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Microplastics Origins and Risks: A Methodological Protocol for Source Identification, Apportionment, and Environmental Risk Assessment in Ahvaz Metropolis, Iran

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A R T I C L E I N F O

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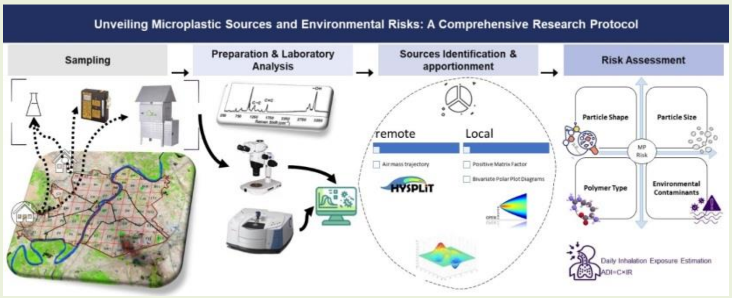
GRAPHICAL ABSTRACT

Introduction: Airborne microplastics (MPs) pollution poses substantial risks to environmental and human health. This research protocol outlines a framework for investigating airborne MPs in Ahvaz, Iran, with objectives including their identification, characterization, and source attribution using chemical signature tracing and receptor modeling.

Methods and Analysis: The study employs a dual approach of passive and active sampling supported by laboratory analysis and multivariate modeling. Sampling locations were selected to represent diverse urban settings, ensuring comprehensive spatial coverage and data reliability. The protocol included meticulous sample collection, volume estimation, and laboratory analysis to ensure the accurate identification and assessment of MPs pollution.

Expected Outcomes: This protocol aims to reveal types, distribution, sources, and potential health risks of airborne MPs in Ahvaz. Insights provided strategies to mitigate MPs emissions in urban areas and a foundation for future research.



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Introduction

Microplastics (MPs) pollution has emerged as a pressing environmental challenge, permeating various ecosystems and eliciting significant global concern [1]. Airborne MPs, specifically, represent a complex and inadequately understood aspect of this issue [2, 3]. These minuscule particles not only pose ecological risks but also potentially impact human health through inhalation exposure [1, 4, 5].

Emerging evidence suggests that inhaled MPs

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can deposit in the respiratory tract, potentially leading to inflammation, oxidative stress, and exacerbation of pre-existing respiratory conditions, such as asthma and chronic obstructive pulmonary disease (COPD) [6, 7]. Additionally, MPs are known to adsorb toxic substances, including heavy metals and persistent organic pollutants, on their surfaces [8]. Once inhaled, these chemical contaminants may desorb in lungs, further increasing the toxic burden on the human body. Long-term exposure to airborne MPs is a growing public health concern, particularly in urban and industrial environments where particulate pollution is already prevalent [9].

It is imperative to understand the origins, distributions, and hazards associated with airborne MPs to effectively manage the environment and protect public health.

This study introduces a novel framework by integrating chemical fingerprinting and receptor modeling approaches to identify and apportion local and remote MPs sources. Unlike earlier studies, our methodology incorporates both passive and active sampling techniques across diverse indoor and outdoor environments tailored to unique urban characteristics of Ahvaz, a city subject to severe environmental challenges.

This protocol combines detailed spatial and seasonal analyses with advanced receptor models, such as Positive Matrix Factorization (PMF), and leverages bivariate polar plots to map emission dynamics with meteorological determinants. Furthermore, this is the first study in the region to estimate the ecological and health risks associated with inhaled MPs, providing a comprehensive risk assessment aligned with global standards.

Nonetheless, there is a dearth of comprehensive methodologies for accurately identifying and attributing sources and assessing risks. The objective of this research protocol is to bridge these critical gaps by presenting an integrated framework that aims to comprehensively evaluate airborne MPs in Ahvaz City, Iran. These innovations make this study a critical contribution to addressing urban MPs pollution and its implications for environmental health. This study aims to illuminate the intricate nature of MPs pollution and contribute to well-informed strategies for mitigation and environmental preservation.

Method details

Study Design

The primary focus of the study was to comprehensively identify and characterize MPs, considering their size, concentration, shape, and polymer types. This investigation encompassed various environments in different areas of Ahvaz City, including both open and closed spaces. The study also assessed the impact of climatic and seasonal factors on the sedimentation rate of MPs. Furthermore, potential sources of MP production were identified and their contributions to emissions were evaluated. An essential aspect of the study was estimating human exposure levels and assessing the health risks associated with exposure to MPs.

To achieve these objectives, two distinct methods of sampling can be employed. The first method is passive dry particle deposition, which involves collecting naturally deposited MPs from various locations without any external manipulation. The second technique is active sampling, which involves using a pump to actively draw air samples. These methods will be systematically applied across different locations, including riverbanks, city centers, industrial zones, and specific enclosed spaces such as residential buildings, service establishments, commercial premises, offices, and production units. By applying these methodologies and sampling techniques, we aimed to comprehensively investigate the presence and characteristics of MPs in diverse settings within Ahvaz City, enabling us to effectively achieve our research objectives.

Detailed insights into the selection of sampling locations, the determination of sample sizes for each method (passive and active), and an outline of the sampling methodology are presented. This approach was designed to ensure the robustness and representativeness of the collected data.

Passive Sampling Methods and Sample Size

A. Selection of Passive Sampling Points

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In the context of PMF models, meticulous selection of sampling locations is pivotal for identifying potential pollutant sources. Typically, this entails choosing sites that represent distinct source compositions in specific geographic areas [10, 11]. Multiple locations, each characterized by varying attributes such as traffic intensity or urban–rural backgrounds, are often considered to discern emission contributions [12]. However, given the absence of prior studies addressing MPs emissions in Ahvaz, a unique approach was adopted for this investigation.

The passive MPs sampling strategy encompasses diverse areas across Ahvaz city, ensuring the representation of the regional source distribution. To achieve this goal, the city was methodically divided into 65 regions, each measuring 2.5 ×2 km, employing ArcGIS version 10.8. Subsequently, the minimum required samples for each of these defined areas were calculated. Subsequently, 30 sampling points were carefully selected across these designated areas on the map, denoted from A to G (Figure 1).

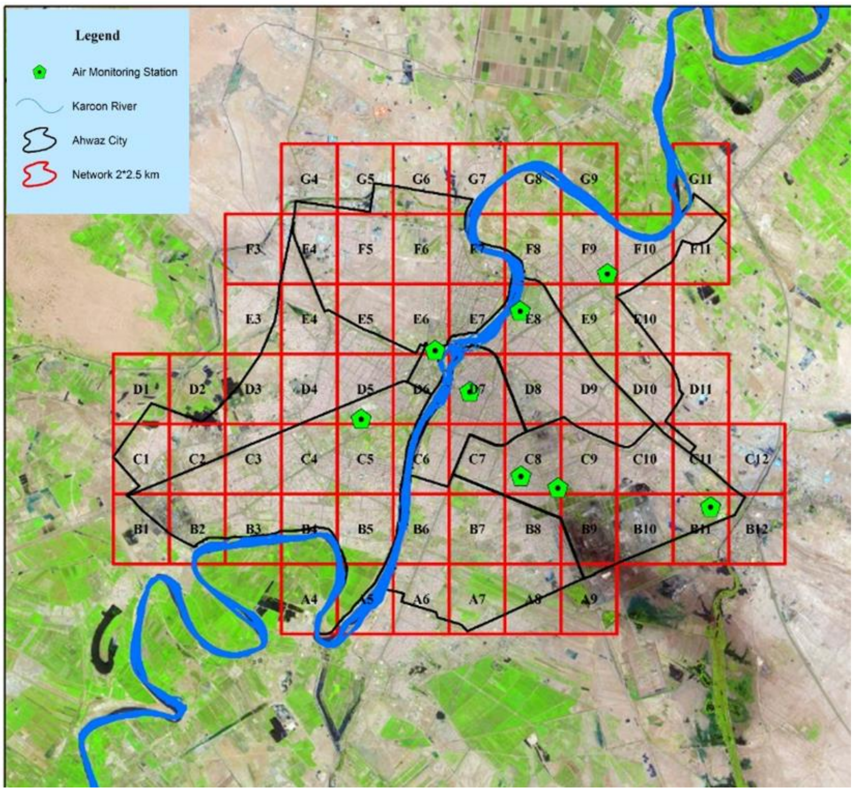

Figure 1: Map depicting eight major districts of Ahvaz city, indicating the anticipated locations for passive sampling.

At each of these designated points, sampling was done during both cold (February) and warm (August) seasons, and this process continued over a continuous week (7 days), with sampling taking place during the first and last weeks of each month. The selected sites (outdoor) for sampling was typically located on building rooftops strategically positioned away from large structures such as tall towers that might unduly influence MPs deposition in their immediate vicinity [13, 14]. The sampler height was meticulously considered to ensure a comprehensive assessment of the MPs concentration, morphology, and type [15-18]

Additionally, a fixed sampling site that is operational throughout the year was established to gauge the average annual MPs sedimentation rate, reported as MPs/m^2/day. This approach also enabled the evaluation of the influence of climatic variables on MPs deposition. Ideally, this fixed location was situated in a building at Ahvaz

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Jundishapur University of Medical Sciences, Ahvaz. Furthermore, in an effort to comprehensively identify and analyze MPs in enclosed and sensitive spaces such as residential, service, commercial, office, and production areas, the sampling strategy encompassed locations near open-space sampling points within the city.

B. Estimation of Sampling Intervals (Number of Samples) for the Passive Method

The sampling duration at each station was directly influenced by the rate of MPs deposition. Optimizing for a shorter sampling period not only increases the sensitivity and accuracy, but also allows for the detection of variations induced by meteorological fluctuations. In the realm of multivariate techniques, such as PMF, an increased number of samples enhances the capacity to identify comprehensive sources over time. According to the recommendations of the PMF software, a minimum of 50 to 100 samples is considered prudent [19]. Determining the sampling intervals requires considering factors such as pollutant concentration, precipitation events, dust levels, and logistical access to the sampling site. These intervals are proportionally linked to local pollutant concentrations and other site-specific factors, making them inherently variable. The optimal duration of sampling intervals was ascertained through preliminary tests, recognizing that previous studies have suggested different sampling frequencies, ranging from biweekly to monthly.

Considering the large number of sampling stations (30 points) and the absence of a universal standard for MPs sampling, collecting samples more than twice a month at each station is impractical. Therefore, a sampling approach featuring a seven-day interval will be employed in both cold and warm seasons, with sampling sessions conducted during the first and second halves of each month. This methodology was based on the dual purpose of monitoring within-month changes and amassing 120 samples across two distinct seasons. For a fixed university-based station, sampling was done on a weekly basis, adding to the comprehensiveness of the dataset. Altogether, this approach culminated in the collection of 288 samples drawn from both open and closed environments, facilitating the meticulous analysis of MPs.

C. Procedure and Approach

Sediment samples were collected from both open and closed environments in accordance with the standard method (ASTM D1739-98)[20]. A conventional funnel-bottle design was used for sample collection. Glass bottles or stainless-steel funnels were deployed in open spaces. Glass sampling containers were strategically positioned on building rooftops. In closed environments with substantial human activity, such as homes or office spaces, 250 ml glass containers fitted with metal or foil lids were placed at a height of 1.2-1.8 meters, corresponding to the average breathing zone of individuals[21].

In the passive sampling approach, both in open and closed environments, the glass containers were exposed to atmospheric conditions for precisely seven days, covering all days of the week, including weekends and holidays. During this period, residents were requested to maintain their usual daily routines without altering their activities. After the designated sampling duration, the glass containers were securely sealed with foil covers and carefully transported to the laboratory. Upon arrival at the laboratory, the collected vials were stored in a dark room at room temperature to preserve sample integrity[22].

Active Sampling and Measurement (Using a Pump)

To evaluate the ecological and health risks associated with inhalation exposure to MPs, a combination of passive and active sampling methods may be necessary [23]. Passive sampling offers extended-term insights into the presence of MPs in the environment; however, it may underestimate airborne MPs concentrations [24]. Conversely, active sampling offers detailed data on particle size, shape, and composition [25]. Therefore, in addition to passive sampling, active air sampling was conducted in both open and closed

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environments, employing a pump for measurement. This active sampling approach provided valuable data for assessing MPs concentrations and their potential health implications. Sampling was executed at various heights, including ground level and elevated positions such as rooftops, ensuring a thorough analysis of the airborne MPs.

Active sampling was conducted at four strategically chosen stations representing distinct environments including areas adjacent to the river, high-traffic zones, industrial centers, and residential areas. This multi-pronged approach enables a detailed quantitative and qualitative comparison of MPs collected via both passive and active methods. Active sampling was run concurrently with passive sampling during cold and hot seasons and was conducted weekly.

For ground-level sampling, the sampler was positioned at a height ranging from 1.2 to 1.8 meters, corresponding to adult breathing levels [26]. In some cases, the sampler may be elevated to heights between 30 and 80 m to capture a broader perspective of the MPs distribution [27]. In closed environments, sampling points was strategically chosen in proximity to passive sampling sites (labeled A to G), encompassing both cold and warm seasons to ensure a comprehensive understanding of airborne MPs in various contexts.

D. Sample Collection and Airflow Rate Specification

1.Sampling tools

In both open and closed environments, MPs samples were collected using pumps and sample filters. In open spaces, a Tisch Environmental $PM_{2.5}$ high-volume air sampler equipped with a 2.5-micron nozzle was used for the collection process [28]. For closed space sampling, individual sampling pumps (SKC model, USA) with quartz microfilters (20.3 × 25.4 cm, Pall, pore size: 2.5 µm) were used [17]. The volume and number of samples were critical factors in this process.

2.Airflow Rate and Sample Volume

Sample volumes can vary significantly across studies, ranging from a few cubic meters sampled in one hour to several thousand cubic meters collected over 45 hours. To ensure accurate estimation of MPs concentrations, the minimum recommended sample volume was set at no less than 70 cubic meters. The specific sampling duration depended on various factors including the pump flow rate, pollutant concentration, organic content, and the research question at hand. In this study, 24-hour sampling periods were employed at flow rates ranging from 10 to 100 liters per minute for open spaces [29].

For closed office and commercial spaces, sampling was conducted continuously during regular office hours, following the OSHA Technical Manual Section II, Chapter 1 (https:// www. osha.gov/ otm/section-2-health-hazards/ chapter-1). In residential houses, sampling was taken place for 8 h while residents were present, maintaining a flow rate of 5 L/min. The objective was to gather 60 active samples from closed environments across two seasons, distributing the pumps among five locations. In open spaces, which included four designated points, 24-hour sampling was carried out for seven days at each location and repeated over two seasons, resulting in a total of 56 active samples. Consequently, this study collected a dataset comprising 116 samples from both closed (60 samples) and open (56 samples) environments. The recorded concentrations were reported in terms of MP/m^3 for the pump-based sampling method.

Laboratory Analysis of Samples

E. Sample Preparation

Analysis of atmospheric samples required a mixture of organic and mineral materials. To effectively separate MPs from mineral particles and eliminate organic substances, a precise pretreatment process was implemented [30]. Each sample was appropriately labeled before proceeding. Pretreatment involved the following steps:

1.Hydrogen Peroxide Washing: The samples were subjected to triple washing with a 30% hydrogen peroxide solution (H_2O_2) to eliminate any biological materials other than MPs.

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Thorough washing was crucial to ensure complete removal [27, 31].

2.Incubation: Subsequently, the samples were placed in an incubator at 55°C for 3 days at a rotational speed of 65 revolutions/min. This step was instrumental in removing polymers with lower degradation temperatures while preserving MP particles [32]. It effectively eliminates biological materials and natural organic substances, such as skin and animal hair [33].

3.Filtration: The contents were then filtered using a glass vacuum filter equipped with a mixed cellulose membrane (MCE) filter measuring 47 mm in diameter and featuring a pore size of 5 mm. During this process, the particles on the filter were collected and prepared for MP analysis.

4.Rinsing: The vacuum filter device and glass containers were washed three times with 200 ml of distilled water to prevent any sticking or contamination.

5.Drying: Following filtration, the filters were placed in glass Petri dishes, labeled, and allowed to dry in a sterile hood at room temperature, shielded from light, for a minimum of 24 hours.

F. MP Observation, Identification, and Measurement Procedures

Guantitative measurements and characterization of particle attributes were performed systematically:

1.Examination: A quarter section of each sample filter was randomly selected for examination under a stereomicroscope (Olympus SZX10). The data derived from this subset were considered representative of the entire sample.

2.Particle Observation: Particles were meticulously observed and counted under a binocular stereo microscope, typically operating at 200x magnification. This enables the identification of their color, size, and morphology.

3.Dimension Measurements: Detailed measurements of the particle dimensions, including length and width, were precisely recorded. The shapes of the particles were also documented, with dimensions categorized into specific size ranges [34].

4.Removal of Large Particles: Particles exceeding a length of 5 mm were carefully removed from the filter using metal tweezers. This step simplified the analysis, by focusing on particles that fall within the standard MP size range.

G. Determining and Identifying Chemical Composition

To assess the chemical composition of the MPs in each sample, 20% of the particles were chosen for analysis via µFTIR (Fourier transform infrared spectroscopy). These selected particles were carefully positioned in a diamond compression cell and analyzed in transmission mode by employing a liquid nitrogen cooling system.

H. Quality Assurance and Error Control

The quality assurance and error control processes in this study were crucial to ensure the reliability and accuracy of the methodology. Several measures were incorporated into the study design. The settled dust was collected for the purpose of examination and subjected to filtration by means of glass fiber filters. To ensure that there is no particle contamination within the laboratory air, an inspection was carried out utilizing a stereomicroscope and µFTIR analysis. To detect and track any potential environmental contamination, the ambient air of the laboratory was continuously monitored by employing an empty MCE filter. To minimize the possibility of particle suspension and external interference, work practices were modified by reducing the amount of ventilation coming from windows, doors, and ventilation devices. The analysis was conducted during periods of low activity. The personnel involved in the examination were asked to wear laboratory test gowns made entirely of cotton to mitigate the risk of contamination during the procedures. The accuracy of the examination increased through rigorous calculation of the limit of detection (LOD) and limit of quantitation (LOQ) for the samples. These calculations assisted in the identification and quantification of three different types of MPs.

Origin and Distribution of MPs Emission Sources

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I. Identification of Local Sources

This research utilized a range of methodologies to identify specific local sources of MPs emissions by considering various parameters, including spatial distribution of MPs concentrations, composition of polymers, chemical characteristics, and structure of MPs. Localization of MPs sources were possible through the implementation of models focused on receptors, PMF, and diagrams representing the distribution of the two variables.

1.Spatial Distribution Identification

The analysis of laboratory data and the geographical origin of samples were the fundamental basis for the identification of MPs sources. The process of identification is further augmented by the implementation of three distinct strategies.

First, the comparison of distinctive chemical fingerprints inherent to particular types of plastics with acknowledged origins facilitated their identification. In addition, meticulous examination was conducted on more sizable plastic particles present in the samples, as they have the potential to function as potential origins of MPs. Finally, the determination of the distance from presumed sources was considered, taking into account the disparities in particle concentration observed in various urban areas.

The convergence of multiple observations supported the identification of particular MPs sources. Spatial distribution analysis, encompassing the concentration, polymer type, chemical composition, and morphology of MPs, relies on the existence of potential sources within the city.

2.PMF Model

The identification and allocation of MPs sources within ambient air pollution represented the essential components of the study. To accomplish this objective, we utilized the PMF model, a potent tool based on in situ observations, for source allocation. We meticulously designed our study and sampling protocol, encompassing specific locations, frequencies, and seasonal variations, to ensure the precise functionality of the PMF model.

The data collection process entailed extensive field sampling of airborne MPs across various locations in Ahvaz city. These samples underwent thorough laboratory analyses, examined types of MPs, chemical compositions, and associated pollutants, forming the foundation for the operation of the PMF model.

The operation of the PMF model can be explained through a hypothetical scenario. Samples were collected from multiple locations in different seasons of Ahvaz city. The samples were analyzed to provide detailed data for the PMF model.

The PMF model initiated the process of source apportionment in the following manner:

- Data Input: The data matrix, consisting of concentrations of various types of MPs, chemical properties, and associated pollutants, served as the input.

- Modeling process: The PMF model processed this data matrix, seeking distinct patterns or 'fingerprints' associated with MP emission sources such as traffic, industry, and meteorological factors.

- Source Apportionment: By utilizing these recognized patterns, the PMF model calculated contributions from each source to the observed concentrations of MPs. It quantified the proportion of MPs that can be traced back to specific sources, such as vehicular emissions or industrial discharges.

- Quantifying Contributions: The PMF model provided quantitative values that indicate the proportionate contribution of each source to the total concentration of MPs. This information was crucial for understanding the significance of each emission source in the MPs pollution scenario in Ahvaz city.

Using PMF model, the study allowed for the precise allocation of contributions from different emission sources to MP pollution in the ambient air of Ahvaz. This vital information assisted in evaluating dynamics of pollution and provided practical insights for policymakers, urban planners, and stakeholders in formulating precise strategies for mitigating specific sources and safeguarding the environment.

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3.Bivariate Polar Plot Diagrams

A specialized algorithm designed for interpreting air quality data in both closed and open-air environments was utilized to model atmospheric conditions (https://davidcarslaw.github.io/ openair/). Bivariate polar plot diagrams are instrumental in visualizing pollutant behavior with respect to wind direction and speed. These diagrams were generated by processing meteorological data and data from various sampling locations [35].

The utilization of bivariate polar plot diagrams was a significant strength of the present study in the context of source detection. These diagrams help us visualize how pollutants disperse and behave in response to different wind directions and speeds. This method is instrumental in identifying potential MPs emission sources throughout a city. For example, during the summer season, if we observe high MP concentrations in a particular area during days with specific wind patterns, bivariate polar plot diagrams can help identify potential sources. These sources may include industrial facilities, busy roadways, or other points of interest that align with the wind direction and could contribute to MPs emissions in that specific area. The combination of seasonal variations, sampling points, and meteorological data, when analyzed through these diagrams, enhanced the ability to pinpoint and categorize MPs sources effectively. The unique approach involved thorough sampling across the city in various seasons, and bivariate polar plot diagrams provided a powerful tool for source detection by visualizing how MPs disperse in response to changing wind conditions. This approach strengthened the accuracy of assessments and was a distinctive feature of the present study.

J.Identification of Remote Sources

To trace MPs suspended in the atmosphere and identify distant emission sources, this study employed various models, including Lagrangian models. These models combine meteorological data with measured chemical compositions to pinpoint potential source locations. The HYSPLIT software (https://www.ready.noaa.gov/ HYSPLIT.php), known for its ability to calculate mass transfer in the atmosphere, was used for determining air mass trajectory and concentration, taking into account dispersion, diffusion, and deposition.

For this investigation, the web version of HYSPLIT was to analyze 72-hour air mass data in Ahvaz, integrating global meteorological data from the United States Meteorological Organization[36-38]. This model is especially effective in simulating pollutant transport over extended distances, making it invaluable for scenarios involving the long-range transport of MPs. The study used sample collection sites at heights exceeding 70 m above the ground to minimize the potential influence of local sampling sites.

Ecological Risk Assessment

In the current context, the absence of a standardized model for evaluating health risks linked to MPs pollution has prompted researchers to adapt existing frameworks initially designed for different pollutants or environmental contexts. Recent studies have predominantly focused on ecological risk assessment concerning the type and concentration of polymers found in airborne MPs [39]. The study employed a novel assessment model based on recent work by Bucci and Rochman that integrates four crucial dimensions [40].

K. Bucci and Rochman Multidimensional Ecological Risk Assessment

This risk assessment aims to classify MPs based on their potential harm, taking into account four key factors: particle size, shape, polymer type, and the presence of absorbed environmental pollutants. Each factor was assigned a specific ranking, where lower values indicate a lower level of harm and higher values indicate a greater level of risk. Specific rankings for each factor can be found in Bucci and Rochman (2022) [40].

L. Daily Inhalation Exposure Estimation

To estimate daily exposure via inhalation, this study adhered to the guidelines provided by the World Health Organization. The estimation was accomplished using the formula ADI=C×IR, where

ADI represents the average daily inhalation (MPs per person per day), C represents the MPs concentration per cubic meter, and IR indicates the inhalation rate (m^3/day) [41]. Recognizing that inhalation rates may vary across different age groups, separate exposure calculations were performed for each demographic category.

Statistical Data Analysis

This study was designed to assess MPs pollution levels and trends, considering variables such as size, polymer type, shape, and associated pollutants across various seasons and geographic regions within the city. Appropriate statistical analyses were performed in accordance with the characteristics and distribution of the data. One-way ANOVA or Kruskal–Wallis tests were used to examine variations in concentrations among different geographical locations and seasons. To compare concentrations between closed and open spaces, independent t-tests or Mann-Whitney tests were applied. In addition, descriptive statistics and correlation coefficients were calculated. All statistical analyses were carried out using STATA version 18 software.

Furthermore, the identification of local sources and remote emission points for MPs involved the use of multivariate techniques, including PMF and pollutant dispersion models (Lagrangian). The software tools including PMF5, HYSPLIT, and Open-Air within an R environment were employed for these analyses.

Advantages of the Proposed Approach:

The integration of both passive and active sampling methodologies provided a robust and complementary framework for assessing airborne MPs in diverse environments. This dual approach enhanced the comprehensiveness of the study by capturing both settled and suspended MPs, offering a more holistic understanding of their distribution and dynamics in the urban air. The combination of these methods also enabled the triangulation of data, increasing the reliability and accuracy of the results. This comprehensive data collection was essential for accurately mapping the prevalence and characteristics of airborne MPs, particularly in a complex urban environment such as Ahvaz.

Limitations

While the dual sampling strategy significantly strengthened the study's findings, several limitations should be acknowledged:

Passive Sampling: Passive sampling, although cost-effective and useful for capturing settled particles, may not capture smaller particles that remain airborne for extended periods. These particles, which are often of particular concern for human health due to their potential for inhalation, could be underrepresented in the collected data.

Active Sampling: Active sampling, though more effective in capturing airborne particles across a range of sizes, requires specialized equipment and is resource-intensive. This methodology can be logistically demanding, particularly in large-scale or long-term studies, and may require significant personnel and operational costs.

Sampling Coverage: Although efforts were made to select diverse sampling locations across different environments, the study sampling points (30 sites) may not capture the full spectrum of MP exposure scenarios in an urban setting. Additionally, the limited temporal focus of sampling (summer and winter) may not reflect seasonal variations in MPsconcentrations in greater detail.

Comparison with Alternatives

While simpler sampling methodologies, such as single-stage gravimetric sampling, are less expensive and easier to implement, they provide only a limited view of airborne MPs. These methods typically lack the precision necessary to capture the full spectrum of MPs particles, especially in dynamic urban environments with varying sources and deposition patterns. More advanced and fully automated methods, such as those employing optical or laser-based particle counters, offer high precision but are less feasible in resource-limited settings due to high costs and technical complexity. Our approach struck a balance by combining practicality with analytical rigor. It ensured robust data collection while maintaining cost-effectiveness and feasibility,

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making it suitable for large-scale studies in diverse urban settings.

Patient and Public Involvement

Patients and the public were not involved in the design, conduct, reporting, or dissemination of the research. The current study did not require direct patient or public involvement. Although the importance of patient and public involvement was acknowledged in certain research contexts, this was not deemed applicable for this specific study.

Ethics statements

This research protocol was deeply rooted in ethical principles, with a focus on environmental responsibility and unwavering dedication to academic and scientific ethics. Our commitment to ethical research encompassed several fundamental principles:

1. Environmental stewardship: Great importance was placed on environmental responsibility by meticulously conducting sampling and collection methods to prevent any ecological impact and uphold environmental integrity.

2. Academic Integrity: The highest standards of authenticity were upheld, ensuring that all data were transparently documented with appropriate citations, in accordance with guidelines of external sources.

3. Legal Compliance: The study strictly adhered to local, national, and international laws to ensure the ethical utilization of resources and materials in the research.

4. Ethical Oversight: This study was approved by the Institutional Review Board of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1402.042).

These ethical principles served as a guiding force for research activities, ensuring adherence to rigorous ethical standards. Any deviations from these principles will be documented and reported as needed.

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Conflicts of interest

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

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Ethical Considerations

This research was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.2024.0205).

Code of Ethics

The authors adhered to ethical guidelines and regulations throughout the study, ensuring the integrity and reliability of the research process. Data collection, analysis, and reporting were conducted transparently, with due regard for scientific and ethical standards.

Authors' contributions

Neda Kaydi: Investigation, validation, data curation, visualization, writing–original draft preparation, writing. Morteza Abdullatif Khafaie: Methodology, supervision, writing - reviewing and editing. Neamatollah Jaafarzadeh Haghighi Fard: Conceptualization, supervision. Sahand Jorfi: Reviewing and Editing. Afhin Takdastan: Reviewing and Editing.

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