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Air borne microplastics (MPs) are one of the major concerns due to their possible health risks and also act as a carrier for several toxic air pollutants. PET is the most abundant airborne MPs and in-vitro studies reported that exposure to PET MPs can cause pulmonary disorders ( $>98.6 \mu\text{g mL}^{-1}$ ). Therefore, determination of mass concentrations of airborne PET MPs is needed to understand the various additional health risks associated with their inhalation exposure. In this study, we reported an optimized LC-MS/MS method to determine mass quantification of PET MPs present in the ambient  $\text{PM}_{2.5}$  and carried out a pilot study in Delhi and Mohali. We believe that the findings in this study provide a better understanding of chemical composition of airborne MPs and the associated health risks.



# Determination of mass concentrations of airborne PET microplastics using liquid chromatography coupled to tandem mass spectrometer (LC-MS/MS)

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## Abstract

Microplastics (MPs) have become a growing concern of environmental pollution in recent times. MPs are easily transferred and ubiquitously found in ambient air. MPs in the air can act as carriers for several toxic pollutants and exposure to MPs can lead to pulmonary diseases in humans. Polyethylene terephthalate (PET) is one of the most abundant MPs used in the manufacturing of various fibres and plastics. In this study, we present a method for the determination of mass concentrations of PET MPs in the airborne inhalable fraction of fine particulate matter (PM<sub>2.5</sub>) using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Teflon and Quartz fiber filters were tested for extraction efficiency in measuring the mass concentrations of airborne PET MPs. Teflon filters have shown good recovery (80% – 120%) compared to Quartz fiber filters. Using this method, a pilot study was carried out in Delhi, the National Capital of India and Mohali, a suburban city in Northwest Indo-Gangetic Plain (NWIGP) for the determination of mass concentrations of PET MPs present in airborne PM<sub>2.5</sub>. Observed maximum mass concentrations of PET MPs in airborne PM<sub>2.5</sub> at Delhi and Mohali are 135.20 ng m<sup>-3</sup> and 157.98 ng m<sup>-3</sup>, respectively.

**Keywords:** Airborne Microplastics, fine particulate matter, PET, Quantification, LC-MS/MS.

## 1 Introduction

Microplastics (MPs) are plastic debris whose size ranges from 1 µm to 5 mm.<sup>1,2</sup> There are myriad sources of airborne microplastics in the ambient atmosphere. MPs enter into the atmosphere from fibres of textiles, erosion of rubber tyres, building materials, solid waste burning, resuspension of emissions



from traffic and industries, sewage sludge used as a fertilizer, air blasting media, degradation of larger particles when exposed to UV radiation and tumble dryer exhaust.<sup>3–5</sup> Due to their size and density, MPs are easily transferred to air and transported by wind to longer distances up to 100 km causing a potential health risk when inhaled continuously by humans.<sup>4,6–8</sup> MPs in the air can act as carriers for heavy metals, microorganisms and carcinogenic polycyclic aromatic hydrocarbons due to their large specific surface area and sorption capacity and thus inhalation exposure to MPs increases the potential cancer risk.<sup>9–12</sup> Earlier studies reported that PET is the most abundant microplastics present in the ambient particulate matter in urban sites of India and China.<sup>13,14</sup> The inhalable fraction of airborne particulate matter with an aerodynamic equivalent diameter less than or equal to 2.5  $\mu\text{m}$  ( $\text{PM}_{2.5}$ ) can reach deeper into the human body and pose health risks further.<sup>15–18</sup> Inhalation of microplastics in airborne  $\text{PM}_{2.5}$  can lead to diseases like asthma, extrinsic allergic alveolitis, chronic pneumonia, inflammation, bronchitis, and pneumothorax.<sup>19,20</sup> *In-vitro* toxicity studies have shown that PET nano plastics can result in the formation of reactive oxygenated species causing cellular oxidative stress.<sup>21</sup> PET is also known to cause vascular prostheses due to the suppression of proliferation and migration of endothelial and mesenchymal cells.<sup>22</sup> Recent *in-vitro* studies have shown that exposure to PET MPs can cause pulmonary disorders if the concentrations of PET MPs exceed more than 98.6  $\mu\text{g mL}^{-1}$ .<sup>21</sup> Also, some of the *in-vivo* studies reported that exposure to PET MPs above 200 ppm can cause hepatotoxicity and neurotoxicity in Zebrafish and *Drosophila melanogaster*, respectively.<sup>23,24</sup> Hence, it is important to carry out the measurements of mass concentrations of PET MPs in the airborne  $\text{PM}_{2.5}$  to understand the various additional health risks associated with it.

In most of the studies, measurements of MPs were carried out extensively using Fourier-transform infrared (FTIR) and Raman spectroscopic techniques. However, these spectroscopic techniques suffer from several artefacts due to the interferences with the sample matrix and also become troublesome for the analysis of MPs when the particle size is below 0.5  $\mu\text{m}$ .<sup>25</sup> Other analytical techniques namely



transmission electron microscope, scanning electron microscope, and dynamic light scattering are also being used in MPs studies. Nevertheless, these microscopic techniques can only provide information about particle number, size, and shape and these techniques failed to identify the type of polymer present in the given sample.<sup>26,27</sup> With the rapid development in various mass spectrometric techniques, pyrolysis gas chromatography coupled to mass spectrometry (py-GC-MS) and thermogravimetry coupled with mass spectrometry techniques (TGA-MS) are being used for the characterization of microplastics present in various sample matrices.<sup>28,29</sup> Note that these techniques require bulk sample for analysis, are suitable only for specific polymers, and are semi-quantitative techniques.<sup>30</sup> Analysis time with LC-MS/MS is less than the GC-MS/MS technique. In GC-MS/MS analysis, conditioning of oven temperature is time-consuming compared to LC-MS/MS.

In this study, we have developed a method for the determination of mass concentrations of PET MPs in the inhalable fraction of ambient PM<sub>2.5</sub> using the state-of-the-art instrumentation technique namely liquid chromatography coupled to tandem mass spectrometer (LC-MS/MS). Till date, no studies have reported the mass concentrations of PET MPs in the inhalable fraction of ambient PM<sub>2.5</sub>. Using this method, a pilot study was carried out to screen and measure the mass concentrations of PET MPs in inhalable fraction of ambient PM<sub>2.5</sub> in Delhi, the National Capital of India and Mohali, a suburban city in Northwest Indo-Gangetic Plain.

## 2 Materials and Methods

### 2.1 Chemicals

Polyethylene terephthalate (PET) (CAS No: 25038-59-9, Product No: 429252-250G) was procured from Sigma Aldrich, Saint Louis, MO 63103, USA. Terephthalic acid (CAS No: 100-21-0, purity: > 99.0%) and pentanol (CAS No: 71-41-0, purity: > 99.0%) were purchased from Tokyo Chemical Industry Co., Ltd, Japan. LC-MS grade formic acid (CAS No: 64-18-6) and methanol (CAS No: 67-



56-1) were obtained from Fisher Chemical, Belgium. HPLC grade methanol and potassium hydroxide pellets (1310-58-3) were procured from Merck Life Science Private Limited, Mumbai, India. HPLC grade trifluoro acetic acid (CAS No: 76-05-1) was procured from Spectrochem Pvt Ltd, Mumbai, India. High purity (PicoPure plus grade) hydrochloric acid (CAS No: 7647-01-0) was purchased from Chem-Lab, Canada. Type -1 Milli Q water (18 M $\Omega$ .cm) was purified using a Milli Q IQ 7000 ultra-pure water purification system.

## 2.2 Sample Collection

At Mohali, ambient PM<sub>2.5</sub> samples were collected on Teflon filters (Whatman membrane filters, Cat No: 7585-004, pore size: 0.5  $\mu$ m and diameter: 47 mm) using a USEPA approved Speciation Aerosol Sampling System (Met one Instruments Inc. OR, USA) at a flow rate of 6.7 L min<sup>-1</sup> for 24 h (04:00 PM to following day 04:00 PM, IST) between 16/10/2022 to 11/11/2022 which is installed on rooftop of academic building 2, IISER Mohali (30°39'52"N 76°43'43"E). A detailed description of the sampling site is given in our previously reported study.<sup>31</sup> A total of seven samples were collected and their results are presented in this study.

Ambient PM<sub>2.5</sub> samples in Delhi were collected on a rooftop of Mausam Bhawan, Indian Meteorological Department (IMD), Lodhi Colony, Delhi (28°35'22"N 77°13'14"E) from 16/10/2022 to 04/11/2022. A total of four samples were collected as a part of the pilot study. A custom designed PM<sub>2.5</sub> cyclone (Mars Bioanalytical, India) equipped with a mass flow controller (Bronckhorst, Holland) and low volume vacuum pump was used for the collection of PM<sub>2.5</sub> samples onto Teflon filters (polytetrafluoroethylene (PTFE), Whatman membrane filters, Cat No: 7585-004, pore size: 0.5  $\mu$ m and diameter: 47 mm) at a flow rate of 12.6 L min<sup>-1</sup> for 24 h (02:00 PM to following day 02:00 PM, IST).

All the collected samples were stored at 4 °C until the analysis of PET MPs was performed using LC-MS/MS facilitated at Sri Sathya Sai Institute of Higher Learning – Central Research Instruments



Facility, Puttaparthi, Andhra Pradesh, India. Figure S1 shows the sampling locations, sampling sites and PM<sub>2.5</sub> samplers used for the collection of PM<sub>2.5</sub> samples in this study.

### 2.3 Extraction and depolymerization of airborne polyethylene terephthalate microplastics

In this study, extraction and depolymerisation of PET microplastics present in the inhalable fraction of particulate matter was carried out using the earlier reported method for sediment samples.<sup>32</sup> Filter samples were extracted using 5 mL of HPLC-grade methanol for 15 minutes under ultrasonication. This process was repeated twice and the methanol layers were pooled into a round-bottomed flask. An aliquot of 200 µL was syringe (PTFE syringe filter, 0.22 µm) filtered from the pooled methanol layers for determining the free terephthalic acid (terephthalic acid present in the non-depolymerized sample). The remaining methanol fraction was allowed to dry completely under the gentle flow of N<sub>2</sub> gas. The obtained residue was then refluxed with 35 mg of KOH and 10 mL of n-pentanol at 135 °C for thirty minutes. After the reflux, the reaction mixture was allowed to cool to room temperature and extraction was carried out with 10 mL of HPLC grade water using an orbital shaker (180 rpm; Make: Labnet and Model: Orbit™ 1900 Digital Heavy Duty Shaker) followed by centrifugation (3000 rpm; Rotor radius: 144 mm; Make: Thermo Scientific and Model: Sorvall™ ST 8 Small Benchtop Centrifuge) for ten minutes each. The extracted aqueous layer was adjusted to pH 2-3 using high-purity hydrochloric acid. Further, unwanted compounds were eliminated from pH adjusted aqueous layer using Agilent technologies HF Bond Elut – C18 solid phase cartridges (Part No: 14102052). Note that SPE cartridges were conditioned using 12 mL of HPLC grade methanol and followed by 12 mL of HPLC grade water prior to their use. The solid phase extracted aqueous layer containing the depolymerized fraction of TPA was analysed with LC-MS/MS as described in section 2.4. The remanent fraction of TPA on the SPE cartridge was washed with methanol and the same was analysed with LC-MS/MS. All the samples were syringe-filtered through the 0.22 µm PTFE filter before injecting into the LC-MS/MS. Note that



the  $\text{TPA}_{\text{deploy}}$  used in equation 1 (section 2.5) represents the fraction of TPA obtained from both the SPE treated aqueous and methanol extracts.

## 2.4 Liquid chromatography – mass spectrometer analysis

In this study, an ultra-high performance liquid chromatography (UHPLC) (Model Name: Agilent Technologies, 1290 infinity series) coupled to triple quadrupole mass spectrometer (Model Name: Agilent Technologies, 6490 iFunnel) with electrospray ionization (ESI) source in negative ionization mode was used for the identification and quantification of terephthalic acid present in non-depolymerized and depolymerized fraction of  $\text{PM}_{2.5}$  extracts. Agilent Zorbax eclipse plus Rapid Resolution High Definition  $\text{C}_{18}$  column (internal diameter: 3.0, column length: 100 mm, and particle size: 1.8  $\mu\text{m}$ ) was used for the separation of analytes. Chromatographic separation was carried out with a binary mobile phase system in gradient mode. Mobile phases used in this study are 0.01% formic acid in water and 100% methanol as mobile phase A and mobile phase B, respectively. The volume of the sample injected into the LC-MS/MS instrument is 10  $\mu\text{L}$ . The gradient composition of the mobile phase is given in Table S1. Terephthalic acid is eluted from the column at 5.3 min using the above liquid chromatography parameters. Multiple reaction monitoring (MRM) mode was adopted for the identification of terephthalic acid (TPA) using LC-MS/MS. Source parameters of the mass spectrometer and MRM conditions used for the identification of TPA are given in Table S2. In this method, 165.0 – 121.0 and 165.0 – 77.0 are the transitions used as quantifier and qualifier ions, respectively for TPA identification. Figure S2 shows the total ion chromatogram and extracted ion chromatograms for two targeted transitions of standard terephthalic acid.

## 2.5 Measurement of mass concentrations of polyethylene terephthalate microplastics

Mass concentrations of PET microplastics in any environmental sample matrix can be measured using the equation 1.<sup>32,33</sup>



$$PET_{Conc} = \frac{\left\{ (TPA_{depoly} - TPA_{non-depoly}) \times \frac{MW_{(TPA-H_2O)}}{MW_{TPA}} \right\}}{f_{(TPA-H_2O)}} \quad (1)$$

Here,  $PET_{Conc}$  is the mass concentration of PET microplastics present in ambient air,  $TPA_{depoly}$  is the terephthalic acid (TPA) obtained after depolymerization of airborne PET microplastics,  $TPA_{non-depoly}$  is the free terephthalic acid (actual terephthalic acid present in the ambient fine particulate matter before depolymerization),  $f_{(TPA-H_2O)}$  and  $MW_{(TPA-H_2O)}$  represent the mass percentage and molecular weight of (TPA – H<sub>2</sub>O).  $MW_{TPA}$  is the molecular weight of TPA. From the chemical structure of PET polymer, [-C(O)-C<sub>6</sub>H<sub>4</sub>-C(O)-O-CH<sub>2</sub>-CH<sub>2</sub>-]<sub>n</sub>, (TPA – H<sub>2</sub>O) accounts for approximately 77.4% ( $f_{(TPA-H_2O)}$ , % w/w) in PET (Wang et al., 2017). TPA is also used as a monomer in the production of polybutylene terephthalate (PBT) and polytrimethylene terephthalate (PTT). Nevertheless, the abundance of these two polymers in ambient particulate matter is relatively much lower compared to the abundance of PET MPs.<sup>34</sup> Hence, the contribution of these polymers to the depolymerised fraction of terephthalic acid is insignificant. Reported measurements of PET in this study represent the upper limit for the mass concentrations of observed PET MPs present in the inhalable fraction of ambient fine particulate matter.

## 2.6 Quality Assurance (QA) and Quality Control (QC)

No plastic containers were used throughout the study to avoid contamination during the analysis. Calibrations were performed in regular intervals during the analysis time with five different concentrations of TPA ranging from 10 ng mL<sup>-1</sup> to 200 ng mL<sup>-1</sup>. Terephthalic acid standards used in the entire study were prepared in LC-MS grade methanol. Figure S3 represents the linearity curve obtained for terephthalic acid. This method had resulted in the excellent linearity ( $r^2 > 0.99$ ). The limit of detection (LOD) and limit of quantification (LOQ) of terephthalic acid were determined to be 2.9 ng mL<sup>-1</sup> and 9.9 ng mL<sup>-1</sup>, respectively. The precision error of the method was verified by analyzing the terephthalic acid standard multiple times (n=5) on the same day. The precision error was observed to



be less than 10% and it is calculated by obtaining the relative standard deviation of the peak area of the same sample that was injected into LC-MS/MS and analyzed five times. Recovery tests were performed as a part of QA and QC using Teflon and Quartz fiber filters and the details have been explained in section 2.7. The total uncertainty reported in Table 1 was calculated using the root mean square propagation of individual uncertainties like the 1% accuracy error inherent in the terephthalic acid standard and the instrument precision error determined using the replicates of the samples (n=5). Further, we have extracted and analyzed the blank filters (n=6) as per the procedure described in section 2.3. The concentrations of PET MPs from the blank filters are summarized in Table S3. These concentrations from the blanks were averaged and subtracted from the samples to report the final concentrations.

## 2.7 Detection of terephthalic acid from the depolymerization of PET spiked onto blank filters

In this study, we also have compared the extraction efficiency of PET MPs with two different extensively used filter substrates for the ambient particulate matter collection namely Teflon and Quartz fiber filters.<sup>35</sup> Based on earlier studies reported in the literature from sediment and water samples, we have chosen this concentration of 50 ng and 100 ng for the recovery test.<sup>28,29,36</sup> To spike the corresponding concentrations of 50 ng and 100 ng of PET microplastics, the standard PET pellets were weighed accordingly and dissolved in the chloroform. The dissolved PET solution was spiked onto the blank filters and then allowed to dry at room temperature to remove the solvent. Thus, the test concentrations were spiked onto the filters. This test was carried out by spiking the two known concentrations (50 ng and 100 ng) of PET onto the blank filters of both Teflon and Quartz fiber filters. The spiked filters were extracted and analyzed using the procedure described in sections 2.3 and 2.4. High recovery of PET microplastics was observed with Teflon spiked filters. The obtained recovery percentages with both Quartz fiber filters and Teflon filters are presented in Table S4. The recovery (80% - 120%) of PET microplastics using Teflon filters is within limits set by the USEPA ( $\pm 20\%$



variability).<sup>37</sup> We observed that the sonication had destroyed the quartz filter fabric and made it difficult to separate fibres from microplastic particles when filtering the suspensions. Similar observations were reported in earlier studies too.<sup>38</sup> Hence, Teflon filters are better suited for the collection and determination of mass concentrations of PET MPs in the inhalable fraction of ambient fine particulate matter.

### 3 Results and Discussion

#### 3.1 Concentrations of polyethylene terephthalate microplastics in ambient aerosols of Delhi and Mohali

Using the developed and optimised method a pilot study was performed to screen and measure the mass concentrations of airborne inhalable fraction of PET MPs in Delhi and Mohali (refer to Figure S1). Considering the logistics, ambient aerosol samples were collected using Teflon filters in October and November 2022 for a few days (refer to Table 1).

All the collected ambient  $PM_{2.5}$  samples were extracted and mass concentrations of PET MPs were determined as described in sections 2.3 to 2.5. Figure 1 (a-c) shows the extracted ion chromatogram of terephthalic acid standard, free terephthalic acid (TPA) and depolymerised fraction of terephthalic acid present in the ambient  $PM_{2.5}$  sample collected in Delhi on 31/10/2022 02:00 PM to 01/11/2022 02:00 PM (IST), respectively. The presence of terephthalic acid in the fraction of depolymerized extracts of ambient  $PM_{2.5}$  samples confirms the presence of PET microplastics in the inhalable fraction of ambient particulate matter in Mohali and Delhi. Table 1 shows the ambient mass concentrations of PET MPs in the inhalable fraction of ambient particulate matter at Mohali and Delhi. In Mohali, about 75% of collected samples contained PET MPs in the inhalable fraction of ambient particulate matter. The maximum mass concentration observed in Mohali was about  $158.0 \text{ ng m}^{-3}$  on October 23, 2022, which happened to be one of the festive days in India known as Choti Diwali (a day before Diwali) where



anthropogenic activity like garbage burning consisting of household plastic items is known to be of very high.<sup>39,40</sup> The most known practice is that many households in this region are known to dispose the old household items and eventually they are burnt in garbage.<sup>41,42</sup> During this sampling day, we also observed the enhanced concentration of carbon monoxide (an in-efficient combustion chemical tracer) up to 3.03 ppm. This indicates the co-emission of PET along with inefficient combustion tracer like CO. Hence, pre-Diwali activity could be a plausible reason for the observed highest concentrations. Due to some operation logistic issues, we were not able to carry out the measurements on Diwali day. In Delhi, PET MPs were observed in all the collected ambient PM<sub>2.5</sub> samples. Mass concentrations of PET MPs present in the inhalable fraction of ambient PM<sub>2.5</sub> samples collected in Delhi ranged from 25.9 to 135.2 ng m<sup>-3</sup>.

Recent *in-vitro* studies have shown that exposure to PET MPs can cause pulmonary disorders if the mass concentrations of PET exceed more than 98.6 µg mL<sup>-1</sup>.<sup>21</sup> Hence, it is important to carry out the measurements of mass concentrations of PET MPs in the inhalable fraction of ambient PM<sub>2.5</sub> to understand the various additional health risks associated with it. To the best of our knowledge, this is the first-ever study to report the mass concentrations of PET MPs in ambient PM<sub>2.5</sub> using LC-MS/MS.

Till date, there is no air quality standard for the PET microplastics present in the ambient PM<sub>2.5</sub>. Our study demonstrates the presence of inhalable fraction of PET MPs in ambient air and developed an optimised method for the determination of mass concentrations of PET MPs using the LC-MS/MS method. This method can be extended to determine the mass concentrations of other types of MPs present in the ambient particulate matter. Further long-term regional studies are needed to understand the sources, seasonal variability, and health effects of inhalation exposure to PET MPs.

**Table 1.** Measured mass concentrations of airborne PET MPs in Delhi and Mohali

Sampling Date	Concentration ± Uncertainty (ng m <sup>-3</sup> )
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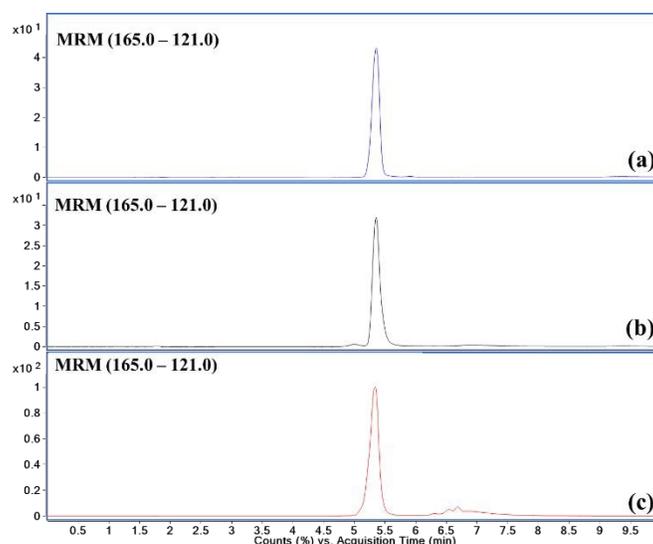
**Mohali Samples**

16/10/2022	$2.8 \pm (0.2)$
19/10/2022	bdl <sup>1</sup>
23/10/2022	$158.0 \pm (13.9)$
26/10/2022	$49.6 \pm (4.4)$
31/10/2022	bdl <sup>1</sup>
04/11/2022	$10.1 \pm (0.9)$
05/11/2022	$13.6 \pm (1.2)$
11/11/2022	$1.9 \pm (0.2)$

**Delhi Samples**

16/10/2022	$135.2 \pm (11.9)$
22/10/2022	$52.1 \pm (4.6)$
31/10/2022	$59.8 \pm (5.3)$
04/11/2022	$25.9 \pm (2.3)$

1: below detection limit



**Figure 1.** Extracted ion chromatograms of **a)** terephthalic acid standard **b)** free terephthalic acid in the aerosol sample of Delhi collected on 31/10/2022 02:00 PM to 01/11/2022 02:00 PM (IST) **c)** depolymerised fraction of terephthalic acid from the inhalable fraction extract of ambient  $PM_{2.5}$  sample collected in Delhi on 31/10/2022 02:00 PM to 01/11/2022 02:00 PM (IST).

**4 Conclusions**

In this study mass quantification of PET microplastics present in the inhalable fraction of particulate matter was achieved using LC-MS/MS. This would help to understand the various additional health risks associated with their inhalation exposure to the mass concentration of microplastics. Further, after validation of the developed method, a pilot study was conducted to determine the presence of



microplastics in the airborne particulate matter and also the first-ever study to report the mass concentrations of PET MPs in ambient PM<sub>2.5</sub> using LC-MS/MS in New Delhi and Mohali in India.

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### Data availability statement

- The data supporting this article have been included as part of the Supplementary Information.

