combined with the SDR (Figure S8), exceeded this threshold (i.e., 32 t/year), the full content of MPs within the topsoil was considered to be mobilized (see Supplementary Material).

For erosion values between 0 and 32 t/year, a linear mobilization function was applied to determine the fraction of MPs transported with the eroded soil. This assumption was made considering the concentration of MPs is approximately homogeneous within the upper 20 cm of the soil (Rillig et al., 2017b; Zubris and Richards, 2005b). The mobilized MP mass from each cell was thus computed as:

$$MP_{mobilized,i,j} = MP_{total,i,j} \cdot \left(\frac{Soil_{eroded,i,j}}{Soil_{total,i,j}} \right) \quad (5)$$

where: $MP_{mobilized,i,j}$ is the mass of MP mobilized from the cell, $MP_{total,i,j}$ is the total mass of MPs present in the top 20 cm of soil, $Soil_{eroded,i,j}$ is the annual soil loss (kg) estimated by RUSLE, $Soil_{total,i,j}$ is the total soil mass in the top 20 cm of the cell.

This approach enabled each cell to contribute a spatially variable, source-specific MP flux, linked explicitly to erosion dynamics. The result is a physically-based and spatially distributed estimate of MP mobilization that can be integrated with topographic routing to evaluate potential transfer toward the fluvial network.

4.3.5 MP transport to river

To simulate the downstream transport of mobilized MPs, we adopted a topographic approach based on the Index of Connectivity (IC) (Borselli et al., 2008), implemented using SedInConnect 2.3 (Crema and Cavalli, 2018). The IC quantifies the potential linkage between sediment source areas and the stream network, based on upslope sediment supply and downslope impedance. Its computation integrates land cover, slope, and contributing area derived from a hydrologically conditioned 10 m DEM. The IC is defined as the logarithmic ratio between the upslope component D_{up} , which accounts for contributing area, slope, and land cover, and the downslope component D_{dn} , representing the impedance to transfer, influenced by slope and distance to the nearest stream, weighted by surface roughness (see Supplementary Material - S2 for full IC and SDR equations, flow accumulation steps, and GIS-based computation details). Input layers included a 10 m DEM and a land cover map, while other components such as slope, flow length, and roughness weights were derived automatically within SedInConnect (Crema and Cavalli, 2018). Values of IC ranged from approximately -13 to +1.7 (Figure S9), with higher values indicating stronger topographic connectivity to the stream network. The IC was

calculated for all cells across the catchment and used as input to define the Sediment Delivery Ratio (SDR) in the subsequent transport model.

The Sediment Delivery Ratio (SDR) was derived using a logistic function (Vigiak et al., 2012), to estimate the fraction of eroded material that effectively reaches the river. SDR values were applied to the soil erosion map from RUSLE to compute sediment yield (SY), which served as a proxy for microplastic delivery.

Transported MPs were then routed using the Flow Accumulation tool in QGIS, allowing the cumulative load from upstream cells to be mapped along the flow network. This approach identifies source zones, flow paths, and potential accumulation hotspots.

All raster-based computations were performed in a GIS environment to maintain spatial consistency. Mathematical formulations and parameter details are provided in the Supplementary Material (Section S2).

4.3.6 Land-to-river MP transport model

4.3.6.1 Model calibration

Based on the preliminary insights from the one-at-a-time (OAT) sensitivity analysis (see Supplementary Material, Section S3), a calibration phase was conducted to identify the parameter set (IC_0 , k) that best reproduces observed sediment yield (SY) values at three hydrometric stations along the Arno River (Paris and Moscatelli, 2005), where suspended sediment transport was monitored during the years 1936-96: Subbiano (738 km²), Nave di Rosano (4083 km²), and San Giovanni alla Vena (8186 km²) (see Supplementary Fig. S10 for the visual representations of sub-catchments). Observed SY values were derived from long-term monitoring and used as a reference to evaluate the model's performance.

Among the tested parameter combinations, the set $IC_0=0.5$ and $k=5$ was selected based on the minimization of absolute error in the two upstream sections (Subbiano and Nave di Rosano). With this configuration, the model reproduced the observed SY at Subbiano and Nave di Rosano with relative errors of 29% and 18%, respectively (see Table 2). Although the prediction at the most downstream station, San Giovanni alla Vena, exhibited a comparable error of 32% (Table 2), the overestimation may still reflect the absence of in-stream retention processes in the model (Schreyers et al., 2024). The underestimations at Subbiano and Nave di Rosano (29% and 18%, respectively), on the other hand, may reflect natural buffering processes occurring along hillslopes and small tributaries, such as sediment storage in minor depressions, vegetated ditches, or subsurface drainage features. Additionally, the eroded material in these areas may include coarser fractions that are less likely to be transported downslope, leading to lower actual delivery than predicted by the SDR function.

Table 4. 2 - Comparison between measured and simulated sediment yield (SY) values at three hydrometric sections of the Arno River catchment used for model calibration. The values refer to average annual sediment transport (t/year) at the outlet of each upstream area. The parameter set $IC0=0.5$, $k=5$ was selected to minimize absolute error in the two upstream stations (Subbiano and Nave di Rosano). The relative error is also reported for each section.

Section name	Area [km ²]	Measured value [t/year]	Calibrated values [t/year]	Error [-]
Subbiano	738	221,400.00	157,237.38	0.29
Nave di Rosano	4083	1,008,501.00	822,703.90	0.18
San Giovanni alla vena	8186	1,555,340.00	2,049,799.20	0.32

This level of discrepancy is consistent with findings from similar watershed modeling efforts (De Vente et al., 2013), where calibration is typically focused on upstream sub-catchments due to their more complex and spatially variable connectivity patterns, and the greater reliability of available monitoring data. As noted by Lee et al. (2010), the spatial and temporal limitations of sediment observations often require model calibration to be selectively applied in data-rich areas, even if this results in moderate residuals across the catchment. Furthermore, the current model configuration does not account for in-stream sediment retention features such as weirs, dams, and artificial barriers, which are widespread throughout the Arno River catchment (Galloway et al., 2020; Rinaldi and Simon, 1998). These structures can significantly reduce sediment delivery by trapping material before it reaches downstream outlets (Chiu et al., 2019), and their exclusion contributes to a systematic overestimation of sediment yield across both headwater and lowland areas. Such simplifications are common in large-scale sediment modeling studies, where the lack of detailed hydraulic and structural datasets makes it impractical to simulate retention processes explicitly.

Overall, the adopted calibration strategy represents a practical trade-off between spatial detail, data availability, and model generalizability, providing an adequate representation of sediment dynamics across the catchment in support of the study's objectives.

4.4 Results

4.4.1 MPs Production

Figure 3 and Table 3 report the estimated MP emissions from the four major terrestrial sources: agriculture, non-urban roads, CSOs, and WWTPs, aggregated at the sub-catchment scale. The maps of Figure 3 reveal distinct spatial patterns, with some areas characterized by intense single-source emissions (e.g., agriculture in Chiana) and others showing a combination of

multiple relevant sources (e.g., Bientina and Ombrone). These values represent the initial release of MPs to the terrestrial or drainage environment, before considering any downstream transport or retention. The urban-related loads (WWTPs and CSOs) are shown as localized inputs at the outlet of each treatment plant, while emissions from diffuse sources are spatially distributed across land-use classes.

Agricultural areas represent significant source of MPs in the Arno River catchment (see Table 3) as the produced annual mass of MPs varies substantially across sub-catchments, ranging from 106 t/year (Greve) to 3127 t/year (Chiana). This variability reflects differences in both agricultural surface area and crop types, which influence the emission factors applied in the model.

The three highest-emitting sub-catchments are Chiana (3127 t/year), Bientina (1993 t/year) and Elsa (1493 t/year). These areas combine intensive cultivation with large cropland extents and are known for high plastic mulch use, particularly in horticulture and irrigated crops.

Table 4. 3 - Annual MP production by source type across the sub-catchments of the Arno River catchment. Values represent estimated MP loads from four main sources: agriculture, urban areas, non-urban roads, and CSOs.

Sub-catchment - name	Agriculture [t/year]	Urban [t/year]	Non-urban road [t/year]	CSO [t/year]
Usciana	772	52717	0.19	4.05
Sieve	850	30285	0.13	1.36
Pesa	277	5087	0.06	0.64
Greve	106	10915	0.08	0.85
Era	1499	10797	0.12	0.84
Elsa	1493	43228	0.18	2.24
Valdarno Med.	615	54213	0.20	5.21
Bientina	1993	50921	0.23	6.64
Bisenzio	145	36305	0.10	3.47
Valdarno Inf.	319	23105	0.08	1.60
Valdarno Sup.	754	38873	0.17	2.24
Casentino	728	19832	0.13	1.12
Ambra	163	2851	0.03	0.27
Ombrone	1051	55641	0.23	6.98
Chiana	3127	69844	0.31	4.72
Outlet	129	7810	0.03	0.53

In contrast, sub-catchments such as Greve (106 t/year), Ambra (163 t/year), and Outlet (129 t/year) show comparatively low MP production, reflecting their predominantly forested or hilly terrain with limited agricultural activity.

Interestingly, Ombrone (1051 t/year) and Sieve (850 t/year) report high emission values despite not having the largest agricultural areas, suggesting that emission intensity (driven by crop type and management practices) may outweigh total surface area in determining MP loads.

MP production from non-urban roads are relatively low but spatially widespread across the Arno catchment. The resulting spatial distribution of MP loads (Fig. 3c) reveals a more limited and homogeneous contribution across the Arno River catchment, with annual values per sub-catchment ranging from 0.03 to 0.31 t/year. The highest emitting areas include Chiana (0.31 t/year), Ombrone (0.23 t/year), and Bientina (0.23 t/year). These catchments are characterized by a relatively dense network of regional and interurban roads intersecting agricultural and peri-urban zones. Despite their relatively small surface extent compared to croplands, non-urban roads contribute detectable quantities of MPs due to continuous traffic flow and particle detachment from tires and asphalt.

In contrast, sub-catchments such as Ambra (0.03 t/year), Outlet (0.03 t/year), and Pesa (0.06 t/year) show lower emissions, consistent with sparser road infrastructure and reduced vehicle flow.

Overall, the contribution of non-urban roads to total MP emissions remains minor compared to agriculture and urban wastewater sources.

The estimated annual production of MPs from CSOs in the Arno River catchment totals 49.94 t/year, distributed heterogeneously across the sub-catchments based on impervious surface coverage. As shown in Figure 3D, CSO-related MP emissions range from less than 0.27 t/year in predominantly rural or forested areas to nearly 7 t/year in highly urbanized sub-catchments such as Ombrone (6.98 t/year), Bientina (6.64 t/year), and Valdarno Medio (5.21 t/year).

The spatial allocation reflects the proxy-based modeling approach adopted in this study, where imperviousness was used as an indicator of stormwater generation and CSO activation frequency. Sub-catchments with extensive built-up areas and dense drainage infrastructure display the highest MP loads, even when their surface area is limited (e.g., Ombrone: 6.98 t/year, Valdarno Medio: 5.21 t/year). In contrast, sub-catchments such as Ambra (0.27 t/year), Greve (0.85 t/year), or Era (0.84 t/year) contribute less than 1 t/year, highlighting the strong correlation between imperviousness and CSO loads.

Although CSO-derived MPs account for a smaller share compared to urban and agricultural sources, their direct discharge into the river system without any filtering or retention makes them particularly relevant from a management perspective. Unlike erosion-driven sources, CSOs bypass soil transport processes, potentially delivering high concentrations of MPs in short timeframes.

MPs coming from urban areas represent the largest source of MP production in the Arno River catchment prior to any transport or treatment. As shown in Table 3, estimated annual production from urban areas range from 2,851 t/year (Ambra) to 69,844 t/year (Chiana), with a total load exceeding 500,000 t/year across all sub-catchments.

The highest-emitting sub-catchments, Chiana (69,844 t/year), Ombrone (55,641 t/year), and Valdarno Medio (54,213 t/year), are characterized by high population densities and extensive urban development (Arezzo, Firenze, Prato and Pistoia). In contrast, rural or sparsely populated areas such as Ambra (2,851 t/year) and Pesa (5,087 t/year) exhibit significantly lower emissions.

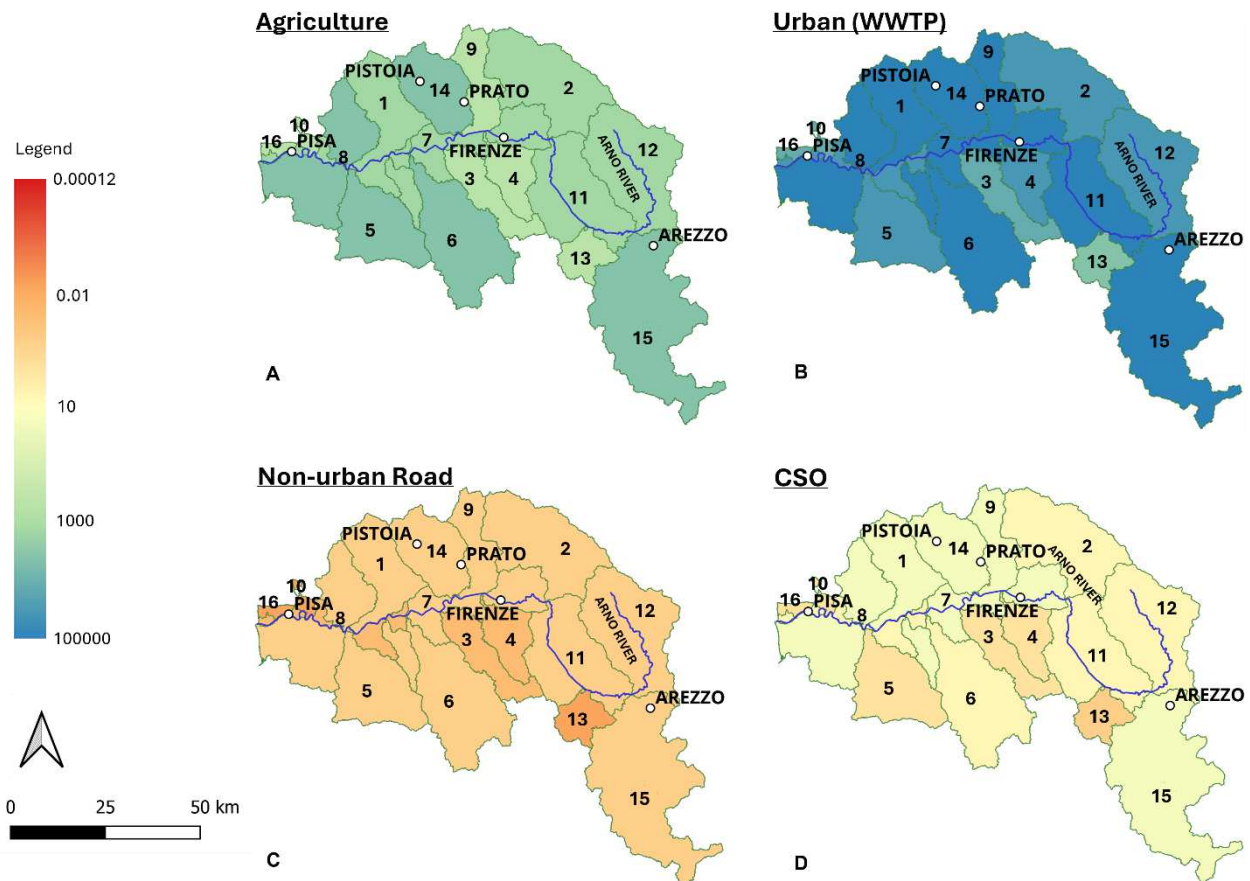


Figure 4. 3 - Annual microplastic (MP) production [t/year] from four terrestrial sources in the Arno River catchment, aggregated at the sub-catchment scale: (a) agriculture, (b) urban wastewater (WWTP inputs), (c) non-urban roads, (d) combined sewer overflows (CSOs). Values are shown on a logarithmic scale to accommodate the wide range of emission magnitudes and to highlight both high-intensity and low-intensity contributors. Agricultural and urban sources dominate in terms of absolute loads, while road- and CSO-related emissions are lower but exhibit distinct spatial patterns. The maps underscore the spatial heterogeneity in MP production across the catchment, reflecting differences in land use, imperviousness, and population density.

4.4.2 Mobilization and delivery efficiency of microplastics from agricultural and road surfaces

We present the results of the transport simulation, highlighting the spatial patterns of MP mobilization and delivery to the river network. To quantify the effectiveness of MP transport from diffuse sources to the river network, we analyzed and compared three main stages for each sub-catchment: (i) the retained fraction of MPs in the soil, (ii) the mobilized fraction due

to soil erosion, and (iii) the amount delivered to the sub-catchment outlet. The sum of these three fractions corresponds to the total MP production from the specific source (agriculture or non-urban roads). This analysis is summarized in Figure 4, which show stacked bar plots for both agriculture and non-urban road sources across all sub-catchments.

As shown in Figure 4A, the delivered fraction of microplastics from agricultural sources varies significantly across sub-catchments, ranging from as low as 3% (e.g., Bisenzio, Ombrone) to a maximum of 32% in the Outlet catchment. Most sub-catchments show delivered values below 15%, indicating limited transfer of mobilized MPs to the river network. Only a few sub-catchments, such as Pesa (18%), Era (18%), and Greve (16%), exhibit slightly higher delivery rates, likely reflecting more favorable connectivity conditions or reduced retention along the transport pathways.

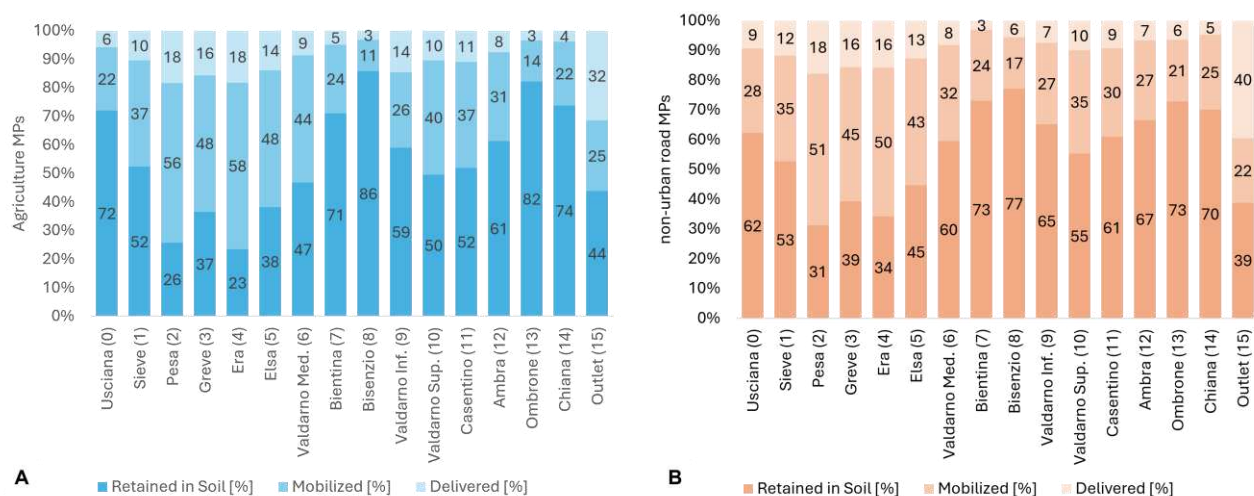


Figure 4. 4 - Contribution of microplastics (MPs) from (a) agricultural land and (b) non-urban roads across the sub-catchments of the Arno River catchment. Stacked bars represent, for each sub-catchment, the retained (darker shade), the mobilized fraction (medium shade), and the amount delivered to the catchment outlet (lightest shade), expressed in tons per year (t/year). Values are normalized to the production to allow direct comparison between catchments. The labels on each bar segment indicate the percentage of each fraction relative to the total MP production in that sub-catchment.

For non-urban roads (Figure 4B), delivered values are similarly low, with most sub-catchments below 15%. The highest value is observed at the Outlet (40%), followed by Pesa (18%), Greve (16%), and Era (16%), suggesting that road-derived MPs may reach the river more efficiently in these areas, potentially due to dense road networks, steeper slopes, or minimal buffer zones. In contrast, catchments such as Bientina, Ombrone, and Chiana show the lowest delivered values (<6%), despite high production, indicating strong attenuation or limited mobilization.

Overall, the model estimates that approximately 1,483 t/year of microplastics are delivered to the surface water network within the Arno River catchment. This includes contributions from agriculture (1,253 t/year), non-urban roads (~0.2 t/year), combined sewer overflows (43 t/year), and wastewater treatment plants (187 t/year). A detailed breakdown of these contributions by sub-catchment is provided in Table S5, which highlights both the spatial heterogeneity of source intensity across the catchment and the overwhelming dominance of agricultural sources in the overall balance.

4.4.3 MPs entering the Arno river

The spatial distribution of MP inputs along the Arno River was reconstructed by integrating both diffuse and point-source contributions. The combination of flow accumulation (for diffuse sources) and localized discharges (for point sources) enables the model to reconstruct the cumulative MP load along the Arno mainstem and to compare the spatial signature of each source category.

Figure 5 shows the cumulative MP loads delivered to the Arno River from four distinct source categories: agriculture, CSOs, non-urban roads, and urban wastewater (WWTP). Values are normalized to the total MP load from each source, allowing comparison of their spatial delivery profiles along the river's longitudinal gradient. The curves illustrate how the cumulative contribution of each source evolves with downstream distance, highlighting differences in the location, intensity, and timing of inputs from each category. This spatial pattern also reflects the longitudinal distribution of population along the Arno River, particularly for urban- and CSO-related sources. The shape of the cumulative curves thus appears consistent with the demographic gradient across the catchment.

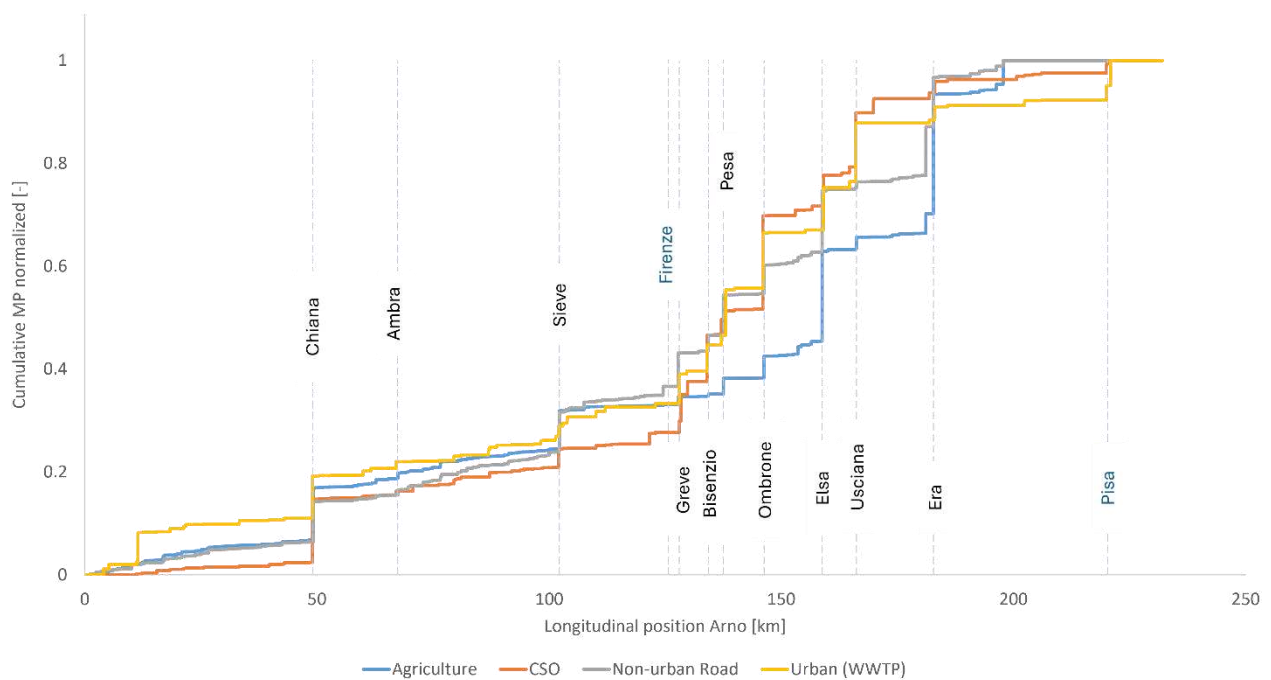


Figure 4. 5 - Cumulative distribution of normalized MP loads delivered to the Arno River from four source categories: agriculture, non-urban roads, combined sewer overflows (CSOs), and wastewater treatment plants (WWTP). Values are normalized with respect to the total annual load for each source type, and plotted against the longitudinal profile of the Arno River (progressive distance from source to outlet). Vertical dashed lines indicate the positions of major tributary confluences and cities.

The cumulative distribution of normalized MP contributions along the Arno River reveals distinct input patterns for each source type, as shown in Figure 5. At the confluence with the Chiana River (~49 km), agriculture, non-urban roads, and WWTPs all show noticeable initial contributions, with normalized values of approximately 0.17, 0.14, and 0.16, respectively. In contrast, CSO-related inputs remain limited at this stage, with a cumulative value below 0.09 and no significant increase observed until further downstream. These early contributions

reflect the spatial distribution of diffuse and point sources in the upstream catchment: while agriculture and roads begin to contribute early via erosion and runoff, and WWTP discharges occur near urban centers already present in the upper catchment, CSO loads are not activated until more densely urbanized areas are reached downstream.

Downstream, between ~50 and ~160 km, all sources show a progressive increase, though with varying intensities. Agriculture displays a consistent and substantial rise, growing from ~0.17 at Chiana to ~0.63 at the Elsa confluence (~159 km), with key increments already visible at Sieve (~0.24) and Greve (~0.35). Non-urban road contributions follow a comparable trend, increasing from ~0.14 at Chiana to ~0.75 at Elsa, with notable jumps at Greve (~0.43), Bisenzio (~0.44), and Ombrone (~0.55), reflecting the influence of connected road networks and efficient runoff pathways in the mid-catchment.

WWTP and CSO contributions display similar cumulative patterns along the river, reflecting their shared discharge locations. Up to the confluence with the Pesa (~138 km), the WWTP curve shows a slightly faster increase compared to the CSO curve. Starting from Pesa and further downstream, the CSO curve exhibits a steeper rise, indicating a more rapid accumulation of normalized load in the lower sections of the river. At Elsa (~159 km), Usciana (~166 km), and Era (~183 km), CSO contributions increase more sharply than those from WWTPs, suggesting a more concentrated downstream delivery relative to their total input.

In the final segment of the river (~170–240 km), all sources continue to increase toward their respective total loads. Agricultural contributions rise from ~0.63 at Usciana (~166 km) to ~0.93 at Bientina (~191 km), showing one of the steepest final increases. Non-urban roads follow a similar pattern, growing from ~0.75 to ~0.97 over the same stretch. CSO and WWTP contributions also intensify downstream, with values at Bientina reaching ~0.89 and ~0.77, respectively. By this point, all source-specific curves approach saturation, indicating that most MP loads from both diffuse and point sources have been delivered to the mainstem before the outlet.

4.5 Discussion

4.5.1 On the MPs production

The spatial heterogeneity of MP production across the Arno catchment emerged as both a critical insight and a modeling challenge. The intensive land use for agricultural activities emerged from high MP production in sub-catchments such as Chiana (3127 t/year) and Bientina (1993 t/year). This ranking is summarized in Table S5, which orders the sub-catchments according to their estimated MP production from each source type (agriculture, urban areas, non-urban roads, and CSOs). These values are consistent with upper-bound estimates reported by Boctor et al. (2025), who found that agriculture inputs can reach up to 730000 t/year across Europe, supporting the likelihood of high local emissions in heavily cultivated areas. By contrast, MPs originating from non-urban roads, though quantitatively minor ($0.01 \div 0.31$ t/year), in sub-catchments as Chiana and Ombrone highlight how even

small diffuse sources may become significant under high connectivity and traffic conditions (Kawecki et al., 2021).

WWTP production concentrate in Chiana (69844 t/year), Ombrone (55641 t/year), and Valdarno Med. (54213 t/year), while CSO-related spikes in Ombrone (6.98 t/year), Bientina (6.64 t/year), Valdarno Medio (5.21 t/year). These localized emission peaks from WWTPs and CSOs align with the most densely populated sub-catchments (e.g., Valdarno Medio, Bientina, Ombrone), supporting the strong influence of population load and sewer network density over land cover percentages or connectivity indices. In particular, elevated CSO emissions in these areas highlight the role of extensive impervious surfaces and suboptimal stormwater management practices, which exacerbate overflow events during rainfall. This suggests that targeted mitigation in these urban hotspots, through improved sewer infrastructure or green stormwater solutions, could significantly reduce point-source MP inputs to the fluvial system (Li et al., 2025; Österlund et al., 2023).

The observed differences between diffuse sources and point-sources indicates the need of an integrated management strategy: diffuse sources require upstream interventions targeting land use practices, such as sustainable agricultural techniques, vegetated buffer strips, and erosion control measures, while point-sources require infrastructure upgrades (such as retrofitting of WWTPs with advanced filtration units or tertiary treatments) and better stormwater management, such as sewer separation, detention catchments, and green infrastructure (e.g., bioswales, permeable pavements). Recent experimental work has demonstrated, for instance, that the application of biochar filters in agricultural drainage systems reduced microplastic concentrations in runoff by over 92%, while field trials with biodegradable mulch films substantially lowered the accumulation of plastic fragments in soils compared to conventional polyethylene films (Akhir and Mustapha, 2022; Yadav et al., 2022). Moreover, field and pilot-scale studies demonstrate that nature-based solutions can substantially reduce diffuse MP inputs: rain gardens and bioretention cells achieved up to 98% removal efficiency (Werbowski et al., 2021), while constructed wetlands and vegetated buffer areas have shown removal rates approaching 100% under certain conditions (Chen et al., 2021; Hassan et al., 2021; Q. Wang et al., 2021). These findings highlight the tangible potential of nature-based and sustainable agricultural practices to mitigate MP emissions at source. The coexistence of both patterns within the same catchment, as observed for the Arno, suggests that integrated, source-specific strategies will be essential for effective MP management.

4.5.2 Transport toward the river network

The spatial analysis of delivered MPs from agricultural and non-urban road sources reveals considerable heterogeneity across the Arno River sub-catchments, reflecting the interplay of land use (Zhang et al., 2022), topography (Rolf et al., 2022), and morphometric characteristics (Gonzalez-Saldias et al., 2024) reported in Table 4. While both sources share erosion-driven mobilization as a common mechanism, their transport behaviors diverge substantially due to differences in spatial distribution and connectivity.

Table 4. 4 - Summary of key morphometric and land use characteristics for each sub-catchment of the Arno River catchment. For each unit, total area, percentage of urban, agricultural, and forested land cover, mean Connectivity Index, mean Sediment Delivery and Population are reported.

Sub-catchment - name	Area [km ²]	Urban Area [%]	Agriculture Area [%]	Woods [%]	Non- urban Road length [km]	IC mean	SDR mean	Population
Usciana	488	17	43	40	366	-6.94	0.06	182816
Sieve	838	7	25	68	568	-7.27	0.05	78495
Pesa	335	8	48	44	337	-6.38	0.07	40873
Greve	282	12	41	47	257	-6.67	0.06	76451
Era	599	6	56	38	292	-6.03	0.07	52853
Elsa	861	9	53	38	1142	-6.25	0.07	123852
Valdarno Med.	587	20	47	33	500	-6.59	0.07	498880
Bientina	804	17	56	28	633	-7.06	0.06	225417
Bisenzio	321	19	18	63	205	-7.80	0.04	182419
Valdarno Inf.	210	17	43	39	161	-6.67	0.06	97031
Valdarno Sup.	780	10	35	55	673	-6.81	0.06	134337
Casentino	882	5	21	74	711	-7.46	0.05	51284
Ambra	206	5	28	67	90	-7.29	0.05	14399
Ombrone	490	23	40	38	340	-7.77	0.05	390078
Chiana	1373	11	62	27	1378	-6.26	0.07	200134
Outlet	74	19	40	41	32	-7.83	0.05	33708

For MPs produced by agricultural sources, the delivered fraction ranged from 3% to 32% (Figure 4A), with the Outlet, Pesa, and Era sub-catchments displaying the highest delivery rates. These values are not directly proportional to the extent of agricultural land, as evidenced by Pesa (48% agriculture), which showed one of the highest delivery percentages (18%), in contrast to Valdarno Inf. (43% agriculture, 14% delivery) or Chiana (62% agriculture, 4% delivery). This suggests that land use extent alone is not a reliable predictor of delivery, accordingly with Corradini (2025) and Hu et al. (2024). Instead, factors such as slope, spatial arrangement of cropland relative to the drainage network, and heterogeneity in sediment connectivity appear to exert a stronger influence. Indeed, some sub-catchments with highest value of mean SDR (e.g., Pesa and Era: SDR = 0.07) coincide with elevated delivery, while others (e.g., Ombrone, SDR = 0.05) remain inefficient transporters, pointing to the role of other landscape-level constraints.

In the case of non-urban roads, delivery percentages varied from 3% to 40%. Notably, the Outlet sub-catchment again exhibited the highest delivery, with 40% of mobilized MPs reaching the fluvial network (Figure 4B). While the linear structure of roads might suggest higher connectivity, our results indicate that this potential is frequently moderated by the surrounding environment. For example, Ambra and Ombrone, despite moderate mobilized fractions (27% and 21%, respectively), show limited delivery (6% in both cases), likely due to their topographic isolation or vegetative buffers that intercept runoff before it reaches streams. Conversely, Greve and Pesa, with non-urban road delivery rates of 16% and 18%, respectively, reflect a more favorable alignment of road networks with surface hydrological pathways.

A comparative analysis between the two sources underscores this divergence. In sub-catchments like Greve and Pesa, where delivered percentages from both agriculture and non-urban road are consistently high (above 15%), the likely cause is a shared topographic and hydrological profile that enhances particle transfer. On the other hand, Bisenzio, despite its intense urbanization and substantial road infrastructure, shows delivery rates below 6% for both sources, aligning with its steep slopes (IC_mean -7.80) and low SDR (0.04), which might favor particle deposition or dispersion into non-channelized areas (Beheshtimaal et al., 2024).

These observations emphasize the complexity of MP transport from diffuse sources. Even when erosion potential (i.e., SDR) is relatively homogeneous across sub-catchments, delivery outcomes vary widely. This reinforces the need to consider not only morphometric indicators but also the spatial configuration of sources and barriers within each catchment. Sub-catchments such as Pesa, Era, and Outlet emerge as critical contributors due to their combination of mobilization potential and hydrological connectivity, making them priority targets for mitigation efforts. In contrast, areas such as Ombrone, Bisenzio, and Ambra demonstrate the buffering capacity of specific landscape settings, which may naturally limit MP transfer to watercourses despite substantial source presence.

4.5.3 On the MPs distribution along the Arno River corridor

The cumulative normalized profiles (Figure 5) illustrate how MP contributions from the four different sources build up along the longitudinal course of the Arno River. The differing trajectories of the curves reflect the influence of localized point discharges (WWTPs and CSOs) compared to more gradual, landscape-mediated diffuse inputs (agriculture and roads).

CSO-derived MPs display a stepwise increase along the river, with cumulative values rising more abruptly in the mid-to-lower reaches, particularly at the confluences with Pesa (~138 km), Ombrone (~146 km), and Elsa (~159 km). This pattern reflects the model assumption that CSO discharges occur at the same locations as WWTPs, resulting in aligned delivery points and delayed upstream contributions. Rather than diffuse increases, CSO loads are concentrated in a limited number of locations, consistent with the structure of combined sewer systems in more urbanized sub-catchments (Figure 1). These spikes are indicative of intense, localized urban discharges, corroborating experimental studies that show how CSO systems can release

high MP loads during overflow events, especially where infrastructure is densely networked (Hurley et al., 2018; Trusler et al., 2024).

WWTP-related MPs display (Figure 5) a stepwise cumulative increase along the river corridor (Figure 5), with visible increments at discrete locations such as Sieve (~102 km), Greve (~128 km), Pesa (~138 km), and Usciana and Era (~166–183 km). This pattern reflects the spatial distribution of treatment facilities in the catchment and their role as localized point sources. Despite the high retention efficiency of advanced WWTPs, continuous discharges of treated effluent contribute measurable quantities of MPs to the receiving water bodies. These findings are consistent with recent studies by Acarer (2023) and Sadia et al. (2022), authors highlighted that even well-functioning plants remain a significant source of MPs, due to the persistence of fine particles in effluent streams.

Non-urban road contributions show a consistent and spatially significant cumulative increase, particularly in sub-catchments such as Greve, Pesa, Elsa, and Usciana. These areas combine moderate to high road density (e.g., Elsa: 1.3 km/km²; Chiana: 1 km/km²; Greve.: 0.9 km/km²) with relatively moderate forest cover (27–47%) and high agricultural land use (41–62%) (see Table 4), conditions that favor efficient surface runoff and microplastic mobilization from road surfaces. In the downstream sub-catchment of Bientina, the cumulative contribution from roads reaches its maximum, reflecting both hydrological connectivity and the accumulation of upstream inputs. Although road-derived MPs are lower in absolute mass compared to urban wastewater, their spatial distribution and delivery potential make them a relevant diffuse source, especially in areas with limited vegetative buffering and steeper slopes. This observation aligns with recent findings by Myszka et al. (2023) emphasizing that traffic-related microplastics can represent a critical input in connected rural landscapes.

Agricultural MPs, on the other hand, show a more delayed delivery profile (Figure 5). While contributions are increase only slightly in the upper sections, they rise substantially between the confluences with Elsa and Era. This increase likely results from cumulative erosion-mediated transport of MPs adsorbed to soil particles, mobilized from widespread upstream fields during rainfall events. Such dynamics are consistent with recent studies highlighting how MP transport from agricultural landscapes can be influenced by erosion processes, land use patterns, and surface connectivity, which collectively shape the timing and magnitude of downstream delivery (Islam and Cheng, 2024; G. Wang et al., 2021).

The spatial offsets observed among the different source profiles underscore the contrasting environmental drivers underlying MP transport. Infrastructure-related sources are governed primarily by the distribution and operation of sewer systems and treatment facilities, whereas diffuse sources are more dependent on terrain morphology, land use, and surface hydrology. Notably, tributaries such as Chiana, Bientina, Ombrone, and Usciana emerge as recurrent hotspots for multiple source types, reinforcing their importance in spatial planning and mitigation prioritization.

When summed across all sources and sub-catchments, the modeled MPs mass entering the Arno River reaches the value of 1482 t/year. This outcome could be compared to empirical observations by Ghinassi et al. (2023) that analyzed MP concentrations in riverbed sediments sampled from a mid-channel bar within the Arno River. In this study an average of 2.13 plastic items per gram of dry sediment was observed. Assuming a mean particle mass of 5 μg (Besseling et al., 2019) and combining this with long-term sediment transport estimates for the catchment, the corresponding mass of MPs potentially retained in deposited sediments is approximately 1656 t/year. By comparison, Monnanni et al. (2024) measured MPs in surface water samples collected from the same river and found a mean concentration of 11.45 plastic items per cubic meter of water. When scaled by the river's annual average discharge ($\sim 110 \text{ m}^3/\text{s}$), this yields an estimated suspended MP load of roughly 0.2 t/year. Although these empirical values differ by orders of magnitude, they reflect distinct environmental compartments: suspended MPs represent a transient, dynamic fraction, while sediment-bound MPs accumulate over time. These differences underscore the importance of integrating observations from multiple environmental compartments when evaluating MP fluxes at the catchment scale, and they support the plausibility of our modeled estimates as upper-bound input values.

These findings suggest that effective reduction of MP inputs to river systems requires an integrated and source-specific approach. Addressing urban discharges through improvements in CSO retention and WWTP treatment performance must be complemented by landscape-based interventions aimed at reducing runoff connectivity and sediment mobilization in agricultural and road-influenced areas. Understanding not only the intensity of emission sources but also the spatial mechanisms facilitating delivery is therefore essential for designing context-appropriate mitigation strategies.

4.6 Conclusions

This study presents a spatially distributed modeling framework to estimate land-to-river MP fluxes from four sources (agricultural activities, non-urban roads, CSOs, and WWTPs) across the selected Arno River catchment. By coupling emission estimates with erosion-based mobilization and topographic connectivity, the model provides a process-based, spatially explicit assessment of MP delivery.

The results highlight the critical role of landscape use in regulating MP transfer efficiency. Sub-catchments such as Chiana ($\sim 416 \text{ t/year}$ produced, $\sim 26 \text{ t/year}$ delivered) and Bientina ($\sim 352 \text{ t/year}$ produced, $\sim 18 \text{ t/year}$ delivered), despite high production, exhibit limited delivery due to flat terrain and low connectivity, while others like Pesa ($\sim 180 \text{ t/year}$ produced, $\sim 45 \text{ t/year}$ delivered) and Era ($\sim 162 \text{ t/year}$ produced, $\sim 41 \text{ t/year}$ delivered), with steeper topographies and favorable transport paths, show disproportionately higher delivery rates. These findings confirm that MP production alone is a poor predictor of aquatic exposure risk and reinforce the need for models that integrate geomorphological transport processes.

Although agriculture and WWTPs dominate total MP production, our estimates show that erosion-driven delivery from agricultural land can approach or even exceed point-source inputs in certain areas, echoing observations from other rural catchments (Rehm and Fiener, 2024). While only a fraction of agricultural MPs reaches rivers annually, most of the applied MPs remain in the topsoil, acting as a long-term legacy source. This persistent reservoir may sustain emissions for decades, even under declining input scenarios, underscoring the importance of integrating temporal feedbacks into future modeling efforts.

The model also shows that MPs from non-urban roads, though limited in absolute terms, can be efficiently routed to water bodies under favorable slope and land cover conditions. CSOs and WWTPs contribute more directly and continuously, but their relative importance varies with urban density and sewer design. This reinforces the need for source-specific mitigation strategies: erosion control in cultivated land, stormwater retention for CSOs, and improved filtering at WWTPs.

By distinguishing among production, mobilization, and effective delivery, the model offers a flexible and transferable tool for identifying source-specific hotspots and assessing mitigation scenarios under real-world constraints. Its operational simplicity, reliance on open geospatial inputs, and avoidance of continuous hydrological time series make it particularly suitable for supporting river catchment management in data-limited regions. In this sense, the framework complements national-scale inventories and can help operationalize EU Water Framework Directive objectives at the catchment scale. Moreover, by explicitly simulating transport through erosion and topographic connectivity, the model enables the identification of critical sub-catchments where diffuse and point sources converge, thus informing spatially targeted interventions.

Future research should focus on validating these estimates through field monitoring and on incorporating key riverine processes that are currently excluded, such as in-stream retention, sedimentation, and degradation of MPs. While the present outputs represent a reasonable upper bound of annual inputs to the fluvial system, they do not account for storage, fragmentation, or temporary sequestration in riverbed sediments and floodplains—factors that may substantially alter MP fate (Mancini et al., 2023b, 2023a; Martuscelli et al., 2025; Nizzetto et al., 2016). Additional refinements should aim to integrate temporal variability, particularly for infrastructure-driven emissions (WWTPs and CSOs), whose contributions are highly sensitive to rainfall patterns, sewer design, and operational performance (Lawrence et al., 2024; Perry et al., 2024). Finally, the model could be extended to simulate future scenarios, exploring how shifts in land use, climate-driven precipitation regimes, and plastic consumption trends may alter MP emissions and transport over time. These developments would enhance the model's utility as a strategic planning tool, supporting long-term pollution mitigation and infrastructure resilience.

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4.9 Supporting Material

Microplastic emissions in river catchments using a distributed erosion-connectivity model

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S1 - Soil Loss Estimation Using the RUSLE Model

Soil loss was estimated using the Revised Universal Soil Loss Equation (RUSLE), which combines multiple environmental factors into a multiplicative model to assess average annual erosion rates (Wischmeier, 1978). The RUSLE model was implemented within a GIS environment at a spatial resolution of 10 × 10 m.

The R factor (rainfall-runoff erosivity) was calculated from gridded precipitation intensity parameters (a, n, m) derived from regional intensity-duration-frequency curves (LSPPs) provided by Tuscany region. These were converted to R values using the empirical formulation $R = 0.417 \cdot P^{2.17}$, where P is the rainfall depth in mm corresponding to a 6-hour duration and 2-year return period.

The K factor (soil erodibility) was derived from pedological databases. For Tuscany, polygon-based soil maps containing granulometric composition and organic matter were used, applying the (Renard, 1997) equation based on the geometric mean diameter of soil particles. For Liguria, values were extracted from the European Soil Data Centre (ESDAC) maps and resampled to 10 m resolution.

The LS factor (topographic factor) was calculated using the approach of (P.J.J. Desmet and G. Govers, 1996), implemented via the “LS Factor – Field Based” tool in SAGA GIS. Flow accumulation and slope maps were derived from a hydrologically corrected 10 m DTM, integrated with 1 m LiDAR data in flat areas.

The C factor (land cover and management) was assigned based on CORINE Land Cover classes, using coefficients from (Panagos et al., 2015). Values ranged from 0.001 (dense forest) to 0.35 (vineyards and bare soils).

The P factor (conservation practices) was conservatively set to 1 across the basin, assuming no specific erosion control measures.

The final map of annual soil loss E was computed as the cell-by-cell product:

$$E = R \cdot K \cdot LS \cdot C$$

All raster operations were performed using QGIS and SAGA GIS tools. The resulting erosion map represents the gross potential soil loss without accounting for sediment deposition processes, which are treated separately via the SDR module.

S2 - Transportation

To simulate the downstream transport of mobilized MPs toward the fluvial network, we adopted a topographic approach based on the Index of Connectivity (IC) proposed by (Borselli et al., 2008), and implemented using the open-source software SedInConnect 2.3 (Crema and Cavalli, 2018). The IC quantifies the potential hydrological linkage between sediment source areas and the stream network by combining an upslope component (D_{up}), which reflects the sediment supply potential, and a downslope component (D_{dn}), representing the impedance to sediment transfer along the flow path.

The IC was calculated from a hydrologically conditioned 10 m resolution DEM (pit-filled), in combination with slope and land cover data. The RUSLE C-factor (see Supplementary Material – S1) was used as a weighting factor, accounting for land cover resistance to particle movement.

The resulting IC values provide a continuous, spatially distributed indicator of sediment and particle transfer potential across the landscape, where higher IC values denote stronger connectivity. IC values were computed for each pixel following the original formulation:

$$IC_j = \frac{D_{up}}{D_{dn}} = \log_{10} \left(\frac{\bar{C}_j \bar{S}_j \sqrt{A_j}}{\sum_{i=j, \dots, n_j} \frac{d_i}{\bar{C}_i \bar{S}_i}} \right)$$

Where C_j is the value of land use of the j -th cell, S_j is the slope of the j -th cell, A_j is the upslope catchment area referred to the j -th cell, d_i is the hydraulic distance of the j -th cell to the closest river.

where C_j and S_j represent the average cover and slope of the upslope contributing area A_j , and d_i , C_i , S_i refer to the properties of the individual downslope cells along the steepest flow path to the river.

Based on the IC raster, the Sediment Delivery Ratio (SDR) was used to estimate the proportion of eroded soil that effectively reaches the river network, serving as an indicator of sediment and microplastic connectivity. SDR values were derived from the Index of Connectivity (IC) raster using a logistic (sigmoid) function, following the formulation proposed by (Vigiak et al., 2012):

$$SDR_j = \frac{SDR_{max}}{1 + e^{\left(\frac{IC_0 - IC_j}{k}\right)}}$$

where SDR_{max} is the maximum delivery efficiency, IC_0 is the IC threshold at which $SDR = 0.5$, and k controls the slope of the logistic curve. Thus, the SDR modulates the fraction of eroded material that effectively reaches the stream network, capturing spatial variability in sediment and MP connectivity.

This function allows spatial variability in sediment connectivity to be captured across the catchment. Stream channels were excluded from the SDR calculation and were assumed to have full connectivity, i.e., $SDR=1$. The spatial distribution of SDR was then applied to the soil loss raster to estimate sediment yield at the catchment scale. This output, representing mobilized soil per pixel, was used as a proxy for erosion-driven MP fluxes.

To trace the path of transported material, we employed the Flow Accumulation tool in QGIS, using the D8 algorithm and the same 10m DEM. This function accumulates the MP load from each upslope pixel along the flow direction, generating a map of downstream MP accumulation. This allows for identifying not only the source areas but also converging flow paths and potential hotspots of microplastic delivery into the river system.

Once the SDR was calculated for each cell, it was applied to the soil erosion output from the RUSLE model to estimate the sediment yield (SY), defined as the actual amount of sediment leaving each pixel and potentially reaching the river system. While RUSLE estimates the potential erosion (gross detachment), SY represents the delivered fraction, modulated by the landscape's connectivity. The two are related as follows:

$$SY_j = E_j \cdot SDR_j$$

where E_j is the annual soil loss (t/ha/yr) from RUSLE, and SY_j is the sediment yield (t/ha/yr) for pixel j .

This calculation links erosion dynamics to hydrological connectivity, allowing identification of areas where erosion is likely to result in downstream sediment and microplastic transport. All computations were conducted in a GIS environment, using raster-based operations to ensure spatial coherence and facilitate integration with the microplastic mobilization model.

S3 - One-at-a-time (OAT) sensitivity analysis

To evaluate the sensitivity of the sediment yield (SY) at the outlet to variations in the SDR function parameters, a one-at-a-time (OAT) analysis was conducted by independently varying the parameters IC_0 and k over a plausible range. Specifically, values of $IC_0 = [-1.5, -0.5, 0.5, 1.5]$ and $k = [2, 3, 5]$ were tested, while keeping the other parameter fixed. For each parameter combination, the SDR was recalculated across the basin and applied to the RUSLE-derived erosion rates (E) to estimate the total SY at the outlet.

The results (Figure S1) show a clear and consistent sensitivity of SY to both parameters. When varying IC_0 (Figure S1A), an exponential relationship is observed for all fixed values of k , with higher IC_0 values leading to substantially lower SY estimates. The exponential decay is particularly steep for lower k values, indicating a stronger weighting of connectivity in the SDR function. This is reflected in the fitted curves, where $k=2$ yields the sharpest decline and highest sensitivity.

Conversely, varying k while keeping IC_0 constant (Figure S1B) produces a logarithmic increase in SY, indicating that larger k values reduce the influence of IC variations and result in more uniform SDR values across the basin. The response is smoother but still clearly structured, with each IC_0 scenario showing a good fit to a logarithmic trend.

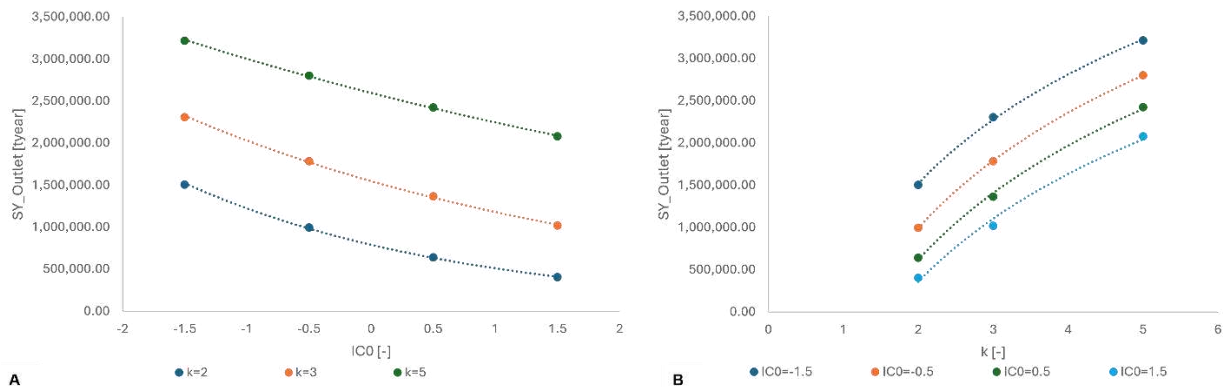


Figure S4. 1 - Results of the one-at-a-time (OAT) sensitivity analysis showing the response of total sediment yield at the basin outlet (SY_Outlet, in t/year) to variations in the SDR model parameters IC_0 and k . (A) Effect of IC_0 on SY for fixed values of k (2, 3, and 5). An exponential decay is observed for all cases, with more pronounced sensitivity at lower values of k . (B) Effect of k on SY for fixed values of IC_0 (-1.5, -0.5, 0.5, 1.5). A logarithmic increase in SY is observed as k increases, with reduced variation for higher IC_0 values. Each curve represents a separate parameter trajectory while holding the other constant.

These findings underscore the critical role of both IC_0 and k in shaping the SDR function and, consequently, the sediment yield (SY) predictions. The pronounced sensitivity of SY to variations in IC_0 suggests that this parameter significantly influences the threshold at which sediment connectivity becomes effective, aligning with observations in similar studies (Abebe et al., 2023; Heckmann et al., 2018; Xiao et al., 2024). Conversely, the parameter k modulates the steepness of the SDR-IC relationship, affecting how rapidly SDR values change with IC. Notably, lower k values result in a steeper response, indicating a more sensitive system where small changes in connectivity lead to substantial differences in sediment delivery.

The interplay between IC_0 and k highlights the necessity for careful calibration of these parameters to accurately represent the spatial variability of sediment transport processes. This calibration ensures that the model captures the nuances of sediment connectivity and delivery, which are essential for reliable SY estimations. The subsequent calibration phase, therefore, focuses on identifying the optimal combination of IC_0 and k that aligns model outputs with observed sediment yield data, enhancing the model's predictive capability and applicability to real-world scenarios.

S4 - Production from agricultural sources (AGR)

The estimated production of MPs from agricultural sources goes to consider the six land cover classes shown in Table S1. The method involves calculating the mass of MPs produced annually in a cell as the sum of ratios between cultivated areas ($aAGR$) and the production rate ($rAGR$) characteristic of the selected crops, as

$$massMPs_{AGR,i,j} = \sum (aAGR \cdot rAGR)_{i,j} \quad (2)$$

Table S4. 1 - Corine Land Cover classes

Corine Land Cover	Description
2101, 2102	Serre e Vivai

2221, 244	Arboricoltura e Aree agroforestali
223	Seminativo con spazi naturali
222	Frutteti e frutti minori
221	Oliveti
210	Vigneti

Applying Equation 2 means that more than one culture could be present in a cell, and all are considered in estimating the total mass of MPs for that cell.

Cultivated areas are identified by working on the Corine Land Cover map provided by Tuscany Region (available at <https://www502.regione.toscana.it/geoscopio/usocoperturasuolo.html>), particularly the 2019 release. While production rates comes from the “Assessment of agricultural plastics and their sustainability a call for action” report provided by FAO in 2019 (available at <https://doi.org/10.4060/cb7856en>).

S5 - Production from road traffic (ROAD)

The estimated production of MPs from road traffic take into account for different road types, different vehicles, and the annual mileage.

The types of roads considered are: rural roads, urban roads, and highways. Since rural and urban roads have traffic flows with similar characteristics (e.g. in terms of vehicle velocity, number of lanes, etc.), it was chosen to merge these two classes. Thus, there are ultimately only two types of roads: (i) rural/urban roads, and (ii) highways.

The method considers six vehicles classes: (i) bus, (ii) moped, (iii) motorcycle, (iv) passenger cars, (v) truck and lorry, and (vi) van.

The annual mileage depends on the type of vehicle and type of road considered. For this information, reference is made to the COPERT database, specifically the 2019 database release (data are available at <https://fettransp.isprambiente.it/#/>). Table S2 reports the considered annual mileage.

Table S4. 2 - Annual mileage for the six vehicle types and the two road types. Values are expressed in vkm/year.

	Rural/urban road	Highway
Bus	1733416	2474850
Moped	4571993	0
Motorcycle	22227025	1169843
Passenger cars	306978129	103931187
Truck and lorry	8557379	14913332
van	33389357	8347340

The estimation of the mass of MPs produced yearly from the road traffic requires few assumption:

- Only roads that fall within the Arno River basin are considered. Starting from the road graph, the lengths of roads in each cell were calculated. If multi-lane roads are present, each lane was counted individually.
- The number of vehicles passing on these roads is calculated on the basis of vehicles registered in the Arno River Basin area. The number of vehicles is assigned to each cell based on the extent of the cell relative to the reference area. In the case of the present application, the reference area is that of the province in which the cell falls. This information is provided by the Automobile Club d'Italia (ACI), here the data from the 2019 has been considered.

- The value of traveled kilometers for each vehicle type is obtained by scaling the annual mileage with the effective length of roads in the cell: $mROAD = \text{mileage} \cdot L_{cell}/L_{basin}$, where L_{cell} and L_{basin} are the total length of roads in the cell and in the river basin, respectively.

The method involves calculating the mass of MPs produced annually in a cell as the sum of the production for each vehicle types. The latter is considered as the ratio between the annual traveled kilometers ($mROAD$), the number of the vehicles passing through that cell (nvC), and the MPs production rate ($rROAD$).

$$massMPs_{ROAD,i,j} = \sum_k (mROAD \cdot nvC \cdot rROAD)_{i,j} \quad (3)$$

$k = (Bus, Moped, Motorcycle, Passenger cars, Truck and Lorry, Van)$

S6 - Production from urban sources (URB)

The estimation of MPs produced in urban environments (in cities), unlike what has been defined for agricultural sources and road traffic, is done indirectly. Starting from the value of MPs contained in the effluent of the waste water treatment plants (WWTP), we proceed by calculating the amount of MPs in the inflow of the same WWTP, and this mass is then distributed throughout the territory connected to the treatment plant. This means that the urban sources are exclusively considered where the sewerage network is identified.

The first step is the characterization of the mass of MPs in the inflows and outflows of the WWTPs located into the Arno River basin. Only the WWTP with a number of equivalent inhabitants (*EI*) greater than 2000 have been considered. For each of them, the sewerage network connected by the Autorità Idrica Toscana, the local waterbodies agency, was provided. The definition of the mass of MPs in the WWTP's outflows begins at the San Colombano WWTP, one of the largest in the Arno River basin. Sampling was done at this plant, which revealed an annual release in the Arno River of 12775 kg of MPs (Becucci et al., 2018). This value was used as a benchmark to calculate the mass of MPs released ($massMPs_{outflow}$) by all other WWTPs through a proportion based on plant size (i.e., number of equivalent inhabitants). Then, the mass of MPs in the inflows ($massMPs_{inflow}$) have been calculated considering the treatments facilities of each WWTP and applying to them a retention percentage (*rp*) of MPs on the basis of literature (Sun et al., 2019):

$$massMPs_k^{inflow} = massMPs_k^{outflow} rp \quad (4)$$

where, $k = 1, 2, \dots, n$ are the WWTPs.

Once calculated the mass of MPs at the inflow of the selected WWTPs, this mass is then distributed throughout the territory connected to the treatment plant. It means that the sewerage network for each plant is considered. All the cells connected to a WWTP receive a quote of the $massMPs_k^{inflow}$ of that WWTP on the basis of the urban land covering of the cell, $aURB_{cell}$ (Corine Land Cover map provided by Tuscany Region).

$$massMPs_{URB,i,j} = massMPs_k^{inflow} \frac{aURB_{cell,k}}{aURB_k}$$

where, $k = 1, 2, \dots, n$ are the WWTPs.

S7 - Monte Carlo Simulations

To test the methodology and provide a better interpretations of results a Monte Carlo simulation is considered. The Monte Carlo simulation is set by investigating the role of the production rates ($rAGR$ and $rROAD$), of the mileage ($mROAD$), and of the retention percentage coefficient (*rp*) on the results. The analysis is conducted by considering 10000 simulation for each of the cells defined to discretize the Arno River basin.

The four selected variables are varied with considering a gaussian-distribution. In particular:

- $rAGR$

This rate depends of many factors, for this reason these rates are usually considered as a range of values. The present study considered rates from the “Assessment of agricultural plastics and their sustainability a call for action” report provided by FAO in 2019 (available at <https://doi.org/10.4060/cb7856en>). Table S3 shows the minimum and a maximum values adopted for $rAGR$ on which the gaussian-distribution was built for the simulations (Figure S2).

Table S4. 3 - Production rates for MPs from agricultural sources; values are in kg/ha/year

	Corine Land Cover

	2101, 2102	2221, 244	223	222	221	210
$rAGR_{min}$	52.01	0.41	0.06	8.41	0.41	8.41
$rAGR_{max}$	221.15	6.15	0.75	106.15	6.15	106.15

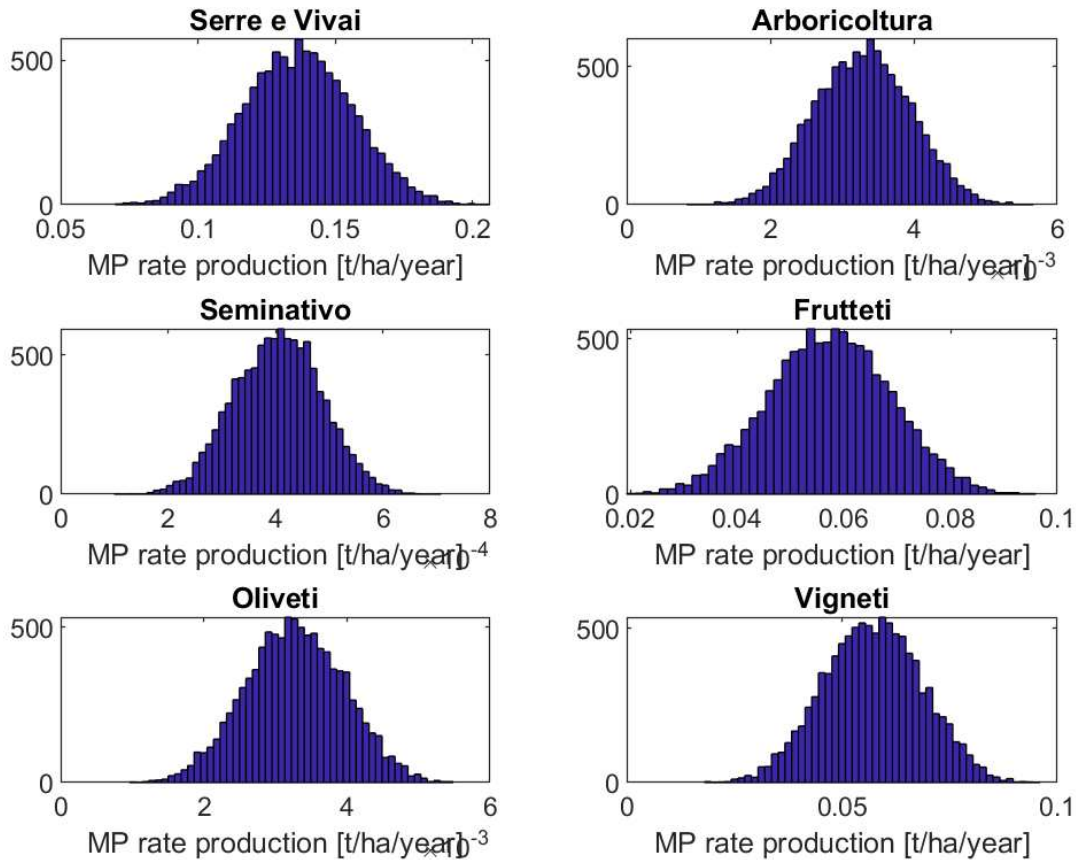


Figure S4. 2 - Gaussian-distribution MPs production rates for agricultural sources in a random cell of the domain.

- $rROAD$

For the estimation of the MPs production from roads the study considers to investigate both the ration of production of MPs ($rROAD$) and the mileage ($mROAD$), considering the six vehicle classes.

Production rates has been taken from COPERT database. In this case two classes of roads were considered (rural/urban roads and highways), for each class a range of values was considered. The COPERT database reports a single value for the different road classes (i.e., rural, urban, and highway), thus, the following two assumptions were considered:

Assumption #1: for urban/rural class the minimum (maximum) value of the rate was adopted as the minimum (maximum) between the one for urban and the one for rural;

Assumption #2: for highways the minimum and maximum values of the ratio were defined to have a gaussian curve with a mean value equal to the value reported by COPERT database and a standard deviation of 0.15.

Considering the two above assumptions the adopted range of values for $rROAD$ are showed in Table S4, while the gaussian-distribution built for the simulations are showed in Figures S3 and S4.

Table S4. 4 - Production rates for MPs from road traffic; values are in g/km

	Rural/Urban roads		Highways	
	$rROAD_{min}$	$rROAD_{max}$	$rROAD_{min}$	$rROAD_{max}$
Bus	0.054	0.074	0.0298	0.0403
Moped	0.025	0.033	0	0
Motorcycle	0.006	0.01	0.0068	0.0092
Passenger cars	0.004	0.014	0.006	0.0081
Truck and Lorry	0.006	0.08	0.0366	0.0494
Van	0.005	0.022	0.0128	0.0172

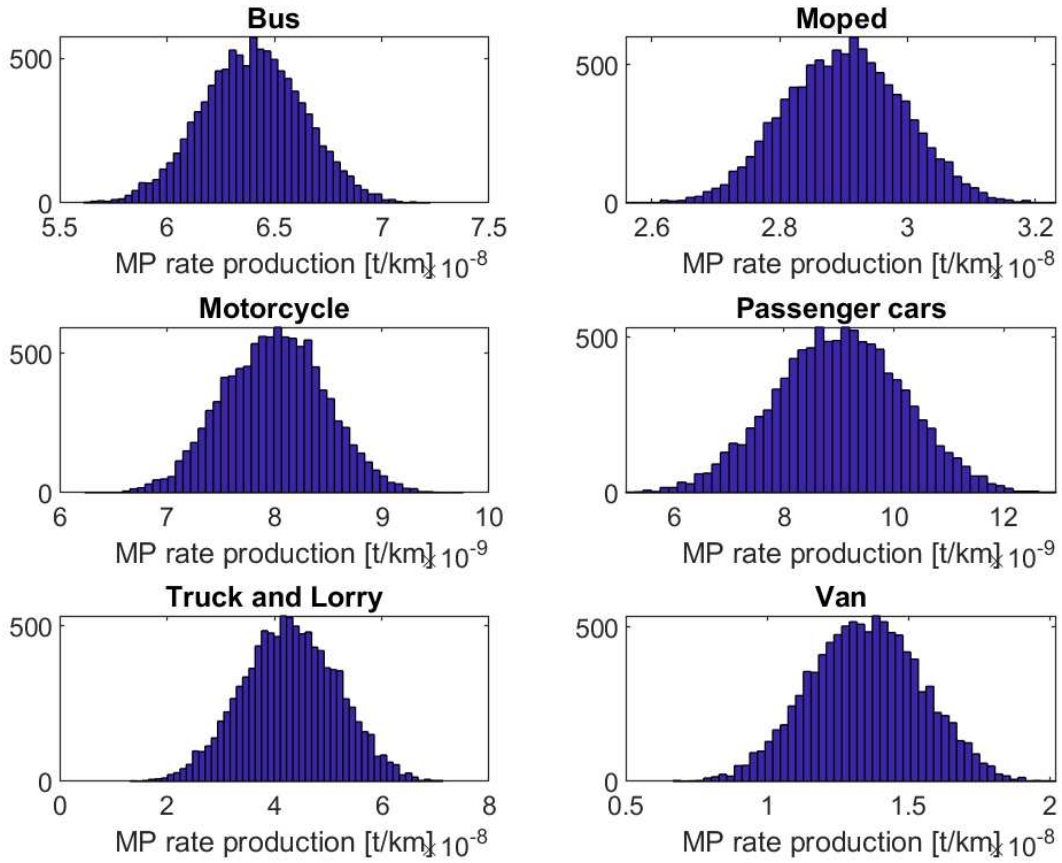


Figure S4. 3 - Gaussian-distribution MPs production rates for the considered vehicle classes in case of urban/rural roads in a random cell of the domain.

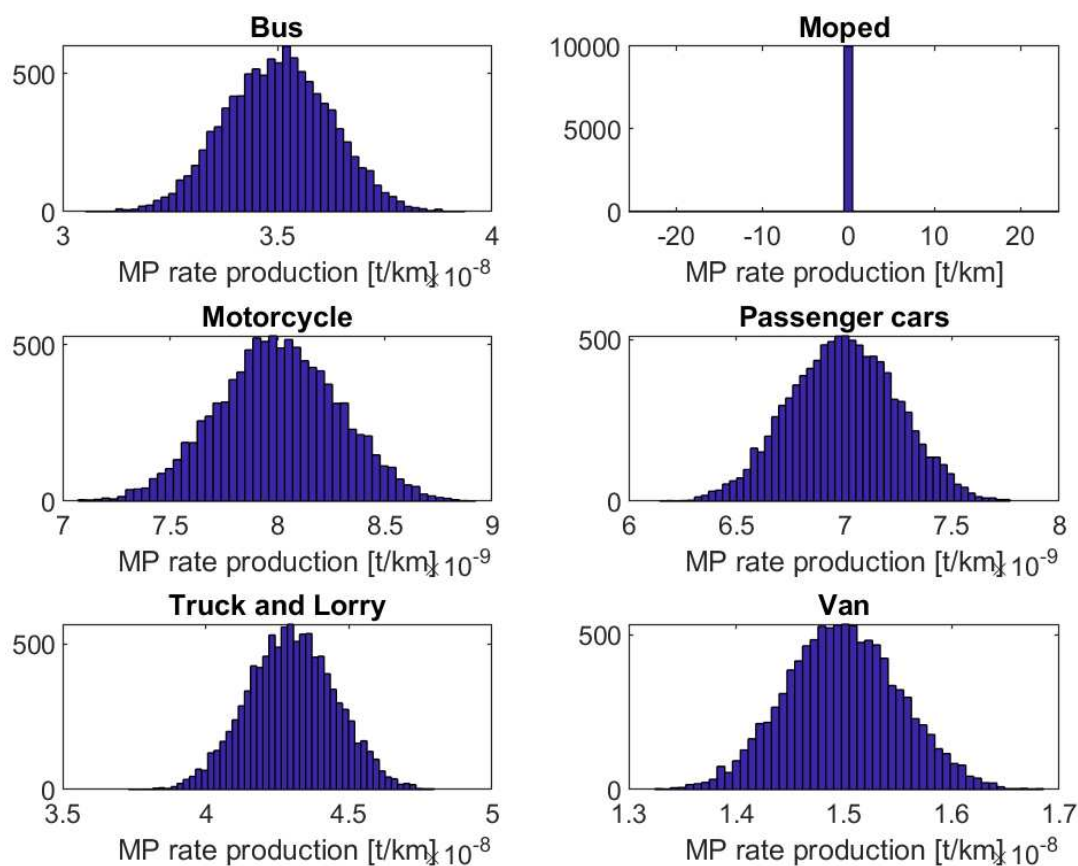


Figure S4. 4 - Gaussian-distribution MPs production rates for the considered vehicle classes in case of highways in a random cell of the domain.

A similar approach as for the *rROAD* for highways was adopted for the mileage (*mROAD*). The COPERT database reports a mean annual value that was used as the mean value of the gaussian-distribution, while the adopted standard deviation was 0.1. Figures S5 and S6 reports the gaussian-distribution built for the simulations.

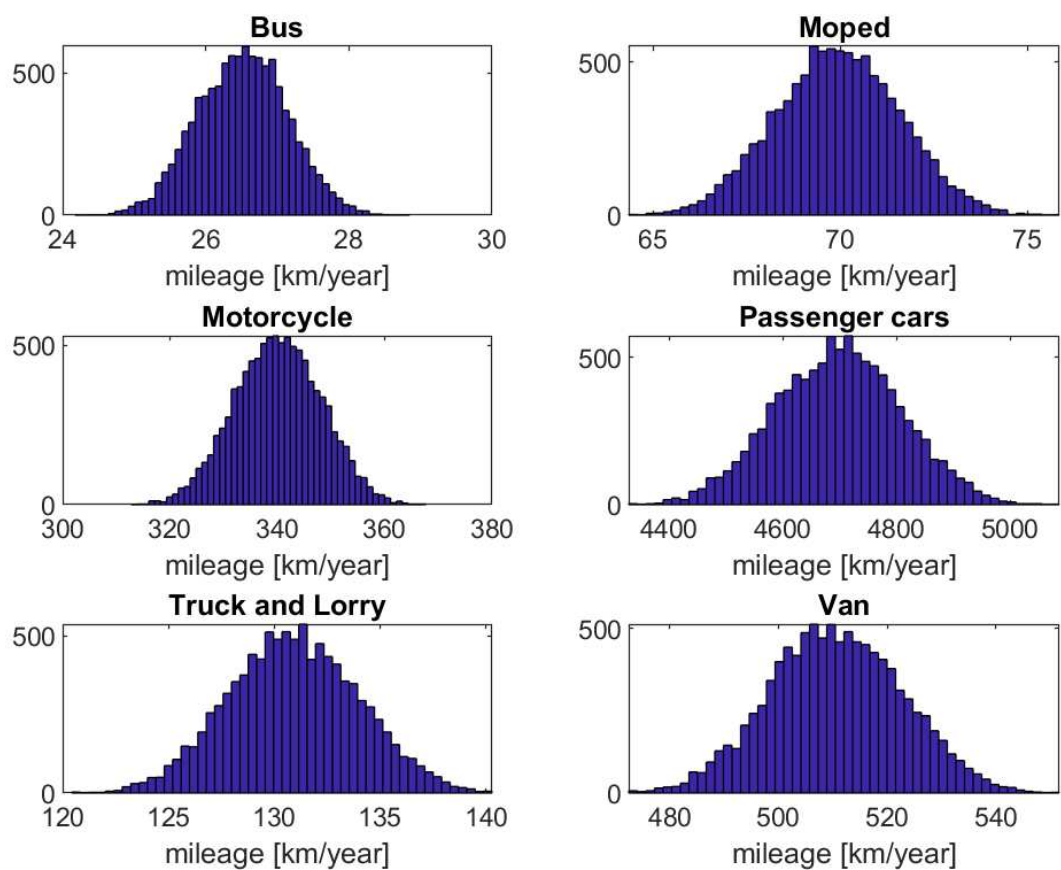


Figure S4. 5 - Gaussian-distribution mileage for the considered vehicle classes in case of urban/rural roads in a random cell of the domain.

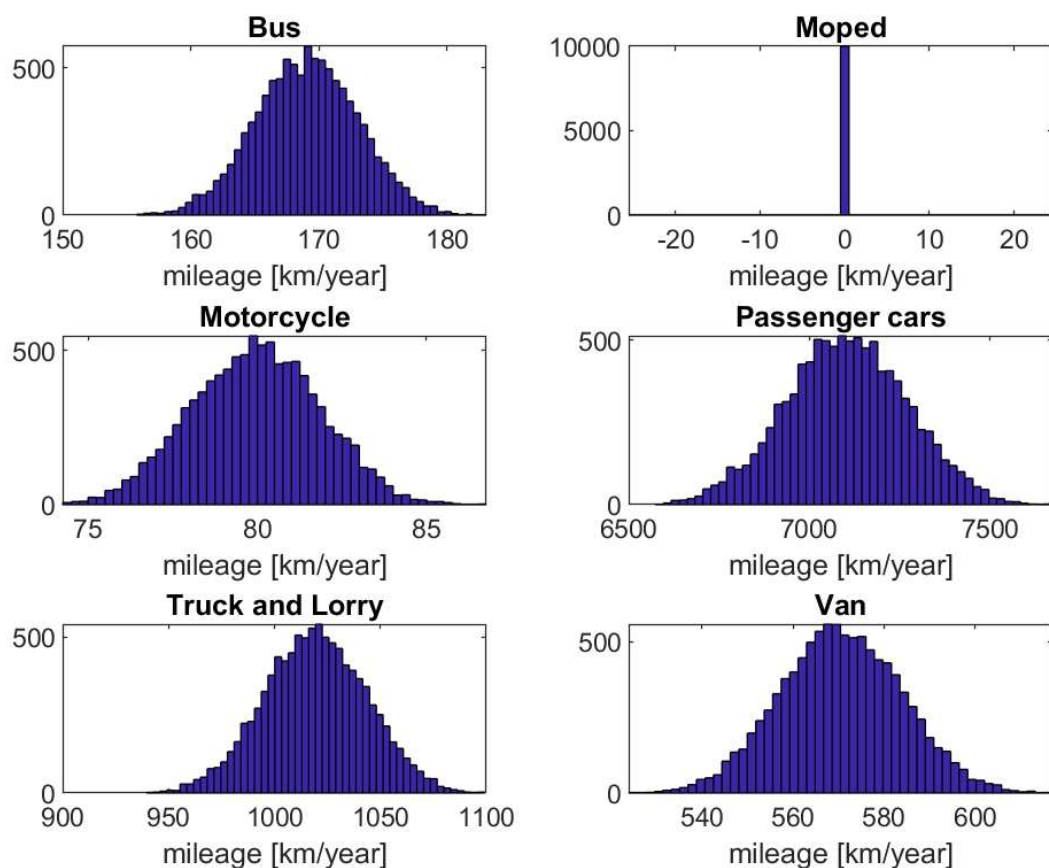


Figure S4. 6 - Gaussian-distribution mileage for the considered vehicle classes in case of highways in a random cell of the domain.

- rp

The values of the retention percentage for the urban source were derived from the literature (Sun et al., 2019). This value strongly depends on the WWTP facilities and could range between 2% and 98%, i.e., 2% for pre-treatments and 98% in case of advanced treatments. Figure S7 shows the gaussian-distribution built for the retention percentage coefficient for the San Colombano WWTP.

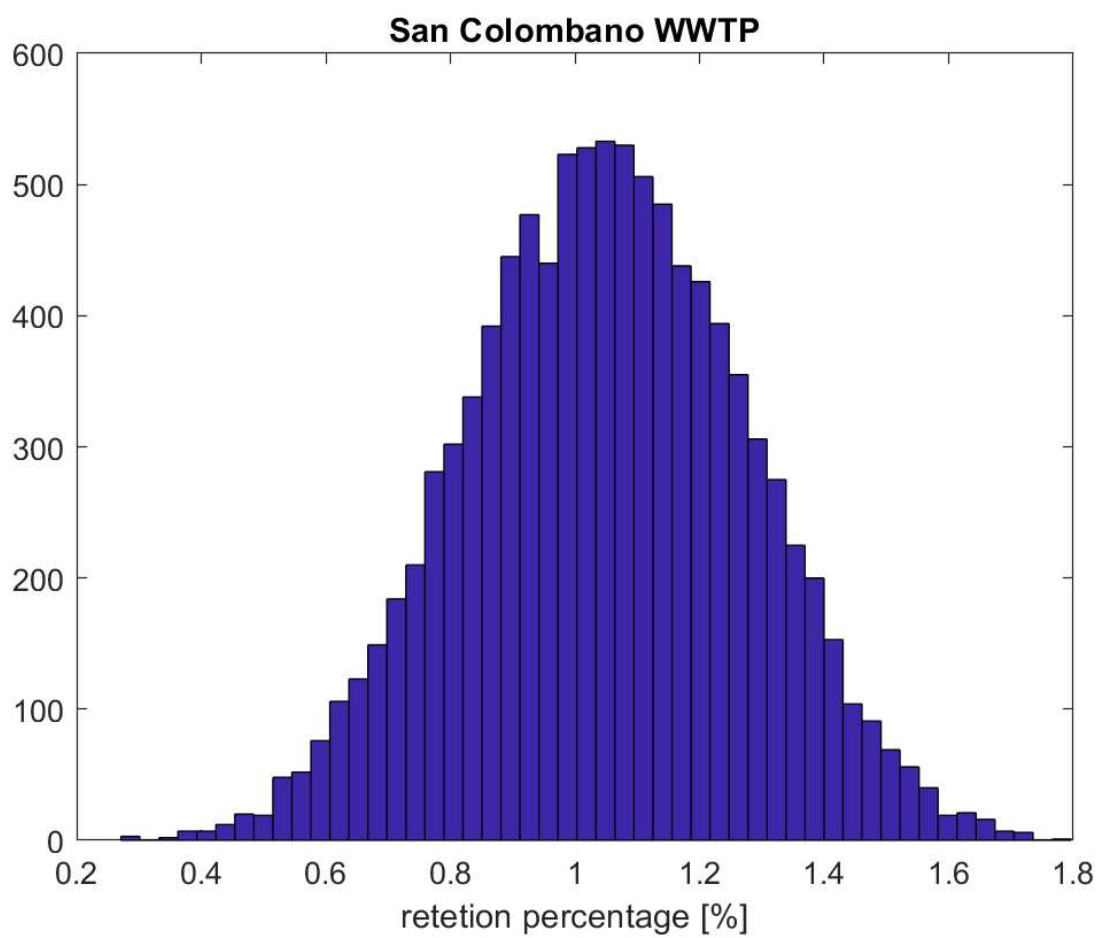


Figure S4. 7 - Gaussian-distribution of the retention percentage coefficient for the San Colombano WWTP.

Table S4. 5 - Annual microplastic (MP) loads delivered to surface waters from each sub-catchment of the Arno River basin, disaggregated by source type. Values are expressed in tonnes per year (t/year) and include contributions from agriculture (surface erosion), non-urban roads (diffuse runoff), combined sewer overflows (CSOs), and wastewater treatment plants (WWTPs, direct discharges). These source-specific inputs represent the values used in the transport model and cumulative analysis

Basin	Agriculture [t/year]	Non-urban Road [t/year]	CSOs [t/year]	Urban (WWTP) [t/year]	Total [t/year]
Usciana	46	0.018	4	18	67
Sieve	88	0.015	1	3	93
Pesa	51	0.011	1	14	65
Greve	17	0.013	1	9	27
Era	276	0.018	1	4	281
Elsa	207	0.023	2	13	223
Valdarno Med.	54	0.016	5	11	70
Bientina	98	0.007	7	30	135
Bisenzio	5	0.006	3	8	16
Valdarno Inf.	45	0.006	2	7	54
Valdarno Sup.	79	0.017	2	13	94
Casentino	80	0.012	1	17	99
Ambra	12	0.002	0	2	15
Ombrone	36	0.015	7	17	60
Chiana	119	0.015	5	13	136
Outlet	39	0.012	1	8	47
Tot [t/year]	1253	0	43	187	

Table S4. 6 - Sub-catchments ranked according to MPs production from different sources: agriculture, urban areas, non-urban roads, and CSOs. Values are expressed in tons per year (t/year).

Sub-catchment	Agriculture [t/year]	Sub-catchment	Urban [t/year]	Sub-catchment	Non-urban road [t/year]	Sub-catchment	CSO [t/year]
Chiana (15)	3127	Chiana (15)	69844	Chiana (15)	0.31	Ombrone (14)	6.98
Bientina (8)	1993	Ombrone (14)	55641	Bientina (8)	0.23	Bientina (8)	6.64
Era (5)	1499	Valdarno Med. (7)	54213	Ombrone (14)	0.23	Valdarno Med. (7)	5.21
Elsa (6)	1493	Usciana (1)	52717	Valdarno Med. (7)	0.20	Chiana (15)	4.72
Ombrone (14)	1051	Bientina (8)	50921	Usciana (1)	0.19	Usciana (1)	4.05
Sieve (2)	850	Elsa (6)	43228	Elsa (6)	0.18	Bisenzio (9)	3.47
Usciana (1)	772	Valdarno Sup. (11)	38873	Valdarno Sup. (11)	0.17	Elsa (6)	2.24
Valdarno Sup. (11)	754	Bisenzio (9)	36305	Sieve (2)	0.13	Valdarno Sup. (11)	2.24
Casentino (12)	728	Sieve (2)	30285	Casentino (12)	0.13	Valdarno Inf. (10)	1.60
Valdarno Med. (7)	615	Valdarno Inf. (10)	23105	Era (5)	0.12	Sieve (2)	1.36
Valdarno Inf. (10)	319	Casentino (12)	19832	Bisenzio (9)	0.10	Casentino (12)	1.12
Pesa (3)	277	Greve (4)	10915	Greve (4)	0.08	Greve (4)	0.85
Ambra (13)	163	Era (5)	10797	Valdarno Inf. (10)	0.08	Era (5)	0.84
Bisenzio (9)	145	Outlet (16)	7810	Pesa (3)	0.06	Pesa (3)	0.64
Outlet (16)	129	Pesa (3)	5087	Ambra (13)	0.03	Outlet (16)	0.53
Greve (4)	106	Ambra (13)	2851	Outlet (16)	0.03	Ambra (13)	0.27

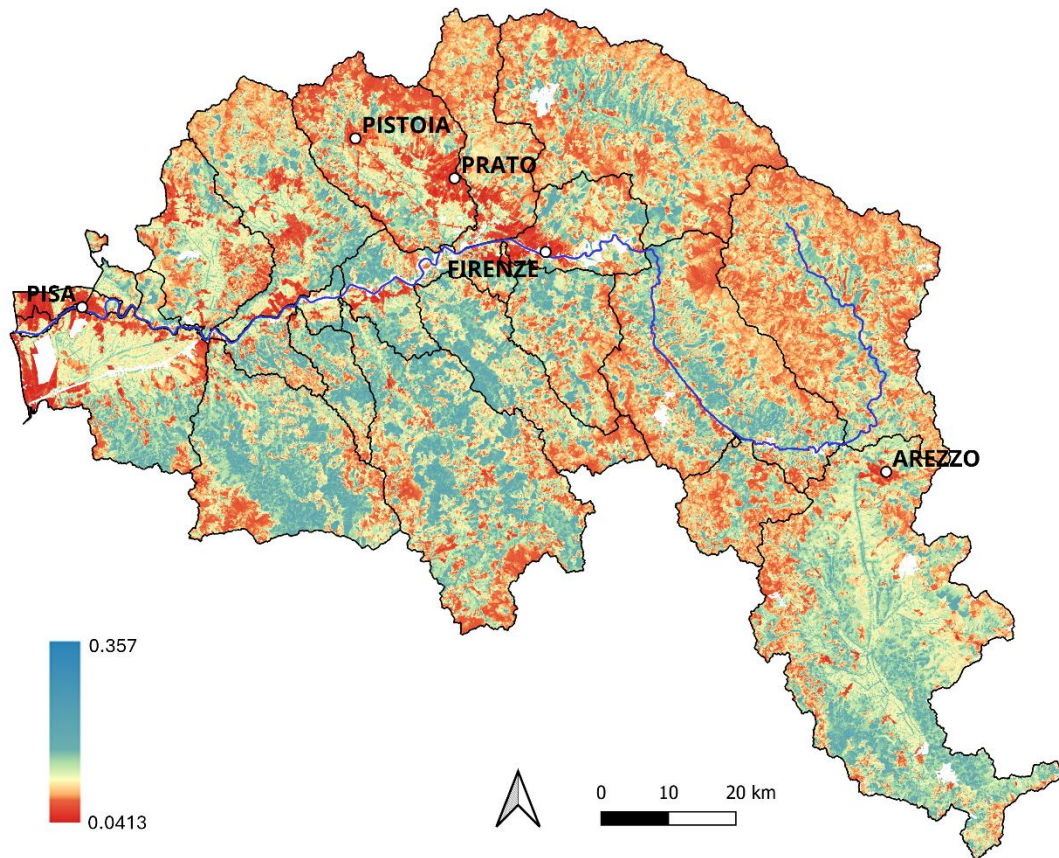


Figure S4. 8 - Spatial distribution of the Sediment Delivery Ratio (SDR) across the Arno River basin, computed using an IC-based model on a 10×10 m raster resolution. Cooler colors (blue) indicate areas with higher SDR values, reflecting greater sediment connectivity and delivery potential, while warmer colors (red) denote areas with lower delivery efficiency. The blue line represents the Arno River, the main watercourse draining the entire basin. Black thin lines delineate the boundaries of the individual sub-catchments. Major urban centers (e.g., Florence, Pisa, Arezzo) are shown for spatial reference.

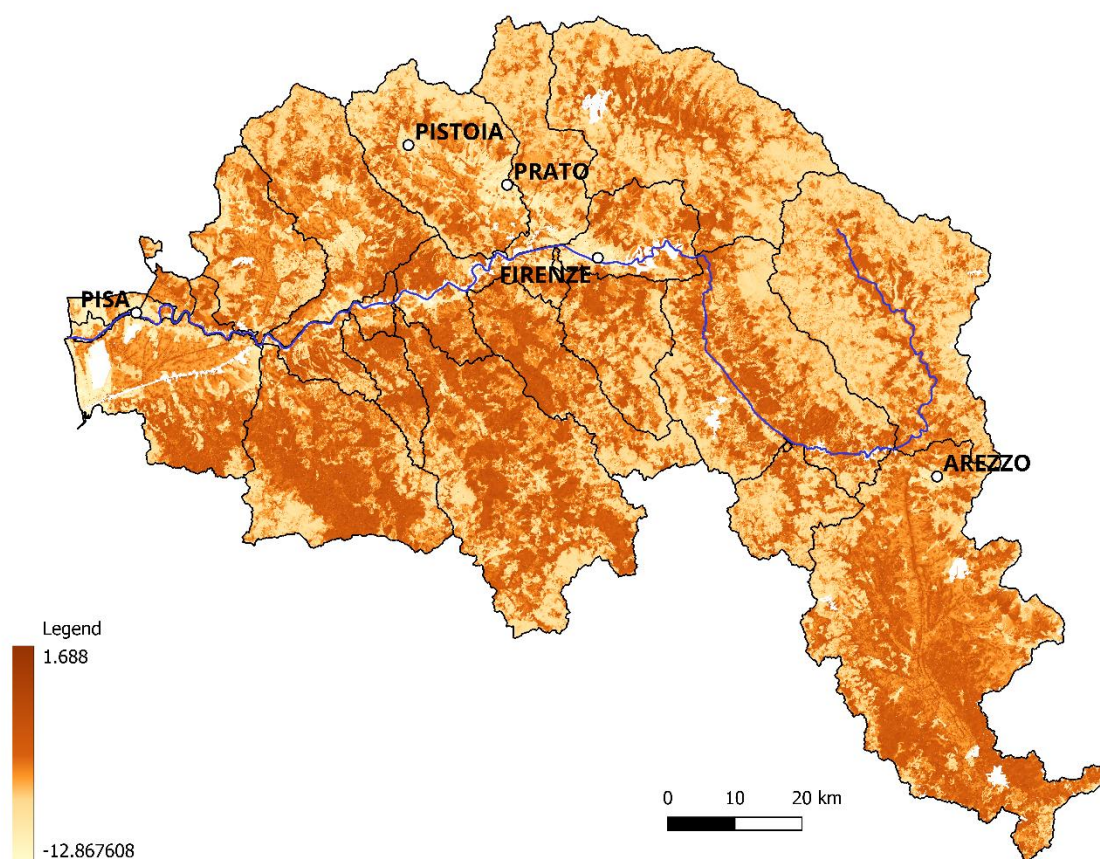


Figure S4. 9 - Spatial distribution of the Index of Connectivity (IC) across the Arno River basin, calculated at 10×10 m resolution. Higher IC values (lighter tones) indicate stronger potential sediment (or microplastic) connectivity toward the drainage network, while lower values (darker tones) identify more disconnected areas. The blue line represents the Arno River, the main channel draining the basin. Black thin lines delineate the sub-catchment boundaries, and major cities (e.g., Florence, Pisa, Arezzo) are included for reference.

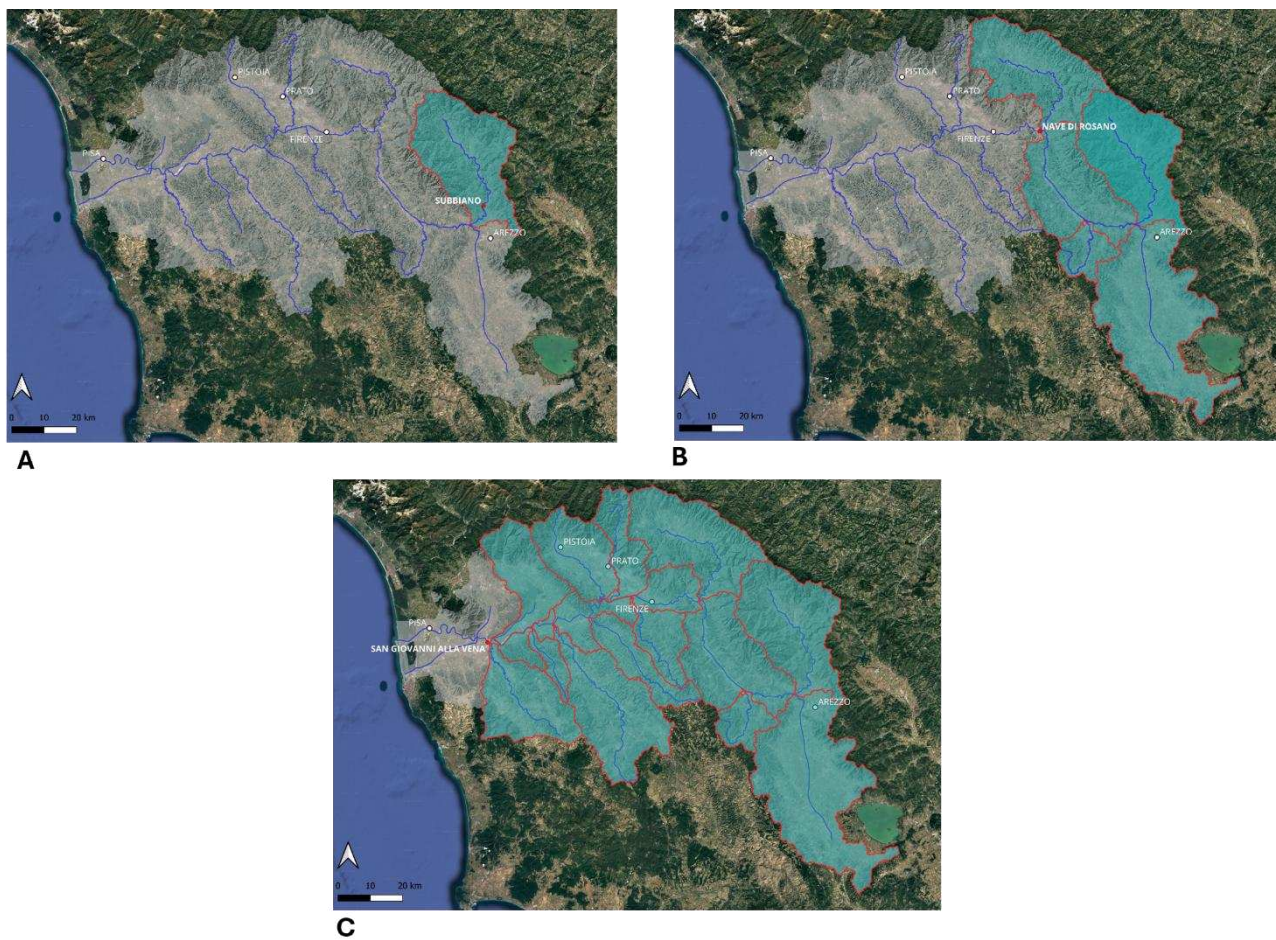


Figure S4. 10 - The figure highlights the sub-catchments of (A) Subbiano , (B) Nave di Rosano, and (C) San Giovanni alla Vena, which were used for model calibration as they provide observed sediment yield data from different sectors of the Arno basin.

5 Conclusion

This final chapter provides a broader reflection on the research presented in this thesis. The thesis was conceived as a deliberately multidisciplinary and multi-scale investigation: spanning from controlled laboratory experiments that isolate the mechanisms of particle retention, to the development of reliable analytical protocols for polymer-specific quantification, and finally to spatially explicit modeling of emissions and delivery across an entire catchment. These levels of analysis are not separate exercises, but complementary disciplinary and scale-dependent perspectives that, when combined, advance a more comprehensive and transferable understanding of MP pollution across freshwater systems (Burns and Boxall, 2018; Koelmans B et al., 2019).

The sections that follow revisit each study not simply as summaries, but as critical reflections on their main contributions, novelty, and limitations. Chapter 2 is reconsidered as a way of unraveling the mechanisms of MP retention by riparian vegetation, situating these results within a broader body of work on sediment and organic matter retention. Chapter 3 is discussed from the standpoint of analytical rigor, addressing the well-recognized need for reproducible and polymer-specific methods that can strengthen the reliability of environmental emission estimates. Chapter 4 is revisited as an attempt to scale up toward basin-level processes, adapting connectivity-based sediment delivery models to the problem of diffuse and heterogeneous MP transport.

After reflecting on each of these contributions individually (Sections 5.1.1–5.1.3), the chapter then turns the global significance (Section 5.2), followed by policy implications (Section 5.3) and future perspectives (Section 5.4). In this way, the conclusion serves not only as a synthesis but also as an interpretive lens, offering a personal perspective on the contribution of this work to the wider scientific and policy debates on freshwater plastic pollution.

5.1 Concluding remarks

5.1.1 Riparian vegetation as a natural barrier

This study demonstrated that riparian vegetation can act as an effective natural barrier for MPs transport, but in ways that are strongly mediated by particle traits, hydrodynamics, and vegetation architecture. The experiments showed that particle density represents a threshold condition: denser MPs ($\rho \approx 1.4 \text{ g/cm}^3$) are predominantly controlled by interactions with the bed and dune-induced recirculation zones, behaving in many respects like coarse sediment. In contrast, lighter MPs ($\rho \approx 1.04\text{--}1.1 \text{ g/cm}^3$) are more sensitive to turbulence and vegetation-induced vertical fluxes, with a greater likelihood of being trapped in higher canopy positions. This distinction emphasizes that retention is not a uniform process, but one that shifts from vegetation-dominated to bedform-dominated as density increases, in agreement with previous findings on sediment and particle transport (Russell et al., 2023; Venditti et al., 2005).

The vertical distribution of trapped MPs further highlighted the interplay between particle buoyancy and canopy structure. Regardless of type, most particles accumulated near the bed and in the lowest canopy layers, but when lower branches were removed, lighter particles were displaced upward and intercepted in higher vegetative zones. This pattern reflects how vegetation architecture can modulate turbulence and vertical fluxes: dense, low-lying structures suppress large eddies and favor near-bed trapping, while sparse or elevated foliage allows particles to be entrained upwards. Such insights resonate with canopy-flow studies showing the role of plant rigidity and complexity in modulating retention efficiency (de Smit et al., 2021; Nepf, 1999).

Turbulence proved to be another critical factor. Retention efficiency declined sharply at higher normalized TKE values, indicating that turbulence not only reduces the probability of particles contacting stems but also promotes resuspension of already retained MPs. In other words, vegetation enhances trapping under low-energy conditions but becomes less effective as turbulence increases, a finding consistent with observations of canopy-induced turbulence affecting particle motion (Wingenroth et al., 2021).

Taken together, these results provide one of the first mechanistic frameworks for understanding MP retention in riparian vegetation. They suggest that vegetation is most effective in filtering MPs when it presents rigid, complex structures close to the ground and when hydrodynamic conditions favor low turbulence and deposition. This has direct implications for nature-based solutions: riparian buffers, already valued for controlling sediments and nutrients, can also be optimized for MP mitigation by prioritizing dense shrubs, juvenile woody species, or designs that replicate their structural traits, especially near banks and depositional areas.

Overall, the study highlights that the role of riparian vegetation in MP retention cannot be reduced to a simple percentage of trapped particles. Instead, it reflects a dynamic interplay between particle density and morphology, canopy architecture, and local turbulence. Recognizing this complexity strengthens the scientific basis for including MPs in the broader discussion of ecosystem services provided by riparian zones and for integrating vegetation into holistic strategies for freshwater plastic pollution mitigation.

5.1.2 Analytical advances with PLE + Py-GC/MS

This study addressed one of the most persistent bottlenecks in MPs research: the reliability of polymer-specific quantification in complex environmental matrices. While spectroscopic methods remain widespread, they are prone to misidentification and often struggle with small particles or organic-rich samples. Thermal approaches such as Py-GC/MS provide greater specificity, but their reproducibility is frequently compromised by sample heterogeneity, matrix interferences, and carry-over effects. Chapter 3 tackled these challenges through a combination of Pressurized Liquid Extraction (PLE) and double-shot Py-GC/MS, focusing not only on developing a workable protocol but also on understanding why certain conditions enhance or compromise analytical reliability.

The experiments revealed that reproducibility begins well before the pyrolyzer: at the stage of sample preparation. Because thermo-analytical methods rely on very small amounts of material, even subtle aggregation or uneven distribution of polymers can generate high variability across replicates. Introducing simple homogenization steps significantly improved consistency, underscoring the importance of sample handling as the foundation for reliable MP datasets. Similarly, the choice of matrix proved decisive. Among the supports tested, Hydromatrix consistently produced the most stable signals and lowest relative standard deviations, avoiding the signal suppression observed with silica or other materials. These findings highlight that “matrix effects” are not abstract artefacts but concrete variables that shape how polymers decompose and are detected during pyrolysis.

At the instrumental level, the optimization of pyrolysis conditions showed that temperature and time strongly influence both sensitivity and polymer-specific responses. The selected condition of 625 °C for 40 seconds maximized the detection of polyethylene while ensuring consistency across other polymers. PET, however, exhibited marked sensitivity to temperature, with some degradation products increasing several-fold across the tested range, a reminder that even under optimized conditions, different polymers respond in different ways. Injection parameters such as split ratios and TDU hold times further shaped the results, particularly by modulating carry-over between runs. Lower split ratios increased peak areas but risked non-linear responses and overestimation of certain analytes, especially for PET and PS. Extended TDU hold times, conversely, reduced carry-over of late-eluting compounds, enhancing reproducibility at the cost of throughput.

Calibration emerged as another critical determinant. Comparing calibration strategies revealed that no single approach is universally optimal. Solid-phase calibrations yielded more consistent results for polyethylene and polypropylene, while solubilized standards provided superior accuracy and linearity for PET and PS. This polymer-dependent behavior emphasizes the need for flexibility and transparency in analytical workflows: researchers must explicitly state which calibration was used and why, rather than treating calibration as a neutral or interchangeable step.

Applied to real samples, the method produced polymer-specific concentration data from wastewater effluents, CSOs, and surface waters. Across all sites, polyethylene and PET dominated, with concentrations in Pontedera CSO samples reaching nearly 750 µg/L for PE and over 50 µg/L for PET. By contrast, polypropylene and polystyrene were consistently near or below the limit of quantification, a finding that aligns with their buoyancy, degradability, and reduced persistence in aquatic environments. More importantly, these applications demonstrated how analytical choices, sample homogenization, matrix selection, pyrolysis parameters, and calibration strategy, directly affect the robustness of concentration data. Without acknowledging these factors, comparisons across sites or between studies risk being misleading.

The significance of this chapter lies in shifting the focus from simply establishing “a method that works” to understanding when and why the method works, and under what conditions it may fail. By systematically identifying and mitigating sources of bias, from sample preparation to calibration, the study contributes to building polymer-specific datasets that are reproducible, transparent, and comparable across laboratories. This is precisely the type of analytical foundation required for scaling up to catchment-wide emission models and for informing regulatory debates on monitoring and mitigation. In this sense, Chapter 3 does not only provide a protocol, but also a framework for analytical accountability: measurements that explicitly incorporate uncertainty and variability, thereby enabling models and policies that rest on firmer empirical ground.

5.1.3 Spatial modelling of MP emissions and delivery

This study demonstrated that MPs dynamics at the catchment scale are not governed by a single source or transport mechanism but by the interplay between local production, geomorphic connectivity, and urban infrastructure. The value of the modelling effort lies not only in estimating fluxes but also in disentangling the problem into three conceptual stages – production, mobilization and effective delivery - and linking these stages to the real geography of the Arno basin.

From the perspective of emissions, the picture that emerged was highly heterogeneous. Intensively cultivated sub-catchments such as Chiana and Bientina generated very high annual production values, in line with European-scale estimates that place agriculture among the dominant diffuse MP sources. At the other end of the spectrum, non-urban roads contributed only fractions of a tonne per year, but in certain basins with high connectivity and traffic intensity, they acted as locally efficient diffuse sources. Urban emissions showed localized peaks: WWTPs provided continuous but spatially concentrated inputs, while CSOs generated episodic but sharp pulses, particularly in highly urbanized sub-catchments such as Ombrone, Bientina, and Valdarno Medio. When normalized by the basin’s population (~2.4 million inhabitants), the total estimated MP delivery corresponded to several hundred grams per inhabitant per year, providing an upper-bound benchmark against national-level estimates of mismanaged plastic waste.

The transition from production to delivery proved to be highly non-linear. Here, the IC–SDR framework offered critical interpretive power: it showed that land use extent is not a reliable predictor of delivered fluxes. Sub-catchments such as Pesa and Era, characterized by steeper slopes and higher SDR values, delivered disproportionately high fractions of their production to the river system. Conversely, flat and poorly connected areas such as Chiana and Bientina, despite high emissions, delivered comparatively little. Delivery efficiencies for agriculture ranged from ~3% to 32%, depending on slope, crop location relative to drainage, and connectivity. For roads, efficiencies reached up to 40% in highly connected basins, but their overall contribution in mass terms remained negligible. By contrast, WWTP and CSO inputs

maintained their relevance where sewer network density was high, highlighting the importance of addressing both diffuse and point sources in a coordinated way.

This “three-stage” perspective (production–mobilization–delivery) carries two main implications. First, it enables a fair comparison across sources: agriculture dominates in terms of annual loads, CSOs define the short-term peaks, WWTPs provide the continuous baseline, and non-urban roads, although negligible in mass, can be locally important where connectivity is high. Second, it offers a decision-making lens: reducing annual loads requires landscape-based measures (erosion control, conservation agriculture, vegetated buffers), mitigating peaks demands urban hydraulic solutions (CSO retention, sewer separation, detention basins), and improving baseline control depends on WWTP upgrades (advanced filtration, tertiary treatment). The hotspot maps produced by the model, identifying Chiana, Bientina, Era, Elsa, and Ombrone as priority areas, are thus directly actionable for management.

Methodologically, the novelty of this work rests on three aspects: (i) a multi-source comparison within a single process-based framework, allowing diffuse and point sources to be consistently evaluated; (ii) the identification of source-specific trade-offs (annual vs episodic, continuous vs pulsed, diffuse vs localized), providing a foundation for portfolio mitigation strategies; and (iii) the spatial prioritization of interventions using physically interpretable indicators (RUSLE, IC, SDR), which move beyond static inventories toward operational hotspot mapping.

At the same time, the study is transparent about its limitations. The modelled estimates represent upper bounds of inputs to the river network, as they do not account for in-stream retention, storage in sediments and floodplains, or degradation and fragmentation during transport. The large discrepancies between sediment-based estimates (cumulative stocks) and water-based measurements (transient fluxes) highlight the need to consider multiple environmental compartments with different residence times. Future refinements should therefore focus on targeted validation in critical sub-catchments, explicit integration of analytical uncertainty (from Chapter 3) into model outputs, inclusion of retention processes in riparian and floodplain zones (from Chapter 2), and the development of event-based or scenario models that capture temporal variability and climate/land-use change.

In summary, this chapter advances MP modelling from static “sum-of-sources” inventories to a spatial diagnostic of delivery pathways, where sources are evaluated through the lenses of connectivity and morphology. This structure allows managers to identify where to act to reduce annual fluxes, when to intervene to dampen episodic peaks, and how to combine green and grey infrastructures. Most importantly, it complements the other two pillars of this thesis: the polymer-specific, uncertainty-aware datasets of Chapter 3 and the mechanistic insights into vegetation retention from Chapter 2. Together, these elements can transform delivery estimates from broad approximations into scenario-sensitive, policy-relevant assessments.

5.2 Global significance of the thesis

Over the last decade, research on MPs has grown exponentially, yet knowledge remains fragmented. Laboratory experiments often provide mechanistic insights but struggle to translate to natural systems; analytical protocols have advanced in sensitivity but remain inconsistent across laboratories, limiting comparability; and models offer large-scale perspectives but are frequently built on uncertain or poorly constrained data. These silos mean that results are often not directly comparable and cannot easily be integrated into coherent frameworks. This challenge has been identified as one of the central obstacles to progress in the field (Horton et al., 2017; Koelmans B et al., 2019) and has been reiterated in more recent reviews calling for system-level, interdisciplinary approaches to plastic pollution (Thompson et al., 2024). In freshwater contexts, (Talbot and Chang, 2022) emphasize that MP knowledge remains scattered across sources, transport, and impact, underscoring the need for systematic methodologies that bridge these gaps. Pal et al., 2025 highlight the importance of linking MPs' distribution, transformation processes, and ecotoxicological risks through integrated analytical frameworks. This studies addresses that gap by juxtaposing three methodological pillars, experimental, analytical, and modelling, each essential but usually disconnected.

The originality of this thesis is not only in the novelty of each individual chapter, but in their juxtaposition and conceptual integration. The experimental study clarified the mechanisms by which vegetation structures influence MP retention, showing that particle density, morphology, and turbulence shape which fractions are immobilized and where. The analytical study provided a reproducible and transparent protocol for polymer-specific quantification, explicitly exposing recovery rates, calibration effects, and matrix interferences that are often overlooked. The modelling study scaled these perspectives up to a large river basin, revealing how different sources interact across space and time and how the trade-offs between continuous (WWTPs), episodic (CSOs), and diffuse (agriculture) emissions generate heterogeneous delivery pathways. Each of these strands offers an important piece of evidence; together they illustrate how MPs must be understood as both source-specific and scale-dependent.

Seen in this light, the thesis contributes to what Thompson et al., 2024 and others describe as a necessary whole-system perspective on plastics. MPs are not a single-source contaminant; they are the result of interactions between production, use, environmental transport, and retention processes (Horton and Dixon, 2018). Policies that aim to mitigate their impacts require evidence that links processes at the particle and habitat level to dynamics at the landscape and societal scale (Thacharodi et al., 2024). This thesis does not claim to close that circle, but it demonstrates concretely how different methodologies and scales can be brought into dialogue, and it identifies the pathways by which stronger integration could be pursued in the future.

The global significance of this work therefore lies in its ability to frame MPs as a cross-scale, multidisciplinary problem that demands integrated approaches. Horton et al. (2017) and

Koelmans et al. (2019) emphasize that the lack of integration between laboratory studies, monitoring campaigns, and modelling frameworks is one of the main reasons why estimates of MP fluxes vary by orders of magnitude. Besseling et al., 2019 similarly stress that local processes such as retention and resuspension are rarely connected to basin-scale mass balances. Recent reviews (Imbulana et al., 2024) call explicitly for multi-scale, multidisciplinary approaches that can link particle-scale behavior with system-wide assessments. In this context, the originality of the present thesis lies in offering a concrete example of how such integration can be pursued, even if only conceptually, within a single research project. By providing a case study of how experimental, analytical, and modelling perspectives can complement and reinforce each other, the thesis responds directly to this challenge.

From a broader viewpoint, this integrative stance also carries implications for environmental governance. Without robust, interdisciplinary evidence, mitigation measures risk being partial or misdirected. For example, end-of-pipe solutions alone will not address diffuse sources; conversely, diffuse-source control without understanding episodic discharges may miss critical peaks of contamination. By linking mechanistic insights with polymer-specific quantification and catchment-scale modelling, the thesis contributes to the type of evidence base that policymakers increasingly require: not isolated datasets, but frameworks that can inform both annual load reductions and event-based risk management.

Finally, the thesis also has significance for the trajectory of MP research itself. It demonstrates the feasibility, and the necessity, of workflows that move iteratively between process studies, measurements, and models. Even where the three strands were not fully coupled in practice, the conceptual and methodological groundwork for such integration has been laid. This echoes the argument of Thompson et al. (2024), who stress that advancing the field will require connecting particle-scale processes, analytical reliability, and system-wide assessments into coherent, transferable frameworks. By articulating these connections within a single doctoral project, this work provides a step toward that vision.

In summary, the contribution of this thesis lies not only in the results it presents, but in the framework it represents: a multidisciplinary and multi-scale approach that highlights the need for integration across methods, disciplines, and scales. Such integration is essential if research on MPs is to evolve from fragmented studies to comprehensive, evidence-based strategies capable of informing policy and guiding mitigation. In this way, the thesis provides both a scientific contribution and a conceptual blueprint for how MP research can continue to develop in the coming years.

5.3 Policy and Environmental Implications

The findings presented in this thesis carry several implications for environmental policy and the design of mitigation strategies. Microplastic pollution, especially in urbanized freshwater

systems, is not only a scientific challenge but also an emerging priority for regulatory frameworks aimed at protecting water quality, ecological integrity, and public health (OECD, 2019; “UN Environment Programme,” 2021).

One of the most relevant contributions is the demonstration that natural features, such as riparian vegetation and sedimentary structures, can significantly influence the retention and fate of MPs within river corridors. The results obtained in Chapter 2 support the idea that vegetation strips, already widely promoted for sediment and nutrient control, may also act as effective nature-based solutions (NBS) for microplastic retention. This highlights an opportunity to repurpose existing green infrastructure guidelines to also mitigate plastic pollution, aligning with the principles of ecological engineering and integrated watershed management (Gallitelli et al., 2020; Martuscelli et al., 2025).

Moreover, the experimental findings on particle-specific retention offer critical input for refining environmental risk assessments. Since not all microplastics behave similarly, it is essential that policy moves beyond general mass-based thresholds and considers polymer type, density, and morphology, parameters that were shown to strongly affect transport and retention (Koelmans et al., 2019; Besseling et al., 2019). This is particularly important when assessing the efficacy of WWTPs or wetlands, where certain polymers may be retained more efficiently than others.

At a strategic planning level, the spatial model presented in Chapter 4 supports the prioritization of intervention areas by identifying critical hotspots for microplastic (MP) delivery to river systems. Such spatially explicit risk assessments are particularly valuable for local authorities and water management agencies. In contexts where financial and operational resources for MP monitoring and remediation are limited, the ability to target high-risk zones enhances both the efficiency and the impact of mitigation strategies (van Emmerik and Schwarz, 2020).

Importantly, this thesis also contributes to the ongoing debate around standardization. The analytical workflow presented in Chapter 3, although not intended as a formal method, aligns with the growing call for harmonized and validated approaches to MP quantification in complex matrices (Lehner et al., 2019; Renner et al., 2018). By systematically assessing extraction efficiency and matrix effects, the study provides a model for how uncertainty can be integrated into environmental reporting and decision-making, which is especially relevant in regulatory contexts.

Finally, by showing how multiple sources contribute simultaneously to riverine MP loadings, including agriculture, roads, CSOs, and WWTPs, this thesis strengthens the case for multi-source, integrated mitigation policies. This supports the view that end-of-pipe solutions alone are insufficient, and that policies must include upstream prevention, such as restricting specific polymers, improving urban drainage, or incentivizing sustainable textile production (Hartmann et al., 2019; Iyare et al., 2020).

5.4 Future Perspectives

While this thesis has addressed several pressing knowledge gaps in microplastic pollution research, it also highlights critical areas where future efforts should be directed. First and foremost, the integration of analytical, experimental, and spatial approaches remains an underexploited avenue. Although this work provided insights into how quantification methods influence emission estimates and spatial modeling, future studies could further strengthen this link by designing workflows in which analytical uncertainty is directly propagated into model simulations, helping to establish error-aware risk assessments for freshwater plastic pollution (Burns and Boxall, 2018; Lehner et al., 2019).

Another important direction concerns temporal dynamics. Most studies operate under steady-state assumptions or fixed hydrological conditions. However, microplastic transport is episodic, often dominated by rainfall-driven pulses, CSO discharges, and flood events (Rehm et al., 2021). Developing event-based monitoring campaigns and transient models capable of capturing short-term variability will be essential to better characterize peaks in MP flux and assess exposure risks for aquatic organisms and water resources.

Further work is also needed to bridge laboratory and field scales. While controlled flume experiments, such as those conducted in Chapter 2, offer a high degree of replicability and mechanistic insight, they cannot fully capture the heterogeneity of natural systems, particularly the interplay of turbulence, sediment sorting, and variable vegetation structures. Multi-scale field validations and mesocosm studies should be encouraged to verify key mechanisms such as retention in riparian zones, depth-specific infiltration, and polymer-specific remobilization during storms (Enders et al., 2020; Ghinassi et al., 2023).

On the analytical front, continued efforts toward method harmonization and inter-laboratory comparisons will remain crucial. In particular, Py-GC/MS protocols, shown in this thesis to yield reliable results across different polymers, could benefit from shared reference materials and calibration standards that facilitate cross-study comparability (Renner et al., 2018; Tagg et al., 2015). The growing recognition of fiber loss during filtration or sieving steps, as well as matrix carry-over and misidentification, underscores the need to design methods that are both polymer- and matrix-aware.

Additionally, the diversity of MP morphologies (e.g., fibers, fragments, foils) should be more explicitly integrated into future modeling frameworks. The spatial model developed in Chapter 4 currently operates under bulk assumptions, but further development could introduce class-specific SDR coefficients, differentiating not only by land use but also by particle behavior, something increasingly feasible with high-performance computing and remote sensing integration (Sullivan et al., 2023).

Lastly, a more explicit effort should be made to align MP research with environmental justice and socio-economic priorities. Urban watersheds, particularly in low-income or high-density

areas, often face disproportionate MP loading due to limited infrastructure or regulatory oversight. Future studies should include equity considerations, ensuring that risk assessments and mitigation strategies reflect both ecological vulnerability and human exposure potential (Rochman, 2018; Wagner et al., 2014).

In summary, this thesis sets the stage for future research that is more interdisciplinary, multi-scale, and systems-oriented, combining methodological rigor with real-world applicability to better address the pervasive issue of microplastics in freshwater environments. Understanding and managing MP pollution depends on integration across scales and disciplines: by linking process-level mechanisms with uncertainty-aware measurements and spatial diagnostics of delivery, this work bridges the gap between local dynamics and basin-wide relevance. The practical consequence is a dual focus for action, green infrastructure and riparian retention to moderate transport locally, and source-specific interventions (WWTP upgrades, CSO control, erosion management) where connectivity amplifies delivery. While uncertainties remain, the framework presented here is transferable and improvable, outlining how process, measurement, and model can iterate toward more reliable assessments and, ultimately, more effective mitigation.

5.5 References

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