

The Influence of polycyclic aromatic hydrocarbons exposure on the gut microbiome composition and inflammatory responses

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ABSTRACT

Particulate matter (PM) is recognized as a contributor to various gastrointestinal diseases, including inflammatory bowel disease. However, the specific impact of polycyclic aromatic hydrocarbons (PAHs), a major component of PM, on microbial community dynamics and immune regulation remains unexplored. In this study, we investigated the effects of PAHs on the inflammatory response of mouse intestinal tissues and microbiota composition using PAHs extracted from PM₁₀ (PM₁₀-PAHs). With increasing duration of exposure to PM₁₀-PAHs, damage to the colonic mucosa and lung tissues was aggravated and the secretion of inflammatory cytokines increased. The composition of the gut microbiota was also significantly altered, with a decrease in Firmicutes and an increase in Bacteroidetes at the phylum level, along with an increase in *Lactobacillus*, *Clostridium*, and *Romboutsia* at the genus level. Notably, despite the cessation of PM₁₀-PAHs administration and the recovery of gut microbial diversity, the microbiota composition and inflammatory cytokine levels did not return to pre-exposure conditions. These results suggest that exposure to PM can have lasting adverse health effects, such as inflammation induced by its main component, PAHs.

1. Introduction

Air pollution is a significant risk factor threatening human health worldwide (Chong-Neto and Rosário Filho, 2025). The number of deaths due to air pollution has nearly doubled from 2.3 million in 1990 to 4.5 million in 2019, with approximately 4.1 million of these deaths (92 %) attributed to particulate matter (PM) (WMO, 2021; OECD, 2023). Among health risk factors, PM ranks as a high priority due to its substantial impact on reducing life expectancy and is projected to cause up to 9 million premature deaths by 2060 (Collaborators, 2017; WMO, 2021).

PM is typically classified by particle diameter, with PM₁₀ and PM_{2.5} (particles with diameters less than 10 and 2.5 μm, respectively) commonly analyzed as benchmarks due to their ability to reach the lungs and alveoli. These particles penetrate deep into the body without filtration, increasing the incidence of various diseases, including respiratory illnesses (Mohammadi et al., 2024), cardiovascular diseases (Brook et al., 2010; Strobl et al., 2024), and type 2 diabetes (Ribble et al., 2023). Furthermore, it was reported that 10 μg/m³ increase in PM₁₀

concentration was associated with an average 0.5 % increase in disease-related mortality (Samet et al., 2000). Seasonal variations in particulate matter levels also play a significant role in the degree of exposure. PM levels soar in the winter due to increased biomass burning for heating and atmospheric inversion layers, while in summer, PM pollution levels decrease, allowing time for the human body to recover from the negative effects of PM (Chauhan et al., 2022). In addition to seasonal factors, geographical characteristics can lead to variations in PM concentrations and PM-related adult mortality rates even within the same region (Arregocés et al., 2023).

Anthropogenic PM is predominantly generated from the incomplete combustion of fuels, leading to inevitable contamination by toxic polycyclic aromatic hydrocarbons (PAHs) (Chaturvedi et al., 2023). Due to their low vapor pressure, PAHs released in the gaseous phase are easily partitioned on PM (Fang et al., 2006). Humans can be exposed to PAHs through inhalation of PM, cigarette smoke, and ingestion of contaminated food (Lee et al., 2016; Lewtas, 2007). Inhaled and ingested PAHs are transported from the lungs to the gastrointestinal tract via mucociliary clearance, exposing not only the respiratory and cardiovascular

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systems but also the gastrointestinal tract to PAHs (Bosch et al., 2023). PAHs disrupt the endocrine system and exhibit mutagenic, carcinogenic, and immunotoxic effects across various organisms (Baird et al., 2005; Qamar et al., 2012; Zhang et al., 2016). Exposure to these compounds and their derivatives has been linked to several gastrointestinal diseases, including forestomach tumors (Wester et al., 2012), colorectal cancer (Diggs et al., 2011), and inflammatory bowel disease (IBD) (Ribi  re et al., 2016). Alarming, ingested PAHs can also be metabolically converted into highly reactive genotoxic metabolites (Cheng et al., 2021).

The gut microbiota is crucial for regulating immune responses and maintaining gut health and homeostasis (Li et al., 2022). Exposure to PM has been associated with gut inflammation and shifts in microbial composition. Numerous studies have reported exacerbated IBD, gut microbiota dysbiosis, and subsequent alterations in metabolic processes due to PM exposure (Kish et al., 2013). While most studies have examined the effects of PM—comprising a mix of PAHs, ions, metals, and more—or the effects of specific PAHs on IBD, the direct impact of PM-derived PAHs on microbial community dynamics and inflammatory responses remains unexamined.

This study aims to directly evaluate the effects of PAHs concentrated in PM. Using PAHs extracted from PM₁₀ standard material (PM₁₀-PAHs), we compared inflammatory responses in intestinal tissue and changes in the gut microbial community based on varying concentrations and exposure durations of PM₁₀-PAHs. This analysis systematically explores the correlation between PAH exposure, intestinal inflammation, and microbial community changes. Additionally, we assessed the resilience of gut microbial composition and inflammatory markers after cessation of PM₁₀-PAHs oral administration for mimicking seasonal variability of PM pollution.

2. Materials and methods

2.1. Animal model and particulate matter treatment

All animal handling and experimentations were in accordance with ethical guidelines and approved by the Animal Care and Use Institutional Review Committee of Korea Research Institute of Bioscience and Biotechnology (approval number: KRIBB-AEC-22254). Eight-week-old female C57BL/6 mice were purchased from Orient (Seongnam-si, Gyeonggi-do, Republic of Korea) and housed in plastic cages in a room with controlled temperature ($22 \pm 2^\circ\text{C}$) and humidity ($55 \pm 5\%$) and maintained on a reverse 12 h light/dark cycle.

PM₁₀ used in this work were obtained by purchasing the European Reference Materials (ERM-CZ100; B-2440, Geel, Belgium). ERM-CZ100 was uniformly dispersed in water by sonication (Sonics & Materials Inc, Newtown, CT, USA), extracted with hexane, and then analyzed by gas chromatography-mass spectrometry (GC-MS) (Agilent Technologies 7890A-GC, Agilent, Santa Clara, CA, USA) (Kozhevnikov et al., 2021). PM₁₀-PAHs included benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, dibenzo[a,h]anthracene, indeno[1,2,3-c,d]pyrene, anthracene, benzo[g,h,i]pyrene, chrysene, coronene, fluoranthene, phenanthrene, and pyrene (Piascik et al., 2010). The extracted PM₁₀-PAHs was dissolved in vegetable oil and subsequently diluted in sterile pyrogen-free saline for oral gavage (Malik et al., 2012; Ribi  re et al., 2016).

Mice were evenly divided into two groups, control and experimental groups. Mice in the control group ($n = 10$) were oral gavage injected with an equivalent volume (100 μl) of sterile pyrogen-free saline. Mice in the PM₁₀-PAHs treatment groups (each group per 10 mice) received a daily oral gavage of 100 μl containing PM₁₀-PAHs at dose of 50 mg/kg ($n = 10$), 100 mg/kg ($n = 10$) and 200 mg/kg ($n = 10$) in a dose-dependent manner. The administered dose corresponded to the amount of PM₁₀ from which the PAHs were extracted. In a separate experiment, mice in the PM₁₀-PAHs group were administrated PM₁₀-PAHs at 200 mg/kg (100 μl by oral gavage daily) for different

durations of 1, 2, and 4 weeks to assess time-dependent effects. 4W-re groups ($n = 10$) were injected PBS for 4 weeks after PM₁₀-PAHs treatment for 4 weeks. After oral gavage injection, each group of mice were all euthanized, their abdomens were opened and the entire colon (from the caecum to the anus), lung tissues and serum were harvested and analyzed by hematoxylin and eosin (H&E) staining and cytokine levels such as TNF- α , IL-1 β , IL-2, IL-6, IL-10 and IL-17.

2.2. Hematoxylin and Eosin (H&E) staining

Mouse tissue in colon and lung was cut into 7- μm thick sections, followed by histochemical staining using H&E. Tissue sections were deparaffinization using graded concentrations of xylene (100, 95, and 70 %) for 5 min each. For nuclear staining, the sections were stained with hematoxylin solution for 5 min and washed with flowing water. Hematoxylin was removed using 1 % HCl and 1 % ammonia solutions, followed by immersion in an eosin solution for 2 min to stain the cytoplasm. Thereafter, the sections were dehydrated using graded concentrations of alcohol (70, 95, and 100 %), followed by treatment with xylene three times (3 min each) to remove alcohol. Finally, the sections were mounted with glass coverslips and viewed using an Olympus BX43 microscope (Olympus, Tokyo, Japan). Photographs were taken with an Olympus DP72 camera (Olympus).

2.3. Determination of the production of TNF- α , IL-1 β , IL-2, IL-6, IL-10 and IL-17

Colon and lung tissues were harvested, washed twice with ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 10 % glycerol, 1 % Nonidet P-40, 5 mM EDTA, 1 mM DTT, 100 mM NaF, 2 mM sodium pyrophosphate, 20 mM β -glycerophosphate, 2 mM sodium orthovanadate) and $1 \times$ protease inhibitor cocktail (Sigma-Aldrich) for 30 min. Fractions were obtained as supernatants after centrifugation at $12,000 \times g$ at 4°C for 20 min. Protein content was determined by the Bicinchoninic Acid (BCA) method (Pierce, Rockford, IL, USA), and the production of TNF- α , IL-1 β , IL-2, IL-6, IL-10, and IL-17 in the colon tissues, sera, and lung tissues were determined using the DuoSet ELISA system (DY410, DY401, DY402, DY406, DY417 and DY421, R&D Systems, MN, USA), according to the manufacturer's instructions. In brief, diluted tissue homogenate and sera samples and the prepared standards were added to the appropriate wells of a pre-coated 96-well microplate. The plate was incubated with primary antibodies for 90 min and after incubation, the wells were thoroughly washed three times with wash buffer and incubated with detection antibodies with Streptavidin-HRP for 60 min. After final washes, the enzyme-substrate reaction produced a colored product proportional to the amount of target biomarker present. The absorbance was measured at 450 nm using a SpectraMax ABS Plus microplate reader (Molecular Devices, CA, USA). All experiments were repeated in triplicate.

2.4. Fecal sample collection and DNA extraction

Fecal samples were collected before (0 week) and 1, 2, 4 weeks after PM₁₀-PAHs treatment. Each animal was removed from its home cage and placed in a clean, clear plastic cage without food or water for 1 h. Stools were collected immediately after expulsion and placed in sealed 1.5 ml tubes and immediately frozen at -80°C , prior to DNA extraction. Microbial genomic DNA was extracted from 200 mg of each fecal sample using a TIANamp Stool DNA Kit (TIANGEN, DP328, Beijing, China) according to the manufacturer's recommendation. Successful DNA isolation was confirmed by agarose gel electrophoresis.

2.5. 16S rRNA sequencing

Total microbial genomic DNA from each fecal sample was extracted

using the FastDNA® Spin Kit for Soil (MP Biomedicals, CA, USA), following the manufacturer's instructions. DNA concentration was determined using the Epoch™ Spectrometer, and purity was verified on a 1 % agarose gel. PCR was conducted in two stages. For the first stage, amplicon PCR, the V3-V4 region of the 16S rRNA gene was amplified using the forward primer 1st 341F (5'-CCT ACG GGN GGC WGC AG-3') and the reverse primer 1st 805R (5'-GAC TAC HVG GGT ATC TAA TCC-3'). In the second stage, index PCR, Forward Index i5 (5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC-XXX XXX XX-TCG TCG GCA GCG TC-3') and Reverse Index i7 (5'-CAA GCA GAA GAC GGC ATA CGA GAT-XXX XXX X-GTC TCG TGG GCT CGG-3') were used. The PCR reaction mixture consisted of: 2.5 µL of 10X buffer, 2.5 µL of dNTP, 1 µL of forward primer (10 pmole/µL), 1 µL of reverse primer (10 pmole/µL), 0.25 µL of *Taq* polymerase, 2 µL of template DNA, and 15.75 µL of ddH₂O, totaling 25 µL. The reaction conditions were as follows: initial denaturation at 95°C for 3 min, followed by 30 s at 95°C for denaturation, 30 s at 55°C for annealing, 30 s at 72°C for extension, and a final extension at 72°C for 5 min. The PCR product was purified using the QIAquick® PCR Purification Kit, and the amplicon library was sequenced on the Illumina MiSeq platform.

2.6. Bioinformatics analysis

The raw sequencing data were stored in FASTQ format, and adapter sequences were removed using Cutadapt. Reads with up to two mismatches and a minimum average quality score of 20 were retained for analysis using DADA2 (Callahan et al., 2016). Additionally, merged sequences were denoised with DADA2 and clustered into operational taxonomic units (OTUs) with 97 % read similarity using UCLUST software (Edgar, 2010). Representative OTU sequences were assigned to bacterial taxa at various classification levels using the SILVA Database. OTU-based alpha diversity was assessed using ACE, Chao1, Shannon, and Simpson indices. While ACE and Chao1 reflect the estimated total

species richness of a sample, Shannon and Simpson indices quantify biodiversity. Principal component analysis (PCA) of OTU relative abundance was performed to estimate beta diversity within the community. Linear discriminant analysis (LDA) coupled with the effect size (LefSe) method was used to identify significantly different bacterial taxa between groups. For metagenome prediction, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2) (Douglas et al., 2020) was employed in combination with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to predict the metabolic functions of the bacterial community.

2.7. Statistical analysis

The significance of all data tests was evaluated using two-way ANOVA or t-tests in Python, with a *p*-value < 0.05 considered statistically significant. The analyzed data were visualized using Origin 2020 (ver 9.7) (OriginLab Corporation, USA).

3. Results

3.1. PM₁₀-PAHs induces damage and inflammation in multiple tissues

To determine whether tissue damage was induced by PM₁₀-PAHs, a histological evaluation of colon and lung tissues was performed using H&E staining after a 2-week oral gavage injection of PM₁₀-PAHs in C57BL/6 mice. In the colonic mucosa of the PM₁₀-PAHs-treated group, infiltration of mixed inflammatory cells, loss of goblet cells, and numerous neutrophils were observed (Fig. 1). This indicates that an inflammatory response was activated in the intestinal tissue. In the lungs, cells in the sterile pyrogen-free saline-treated control tissue displayed a reticular arrangement, whereas in lung tissue exposed to orally administered PM₁₀-PAHs, immune cells were progressively filling the reticular structure. These results demonstrate that exposure to

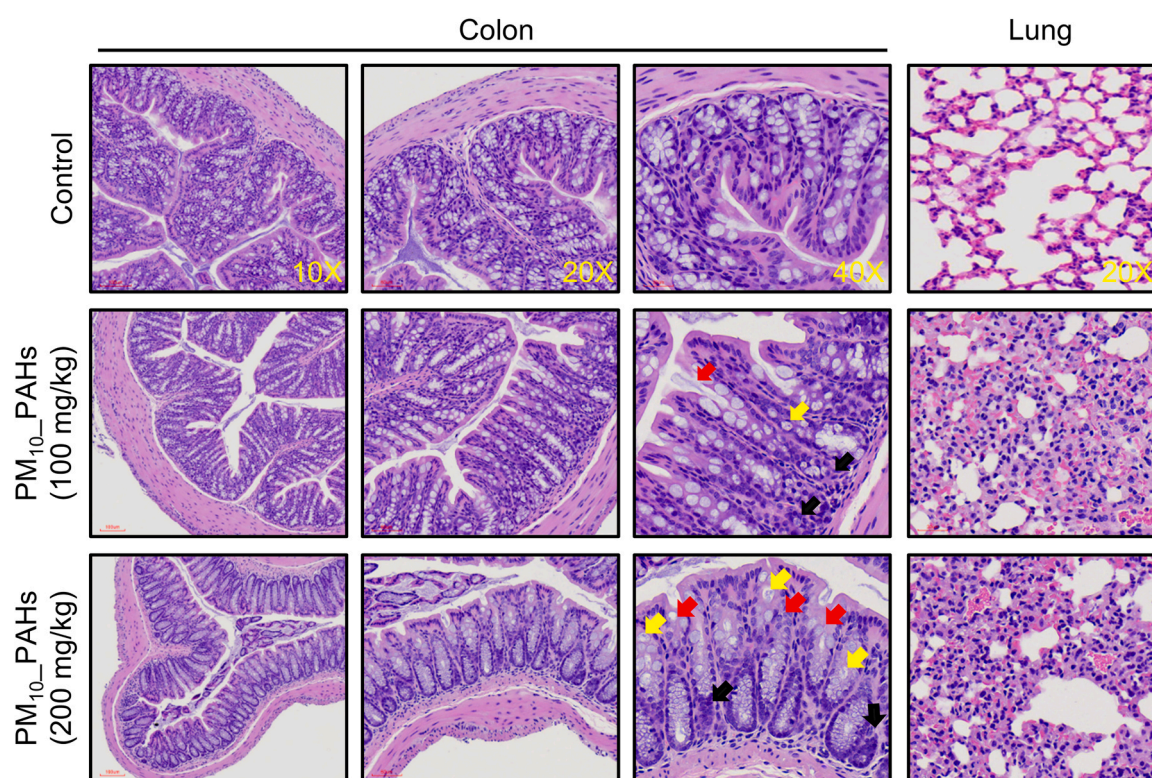


Fig. 1. Histopathological analysis of colon and lung tissues based on PM₁₀-PAHs exposure. H&E-stained tissue sections of C57BL/6 mice orally administered sterile pyrogen-free saline or PM₁₀-PAHs were examined microscopically. Black arrows indicate the infiltration of mixed inflammatory cell, red arrows denote loss of goblet cell, and yellow arrows highlight the presence of numerous neutrophils.

PM₁₀-PAHs caused damage to both the colonic mucosa and lung tissue, with the regions of inflammation expanding in a concentration-dependent manner over the same 2-week period. As the concentration of orally gavaged PM₁₀-PAHs increased, a rise in the production of inflammatory cytokines IL-1 β , IL-17, IL-6, IL-2, and TNF- α was observed in colon tissues, lung tissues, and sera (Fig. 2). Additionally, the anti-inflammatory cytokine IL-10 was also increased in colon tissues, lung tissues, and blood in a PM₁₀-PAHs concentration-dependent manner. These findings indicate that oral gavage of PM₁₀-PAHs induces a systemic inflammatory response in the colon and lungs.

3.2. Ingested PM₁₀-PAHs reduced alpha diversity of gut microbial community

To assess whether ingested PM₁₀-PAHs affected the gut microbial community, fecal samples were collected from mice orally administered PM₁₀-PAHs (200 mg/kg) at 1, 2, and 4 weeks. Fecal DNA was extracted, and microbial communities were analyzed using 16S rRNA gene amplicon sequencing. The Chao1, ACE, Shannon, and Simpson indices were used to evaluate the diversity and richness of the gut microbiome. As exposure duration to PM₁₀-PAHs increased, the Chao1, ACE, and Shannon indices at 1 W, 2 W, and 4 W (indicating the duration of PM₁₀-PAHs exposure for 1 week, 2, and 4 weeks, respectively) decreased compared to control mice, while the Simpson index increased (Fig. 3). Notably, in mice orally administered PM₁₀-PAHs for a 2-week period, all index values showed significant changes. Additionally, to determine whether the diversity and richness of the gut microbiome could be restored to normal, 4 weeks of PM₁₀-PAHs administration were followed by PBS administration for 4 weeks (designated as 4W-re), after which the gut microbiome was analyzed. The results indicated that all four alpha diversity indices in the 4W-re group returned to levels similar to those of the control mice. These findings suggest that the gut microbiome in mice is sensitive to PM₁₀-PAHs but can recover to normal levels in terms of alpha diversity once PM₁₀-PAHs exposure is discontinued.

3.3. PM₁₀-PAHs disturbed the gut microbiota beyond its capacity for resilience

To further assess the effects of PM₁₀-PAHs exposure on the gut

microbiome, the composition of the gut microbial community was analyzed. Prior to PM₁₀-PAH treatment, the gut microbial community of C57BL/6 mice was dominated by Firmicutes and Bacteroidetes at the phylum level (Fig. 4A) and by *Lactobacillus* (29.13 %) and *Bacteroides* (17.90 %) at the genus level (Fig. 4B).

After 4 weeks of PM₁₀-PAH treatment, the relative abundance of Firmicutes decreased from 87.99 % to 77.20 % while the one of Bacteroidetes increased from 10.26 % to 21.62 %, indicating that bacteria within Firmicutes are more sensitive to PAH toxicity than those within Bacteroidetes (Fig. 4A). This PM₁₀-PAH-induced changes in the gut microbiota was attributed to a decline in key genera such as *Eubacterium_g6* and *PAC001043_g* within Firmicutes and increase in genus *PAC001063_g* (11.52 %) and *PAC001074_g* (4.26 %) belonging to family *Muribaculaceae* of phylum Bacteroidetes, which were not detected in the gut microbiota of PM₁₀-PAHs-untreated mice (Fig. 4B and Table S1).

Even 4 weeks after discontinuing PM₁₀-PAH administration, the microbial community did not return to its original community structure. The relative abundance of Firmicutes further decreased from 77 % to 71 %, while the initially dominant genera, *Lactobacillus* and *Bacteroides*, declined to 18 % and 8.3 %, respectively. In contrast, genera that initially had low relative abundance, such as *PAC001063_g*, *PAC002367_g*, and *PAC000198_g*, increased to 10.66 %, 6.55 %, and 4.67 %, respectively. These results suggest that PM₁₀-PAHs may exert a toxicity level severe enough to surpass the resilience of the gut microbiota, indicating that once the gut microbial community is disturbed, it may not return to its original state even without further toxicant exposure. This disturbance allows new bacterial species, previously not dominant, to occupy the gut environment, preventing a full restoration to the initial community structure.

3.4. PM₁₀-PAHs exposure disrupts microbial functional profile with limited recovery

To examine the metabolic pathway changes induced by PM₁₀-PAHs exposure, functional profile of microbial communities were predicted using PICRUSt2 based on the gut microbiota composition and KEGG Orthology (KO) numbers were assigned to the predicted genes, resulting in 6556 annotated KO entries (Table S2). KO numbers that exhibited significant changes in frequency across the 1 W, 2 W, and 4 W samples

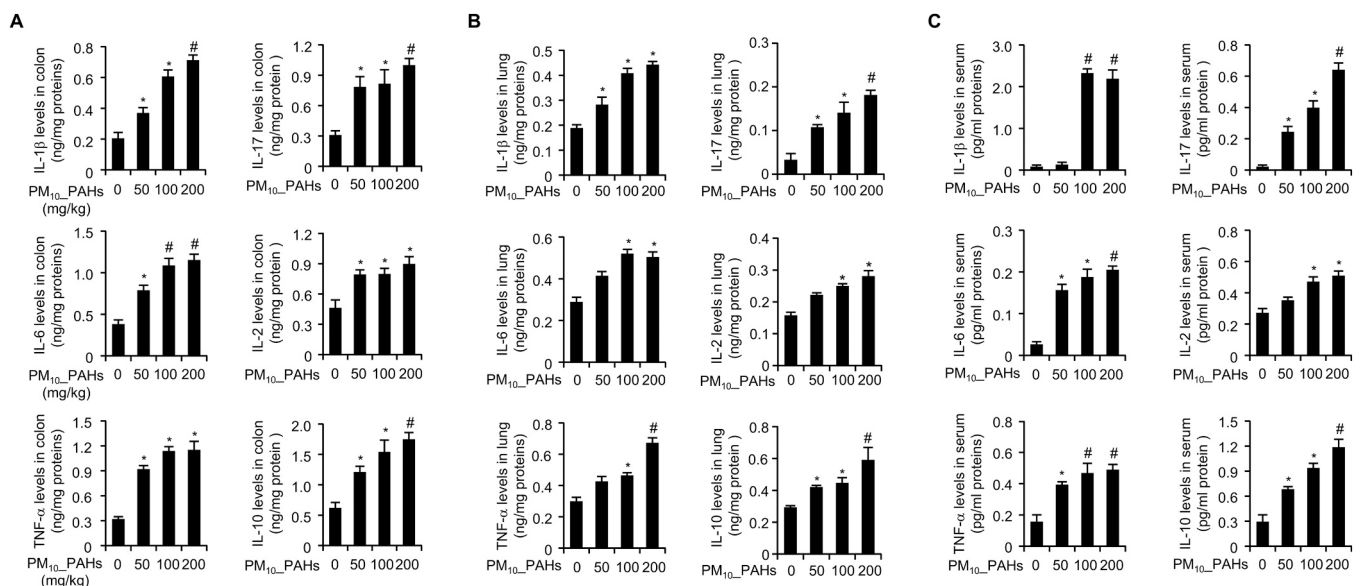


Fig. 2. Effects of PM₁₀-PAHs exposure on immune responses. (A) Concentration-dependent secretion of inflammatory cytokines (IL-1 β , IL-6, TNF- α) in the colon tissues following PM₁₀-PAHs administration (0, 50, 100, 200 mg/kg). (B) Production levels of inflammatory cytokines in lung tissues after oral gavage with PM₁₀-PAHs. (C) Serum levels of inflammatory cytokines (IL-1 β , IL-6, TNF- α , IL-17, IL-2, IL-10) measured after oral PM₁₀-PAHs exposure (* p -value $\leq$ 0.05, # p -value $\leq$ 0.01).

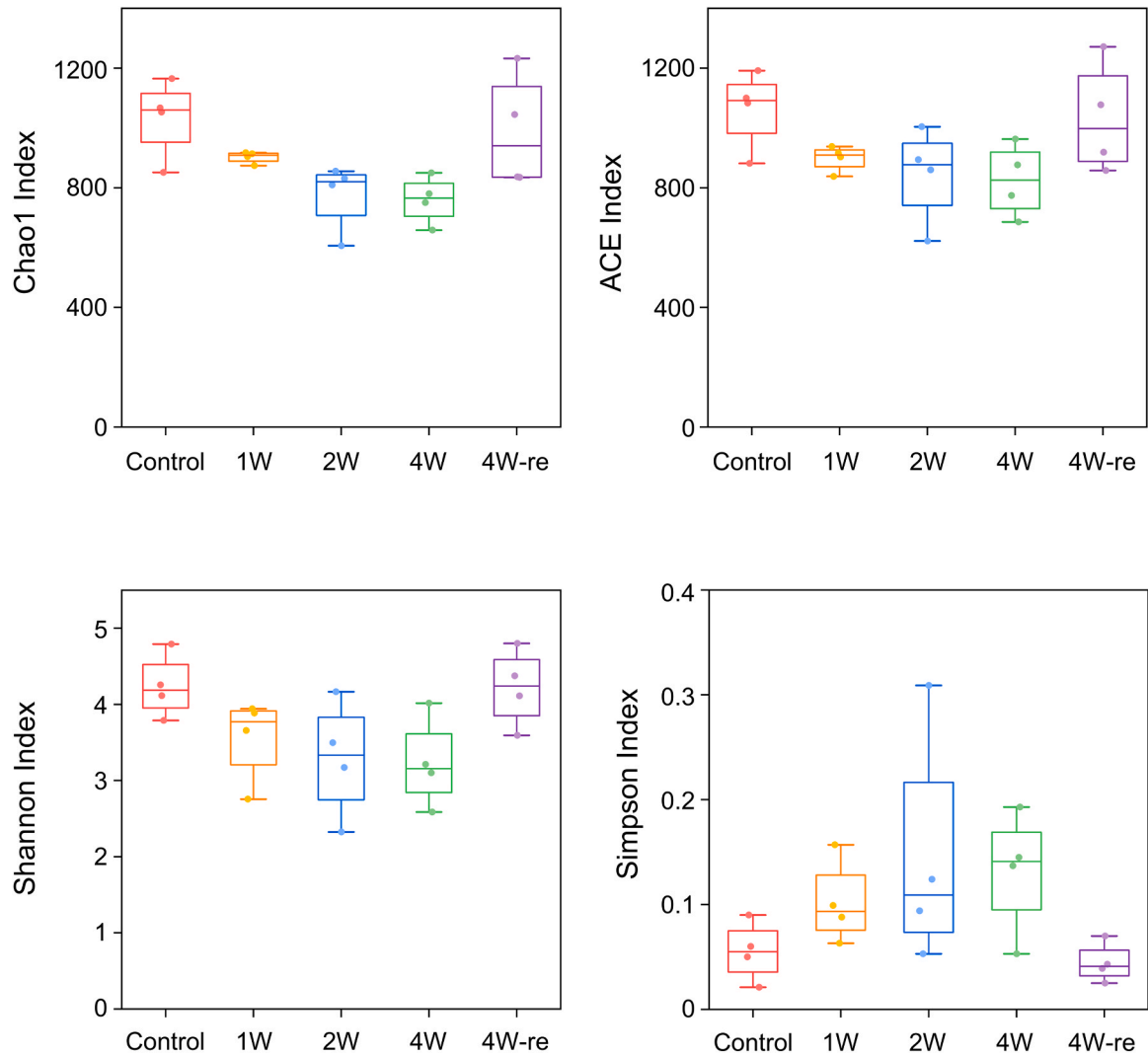


Fig. 3. Alpha diversity indices evaluating the effects of PM₁₀PAHs exposure on the gut microbial community. The box represents the interquartile range, the line indicates the median, and the whiskers show the minimum and maximum values. The control group was orally gavaged with sterile pyrogen-free saline, while 1 W, 2 W, and 4 W represent groups exposed to PM₁₀PAHs for 1, 2, and 4 weeks, respectively. 4W-re indicates the group exposed to PM₁₀PAHs for 4 weeks, followed by PBS administration for an equal duration.

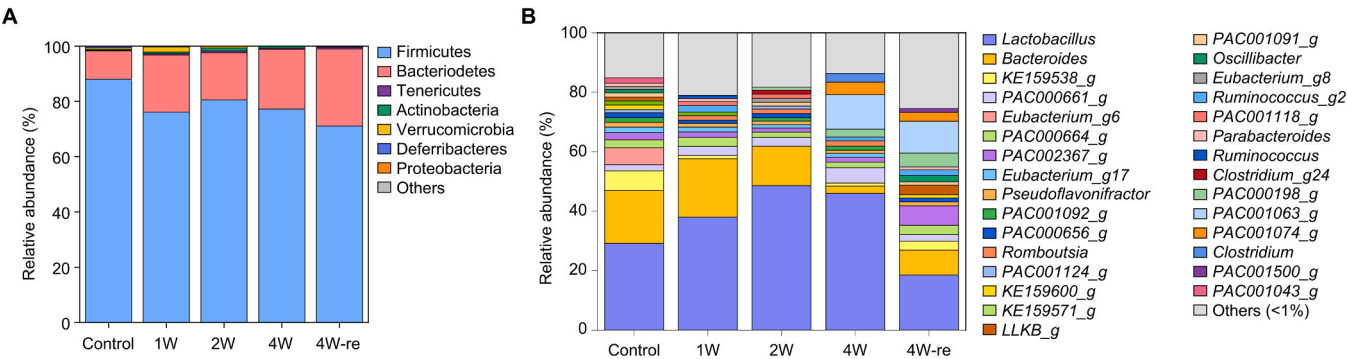


Fig. 4. PM₁₀PAHs exposure alters gut microbiota composition over time. Taxonomic composition of the gut microbiota in mice exposed to sterile pyrogen-free saline or PM₁₀PAHs in a time-dependent manner at the (A) phylum and (B) genus levels. Statistical significance was determined using two-way ANOVA (p -value < 0.05).

were identified and then KO numbers that showed differences between the 4 W and 4W-re (indicating 4 weeks of rehabilitation after 4 weeks of PM₁₀PAHs exposure) samples were summarized in Table S3. Among the 736 KO numbers that exhibited significant frequency changes, 729 showed a decreasing trend with prolonged PM₁₀PAHs exposure,

whereas only six (K06897, K08679, K04720, K03785, K01658, and K13940) increased in the 4W-re sample (Fig. S1). Only five KO numbers exhibited an increasing trend in the PM₁₀PAHs exposure groups (blue-shaded), and among them, three significantly decreased in the 4W-re sample (Table S3 and Fig. S1). However, their impact on overall gut

microbial metabolism appears to be minimal. The overall decrease in the frequency of predicted genes and the lack of functional recovery during the rehabilitation period suggest that once microbial functions were disrupted, they may not be fully restored even after the cessation of PM₁₀-PAHs exposure.

3.5. Prolonged PM₁₀-PAHs exposure leads to sustained inflammation and tissue damage in the gut

The pathological characteristics of intestinal tissues and immune responses in response to PM₁₀-PAHs exposure were examined. In the control group, intestinal cells were regularly arranged, whereas inflammatory responses and tissue damage began to appear in some intestinal tissues in the group orally administered PM₁₀-PAHs for one week (Fig. 2). Inflammatory regions were more widespread, and greater tissue damage was observed in the 2 W and 4 W samples, indicating that prolonged exposure to PM₁₀-PAHs leads to increased inflammation and tissue damage. Specifically, IL-1 β and IL-6 levels continuously increased with longer durations of PM₁₀-PAHs exposure, with the highest levels observed in the 4 W samples (Fig. 5). TNF- α , IL-2, and IL-10 showed their highest levels after one or two weeks of exposure, while IL-17 peaked at two weeks and did not decrease thereafter. Even after cessation of PM₁₀-PAHs exposure, the levels of pro-inflammatory (M1) macrophage-inducing cytokines, such as TNF- α , IL-1 β , IL-6, and IL-17, remained elevated compared to the control. These results suggest that PM₁₀-PAHs exposure increases inflammatory cytokine levels and induces a prolonged inflammatory response, preventing full recovery even after a four-week rehabilitation period.

4. Discussion

PAHs, known for their carcinogenic, mutagenic, and teratogenic properties (da Silva et al., 2021), are ubiquitous environmental pollutants to which humans are exposed through inhalation, ingestion, and dermal contact (Montano et al., 2025). Due to the high lipophilicity, PAHs readily accumulate in visceral organs rich in adipose tissue after entering the body (Abdel-Shafy and Mansour, 2016). Consequently, they are considered major toxic components of PM, with established associations to diseases such as lung cancer, atherosclerosis, hypertension, myocardial infarction, and breast cancer (Holme et al., 2019; Korsh

et al., 2015). In this study, we investigated the systemic effects of orally administrated PM₁₀-PAHs in mice, with a particular focus on gut microbiota and immune responses.

Since ingested particulate matter directly interacts with the gastrointestinal tract—a primary interface between the host and the external environment—previous studies have employed oral administration methods to investigate the effects of particulate matter on gastrointestinal diseases (Kish et al., 2013). Exposure to PM₁₀-PAHs resulted in structural damage to the colonic mucosa, leading to immune cell infiltration in the colon and lung tissues, which in turn activated an inflammatory response (Figs. 1 and 2). Intensive inflammatory response might be attributed to the increased intestinal permeability, allowing PM₁₀ and inflammatory factor such as lipopolysaccharides (LPS) to enter the bloodstream that were identified from the previous study (Kish et al., 2013; Li et al., 2024). In addition, increased expression of inflammatory cytokines was observed in lung tissue. A previous study reported that PM-induced gut microbiota dysbiosis can negatively affect lung function (Song et al., 2024). Similarly, the oral and intraperitoneal administration of phenanthrene, a representative PAH, in female mouse models was shown to significantly increase pro-inflammatory cytokines such as TNF- α and IL-6 in lung tissue (Ma et al., 2020). Analysis of microbial composition revealed a decrease in the relative abundance of Firmicutes and an increase in Bacteroidetes at the phylum level, along with an increased abundance of *Lactobacillus* in the PM₁₀-PAHs exposure groups (Fig. 4 and Table S1). Since our study, which used PAHs extracted from PM₁₀, showed similar inflammatory responses and microbial community changes to those observed in studies using conventional PM₁₀, it is likely that PAHs are the primary components responsible for the previously observed harmful effects of PM₁₀ (Kish et al., 2013; Li et al., 2024; Mutlu et al., 2018; Phillippi et al., 2022).

The gut microbiota plays a crucial role in maintaining human health by regulating immune responses and providing protection against harmful substances. Alterations in the gut microbiota have been linked to serious diseases such as inflammatory bowel disease and colorectal cancer (Afzaal et al., 2022; Xu and Knight, 2015). In this study, we observed a reduction in both the diversity and richness of the mice gut microbiota exposed to PM₁₀-PAHs (Fig. 3). Therefore, dysbiosis in the gut microbiota induced by exposure to PM may contribute to disease development (Kish et al., 2013; Zhao et al., 2024). Bacteria that showed significant changes in frequency following PM₁₀-PAHs exposure were

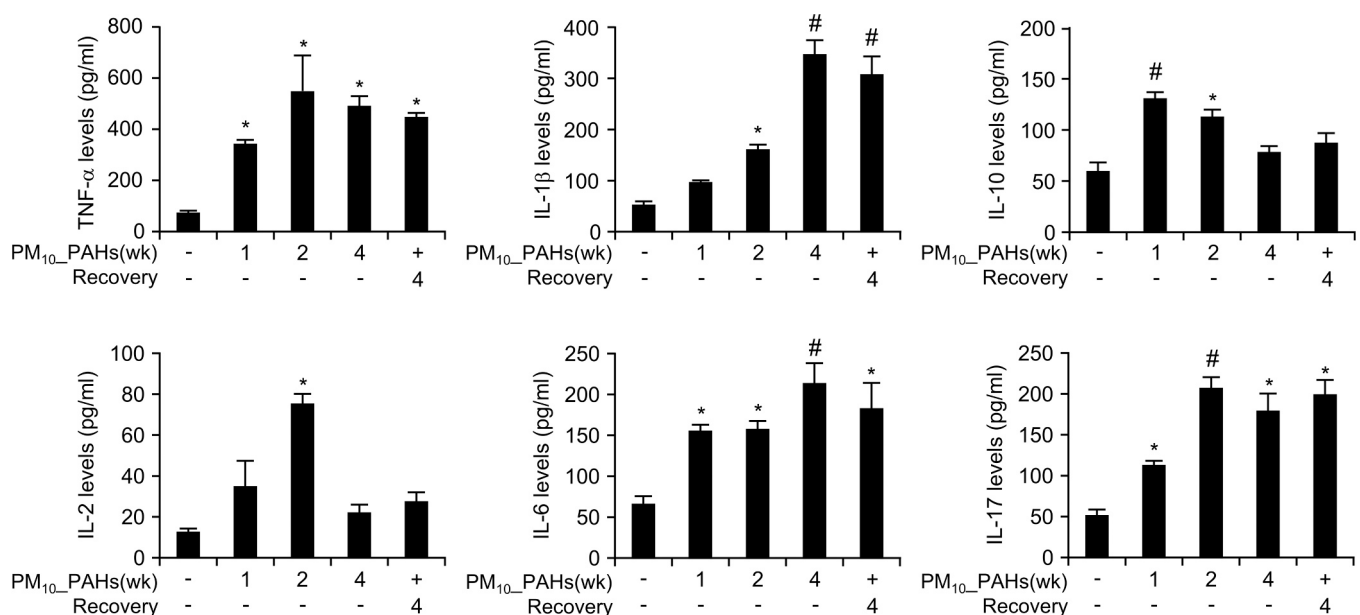


Fig. 5. Examination of inflammatory cytokine levels in serum after cessation of PM₁₀-PAHs exposure. PM₁₀-PAHs(wk) indicates the duration (weeks) of PM₁₀-PAHs exposure. Recovery refers to mice exposed to PM₁₀-PAHs for 4 weeks, followed by PBS administration for an additional 4 weeks. **p*-value ≤ 0.05 , #*p*-value ≤ 0.01 .

determined by analysis of community composition, LEfSe, and LDA (Figs. 4 and 5). *Lactobacillus* species are known to selectively degrade pro-inflammatory chemokines through the secretion of lactocepin, exert anti-inflammatory effects, mitigate intestinal barrier damage in human intestinal epithelial cells (Caco-2) via aryl hydrocarbon receptor (AhR) activation, and alleviate colitis symptoms (Ahmed et al., 2022; Hsieh et al., 2018; Olivares et al., 2006; Wang et al., 2014; Zhang et al., 2022). Additionally, *Lachnospiraceae* and *Ruminococcaceae* families, along with the *Muribaculaceae* genus, are key producers of short-chain fatty acids (SCFAs) such as propionate and butyrate, which serve as major energy sources for intestinal epithelial cells and play a crucial role in maintaining gut homeostasis through their anti-inflammatory effects (Lobionda et al., 2019; Smith et al., 2019; Vacca et al., 2020). However, further research is needed to determine the specific effects of these bacteria on host health under PM₁₀-PAHs exposure conditions.

After the cessation of PM₁₀-PAHs exposure, the elevated inflammatory response levels and the microbial community structure did not return to their original state, even though the diversity and richness of the gut microbiota recovered to levels comparable to those of the control group (Fig. 4A). When PAHs enter the body, they can induce oxidative stress and inflammatory responses, which may lead to a systemic inflammatory response (Xiao et al., 2023; Zhou et al., 2023). In addition, systemic inflammatory responses and gut microbiota dysbiosis can lead to immune imbalance and delayed mucosal healing, thereby creating a vicious cycle in which inflammation and microbial imbalance are sustained (Shen et al., 2025). Consequently, the persistent systemic inflammation at the cellular level may hinder the restoration of gut microbiota composition and inflammatory markers in the bloodstream to normal levels. This suggests that a longer recovery period may be required for full restoration. Also, given the toxicity of PAHs against bacteria, it is likely that a substantial portion of the microbiota was eliminated, leading to a reduction in the overall community size. During the recovery process, changes in microbial growth rates, competition, and symbiotic relationships may have contributed to the formation of a newly structured microbial community. However, despite this structural reorganization, the overall functional capacity of the microbiota, as determined by individual gene functions, does not appear to have been restored. This study demonstrated that PAHs have a significant impact on systemic inflammatory responses and gut microbiota imbalance. However, real-world PM is a complex mixture containing various toxicants beyond PAHs. Therefore, the findings of this study may not fully represent the biological responses that occur under actual environmental PM exposure conditions.

5. Conclusion

This study demonstrated that PM₁₀-PAHs exposure induces tissue damage and inflammatory responses in colonic and lungs while disrupting gut microbiota community. These findings suggest that PM₁₀-PAHs may contribute to the development of inflammatory bowel disease and systemic inflammatory responses. Furthermore, although gut microbial diversity recovered after PM₁₀-PAHs exposure ceased, the overall microbiota composition and immune responses remained altered. These results indicate that environmental pollutants such as PAHs can trigger systemic inflammation, leading to prolonged immune responses independent of the recovery of gut microbiota diversity. This study demonstrated that orally administered PM₁₀-PAHs induce intestinal barrier damage, disruption of the gut microbiota, and systemic inflammatory responses, thereby providing important foundational data for understanding the biological risks of PAHs exposure in living organisms.

CRediT authorship contribution statement

Seon-Jin Lee: Writing – review & editing, Supervision, Funding acquisition. **Sang Jun Lee:** Writing – review & editing, Supervision,

Funding acquisition, Conceptualization. **Young-Jun Park:** Investigation, Funding acquisition, Conceptualization. **Jaejoon Jung:** Writing – original draft, Investigation, Data curation. **Song Hee Jeong:** Writing – original draft, Visualization, Investigation, Data curation.

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Declaration of Competing Interest

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

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2025.118910](https://doi.org/10.1016/j.ecoenv.2025.118910).

Data Availability

Data will be made available on request. Supplementary Table S1 is publicly accessible via Mendeley Data [doi: 10.17632/74mzdwjpp.1].

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