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Supplemental Material

Respiratory Effects of Traffic-Related Air Pollution: A Randomized, Crossover Analysis of Lung Function, Airway Metabolome, and Biomarkers of Airway Injury

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Table of Contents

Questionnaires

Table S1. The p-values for the difference of goodness-of-fit between the linear and non-linear models assessing exposure to traffic related air pollutants and outcome measures in a randomized crossover study, n=56 adults, Shanghai, China.

Table S2. Spearman correlation coefficients among traffic related air pollutants measured in a randomized crossover study, n=56 adults.

Table S3. Respiratory symptom score (mean \pm SD) before and after each exposure session (Road and Park) among 56 young adults participating in a randomized crossover study in China.

Table S4. P values for associations shown in Figure 1 for identified metabolic pathways associated with TRAP and individual air pollutants based on the untargeted metabolome wide-association study (MWAS) conducted for exhaled breath condensate (EBC) metabolomics in a randomized crossover trial in China (n=56 adults).

Figure S1. Flowchart of participants' recruitment and selection for a randomized crossover study on exposure to traffic related air pollution.

Figure S2. Time series of 24-hour sampling for *NanoTracer* (UFP) (5-minute interval), *MicroPEM* (PM_{2.5}) (1-minute interval), *MicroAeth* (BC) (5-minute interval) and *PEK Lite* (NO₂ and CO) (1-minute interval) during the side-by-side tests before the trial.

Figure S3. Time series of 24-hour sampling for *NanoTracer* (UFP) (5-minute interval), *MicroPEM* (PM_{2.5}) (1-minute interval), *MicroAeth* (BC) (5-minute interval) and *PEK Lite* (NO₂ and CO) (1-minute interval) during the side-by-side tests at the middle of the trial.

Figure S4. Time series of 24-hour sampling for *NanoTracer* (UFP) (5-minute interval), *MicroPEM* (PM_{2.5}) (1-minute interval), *MicroAeth* (BC) (5-minute interval) and *PEK Lite* (NO₂ and CO) (1-minute interval) during the side-by-side tests at the end of the trial.

Questionnaires

Basic Information Questionnaire

Dear Volunteers.

Thanks for participating in the "Study on the Acute Health Effects of TRAP Exposure".

Please take a few minutes to fill out this questionnaire carefully, and thanks for your support.

Date: _____
Name: _____
Age: _____
Sex: _____
Tel: _____

1. Are you a current smoker?

yes no

2. Does anyone in your dorm or office smoke?

yes no

3. In the past 12 months, have you resided regularly in Fenglin/medical campus of Fudan University?

yes no

4. Have you ever been diagnosed with any of following allergic diseases (atopic dermatitis, allergic rhinitis, allergic asthma, anaphylactoid purpura, anaphylactic shock)?

yes no other allergic diseases: _____

5. Have you ever been diagnosed with any of following cardiovascular disease

(congenital heart disease, coronary heart disease, pulmonary heart disease, rheumatism of heart, hypertensive heart disease)?

yes no other cardiovascular diseases: _____

6. Have you ever been diagnosed with any of following respiratory disease (asthma, chronic tracheobronchitis, chronic pharyngitis, pulmonary tuberculosis, pneumothorax)?

yes no other respiratory diseases: _____

7. Have you ever been diagnosed with any of the following chronic disease (hypertension, hyperlipidemia, diabetes mellitus, chronic hepatitis, chronic nephritis)?

yes no other chronic diseases: _____

8. Have you ever had any surgery?

yes (time of the surgery (year/month/day): _____; type of surgery: _____)

no

9. Have you taken any medications in the recent 2 months (2019/7/1 - today)?

yes, details of the medication (e.g., names and duration of medication taken): _____ no

10. Have you taken any following dietary supplements (fish oil, vitamins, dietary fiber) in the recent 2 months (2019/7/1 - today)?

yes no other dietary supplements: _____

Information before each exposure session

Date: _____

Name of subject: _____

Tel: _____

Staff signature: _____

1. Have you taken any medications in the past three days?

yes, details of the medication (e.g., names and duration of medication taken): _____ no

2. Have you taken any following dietary supplements (fish oil, vitamins, dietary fiber) in the past three days?

yes no other dietary supplements: _____

3. Is someone smoking in your dorm or office in the past three days?

yes no

4. Have you drunk alcohol in the past three days?

yes no

5. Please indicate which below condition(s) or symptom(s) you have in the past three days (if applicable):

cold symptoms including cough, sore throat, fever and runny nose allergy

injury gastrointestinal disorders other condition(s) or symptom(s):

_____ none of those condition or symptom

Tables

Table S1 The p-values for the difference of goodness-of-fit between the linear and non-linear models assessing exposure to traffic related air pollutants and outcome measures in a randomized crossover study, n=56 adults, Shanghai, China.

	FVC	FEV ₁	FEV ₁ /FVC	MMEF	symptom scores	SP-D	Ezrin	8-isoprostane	TNF- α
PM _{2.5}	0.64	0.89	0.65	0.68	0.97	0.31	0.26	0.10	0.74
UFP	0.62	0.12	0.11	0.22	0.13	0.64	0.70	0.88	0.09
BC	0.25	0.38	0.85	0.84	0.98	0.54	0.55	0.95	0.66
NO ₂	0.90	0.28	0.19	0.21	0.47	0.07	0.51	0.76	0.58
CO	0.79	0.46	0.74	0.15	0.77	0.68	0.83	0.94	0.60

Notes: All the models were adjusted for age (continuous variable), sex (binary variable), and BMI (continuous variable)], temperature (continuous variable) and relative humidity (continuous variable).

Abbreviations: FVC, forced vital capacity; FEV₁, forced expiratory volume in the first second; MMEF, maximal mid-expiratory flow; SP-D, Surfactant Proteins D; TNF- α , tumor necrosis factor- α ; BC, black carbon; PM_{2.5}, fine particulate matter; CO, carbon monoxide; NO₂, nitrogen dioxide; UFP, ultrafine particles.

Table S2 Spearman correlation coefficients among traffic related air pollutants measured in a randomized crossover study, n=56 adults.

	BC	PM _{2.5}	CO	NO ₂	UFP
BC	-	0.55	0.87	0.81	0.78
PM _{2.5}	-	-	0.56	0.4	0.25
CO	-	-	-	0.82	0.73
NO ₂	-	-	-	-	0.71
UFP	-	-	-	-	-

Abbreviations: BC, black carbon; PM_{2.5}, fine particles matter; CO, carbon monoxide; NO₂, nitrogen dioxide; UFP, ultrafine particles.

Table S3 Respiratory symptom score (mean \pm SD) before and after each exposure session (Road and Park) among 56 young adults participating in a randomized crossover study in China.

	Road		Park	
	Before (n=56)	After (n=56)	Before (n=56)	After (n=56)
Irritation of the nose	0.05 \pm 0.23	0.36 \pm 0.67	0.04 \pm 0.19	0.18 \pm 0.47
Itchy nose	0.13 \pm 0.38	0.43 \pm 0.81	0.13 \pm 0.33	0.14 \pm 0.40
Dry nose	0.23 \pm 0.54	0.70 \pm 0.99	0.29 \pm 0.65	0.25 \pm 0.61
Burning nose	0.02 \pm 0.13	0.16 \pm 0.50	0.05 \pm 0.30	0.07 \pm 0.26
Stuffy nose	0.20 \pm 0.48	0.13 \pm 0.38	0.27 \pm 0.65	0.16 \pm 0.46
Running nose	0.18 \pm 0.47	0.38 \pm 0.78	0.13 \pm 0.38	0.14 \pm 0.40
Itchy throat	0.25 \pm 0.61	0.59 \pm 1.14	0.20 \pm 0.48	0.21 \pm 0.46
Irritation throat	0.02 \pm 0.13	0.34 \pm 0.79	0.04 \pm 0.19	0.09 \pm 0.35
Swelling throat	0.02 \pm 0.13	0.25 \pm 0.64	0.04 \pm 0.19	0.04 \pm 0.19
Burning throat	0.00 \pm 0.00	0.20 \pm 0.62	0.04 \pm 0.19	0.05 \pm 0.30
Urge to cough	0.20 \pm 0.59	0.45 \pm 0.95	0.18 \pm 0.39	0.18 \pm 0.39
Pressure on chest	0.05 \pm 0.23	0.04 \pm 0.19	0.04 \pm 0.19	0.16 \pm 0.53
Oppression to breath	0.02 \pm 0.13	0.09 \pm 0.29	0.09 \pm 0.44	0.07 \pm 0.32
Expectoration	0.11 \pm 0.37	0.21 \pm 0.65	0.13 \pm 0.43	0.09 \pm 0.29
Total score	1.70 \pm 2.28	4.91 \pm 6.54	1.89 \pm 3.00	2.09 \pm 3.63

Note: This questionnaire requires participants to rate their self-perceived severity of 14 symptoms in the respiratory tract. A higher score indicates greater symptom severity, with a score of 0 referring to no symptoms and a score of 5 referring to a severe symptom. The total respiratory symptom score was then calculated by summing up the scores across the 14 symptoms.

Abbreviations: SD, standard deviation.

Table S4 P values for associations shown in Figure 1 for identified metabolic pathways associated with TRAP and individual air pollutants based on the untargeted metabolome wide-association study (MWAS) conducted for exhaled breath condensate (EBC) metabolomics in a randomized crossover trial in China (n=56 adults).

Pathway	TRAP	UFP	BC	NO ₂	CO	PM _{2.5}
Positive ionization mode						
Vitamin E metabolism	0.11	0.04	0.06	0.08	0.00	0.99
Vitamin B9 (folate) metabolism	0.01	0.01	0.05	0.06	0.01	0.99
Vitamin B6 (pyridoxine) metabolism	0.22	0.08	0.10	0.02	0.06	0.99
Vitamin B3 (nicotinate and nicotinamide) metabolism	0.04	0.18	0.09	0.04	0.06	0.99
Urea cycle/amino group metabolism	0.00	0.00	0.00	0.00	0.00	0.99
Tyrosine metabolism	0.04	0.11	0.04	0.02	0.05	0.99
Squalene and cholesterol biosynthesis	0.12	0.18	0.22	0.29	0.02	0.99
Saturated fatty acids beta-oxidation	0.01	0.00	0.01	0.00	0.00	0.99
Pentose and Glucuronate Interconversions	0.00	0.01	0.01	0.01	0.00	0.99
Omega-3 fatty acid metabolism	0.03	0.01	0.02	0.01	0.01	0.99
Nitrogen metabolism	0.03	0.44	0.11	0.01	0.06	0.99
Lysine metabolism	0.02	0.00	0.01	0.00	0.00	0.99
Linoleate metabolism	0.00	0.02	0.00	0.00	0.02	0.99
Glycosphingolipid metabolism	0.16	0.02	0.01	0.01	0.00	0.99
Glycine, serine, alanine and threonine metabolism	0.01	0.19	0.39	0.01	0.21	0.99
Fatty Acid Metabolism	0.12	0.03	0.18	0.06	0.12	0.99
Fatty acid activation	0.03	0.01	0.03	0.01	0.02	0.99
Di-unsaturated fatty acid beta-oxidation	0.07	0.03	0.04	0.04	0.02	0.99

Carnitine shuttle	0.00	0.00	0.00	0.00	0.00	0.99
Butanoate metabolism	0.11	0.23	0.27	0.37	0.01	0.99
Beta-Alanine metabolism	0.23	0.22	0.26	0.03	0.37	0.99
Aspartate and asparagine metabolism	0.00	0.00	0.00	0.00	0.00	0.99
Arginine and Proline Metabolism	0.00	0.00	0.00	0.00	0.00	0.99
Negative ionization mode						
Vitamin B9 (folate) metabolism	0.01	0.01	0.00	0.99	0.99	0.99
Urea cycle/amino group metabolism	0.16	0.05	0.01	0.99	0.99	0.01
Tyrosine metabolism	0.17	0.03	0.02	0.20	0.99	0.00
Tryptophan metabolism	0.11	0.08	0.12	0.20	0.99	0.02
TCA cycle	0.34	0.38	0.42	0.99	0.99	0.04
Sialic acid metabolism	0.78	0.96	0.84	0.99	0.99	0.05
Pyruvate Metabolism	0.00	0.00	0.00	0.99	0.99	0.02
Pyrimidine metabolism	0.49	0.05	0.01	0.12	0.99	0.13
Purine metabolism	0.03	0.06	0.01	0.99	0.99	0.00
Propanoate metabolism	0.03	0.02	0.01	0.01	0.99	0.99
Phosphatidylinositol phosphate metabolism	0.29	0.33	0.16	0.99	0.99	0.02
Pentose phosphate pathway	0.09	0.26	0.11	0.99	0.99	0.04
Methionine and cysteine metabolism	0.12	0.21	0.07	0.99	0.99	0.00
Lysine metabolism	0.00	0.00	0.00	0.06	0.99	0.00
Leukotriene metabolism	0.06	0.05	0.15	0.99	0.99	0.99
Hexose phosphorylation	0.16	0.03	0.29	0.99	0.99	0.99
Heparan sulfate degradation	0.05	0.04	0.02	0.99	0.99	0.01
Glyoxylate and Dicarboxylate Metabolism	0.05	0.04	0.02	0.99	0.99	0.01
Glycosphingolipid metabolism	0.00	0.00	0.00	0.11	0.99	0.00
Glycolysis and Gluconeogenesis	0.05	0.04	0.13	0.07	0.99	0.07

Glycerophospholipid metabolism	0.00	0.00	0.00	0.99	0.99	0.01
Glutamate metabolism	0.29	0.02	0.01	0.99	0.99	0.99
Fructose and mannose metabolism	0.00	0.00	0.04	0.03	0.99	0.99
Fatty Acid Metabolism	0.01	0.01	0.00	0.99	0.99	0.00
Fatty acid activation	0.00	0.00	0.00	0.99	0.99	0.00
De novo fatty acid biosynthesis	0.01	0.01	0.00	0.99	0.99	0.00
Chondroitin sulfate degradation	0.05	0.04	0.02	0.99	0.99	0.01
Butanoate metabolism	0.01	0.00	0.00	0.01	0.99	0.00
Beta-Alanine metabolism	0.42	0.03	0.08	0.99	0.99	0.99
Ascorbate (Vitamin C) and Aldarate Metabolism	0.02	0.02	0.07	0.99	0.99	0.06
Arginine and Proline Metabolism	0.01	0.01	0.00	0.99	0.99	0.02
Alanine and Aspartate Metabolism	0.34	0.01	0.00	0.99	0.99	0.04

Abbreviations: TRAP, traffic-related air pollution; UFP, ultrafine particles; BC, black carbon; NO₂, nitrogen dioxide; CO, carbon monoxide; PM_{2.5}, fine particulate matter; FVC, forced vital capacity; FEV₁, forced expiratory volume in the first second; MMEF, maximal mid-expiratory flow.

Figures

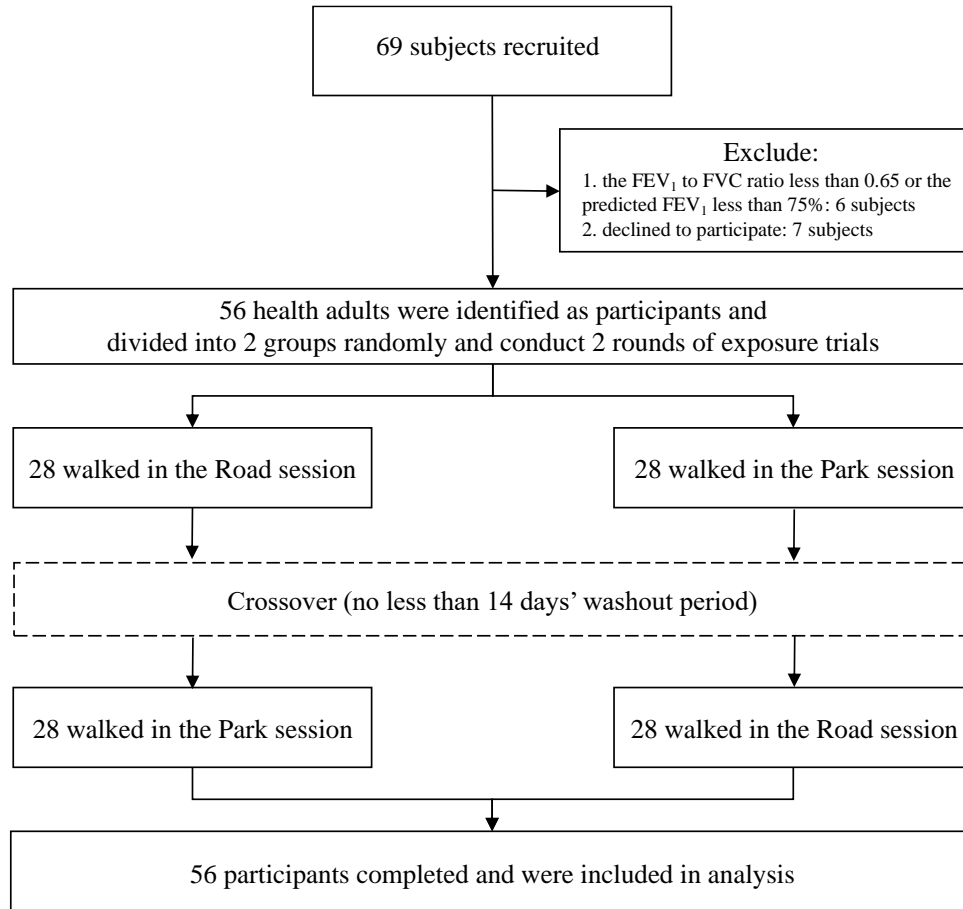


Figure S1 Flowchart of participants' recruitment and selection for a randomized crossover study on exposure to traffic related air pollution.

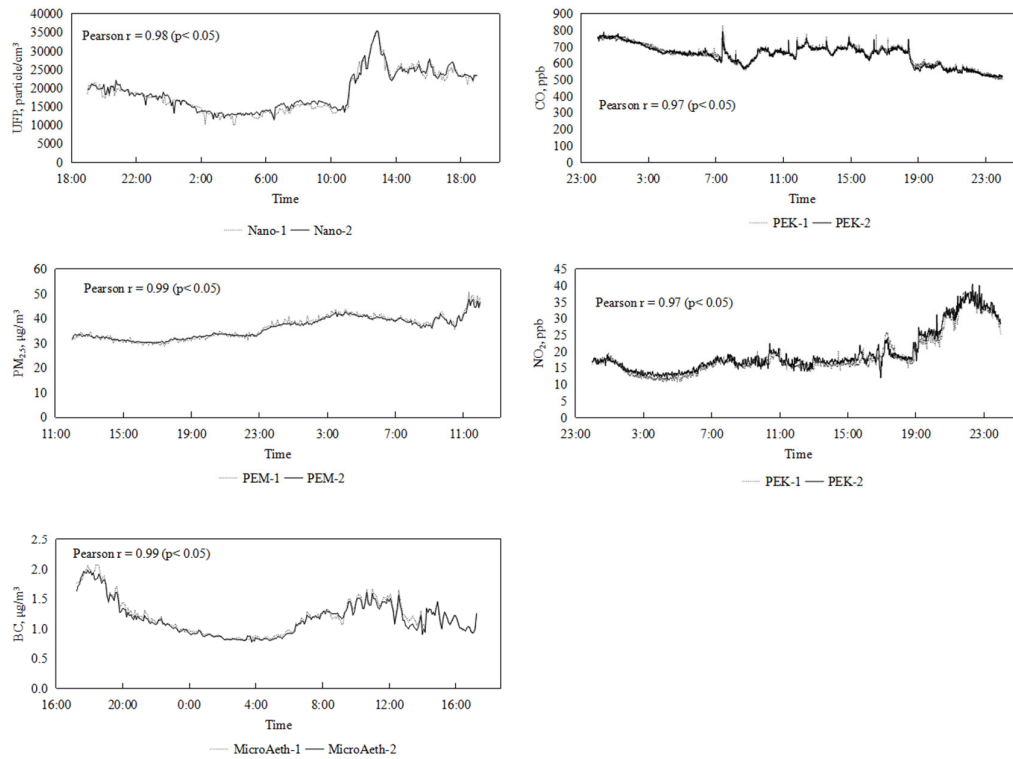


Figure S2 Time series of 24-hour sampling for *NanoTracer* (UFP) (5-minute interval), *MicroPEM* (PM_{2.5}) (1-minute interval), *MicroAeth* (BC) (5-minute interval) and *PEK Lite* (NO₂ and CO) (1-minute interval) during the side-by-side tests before the trial.

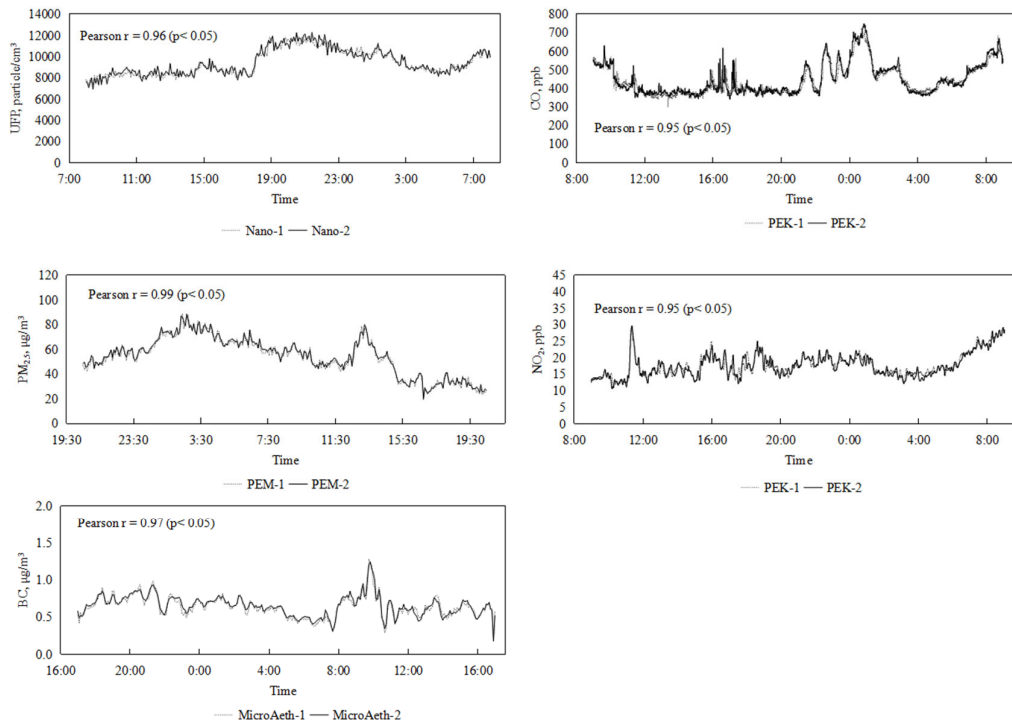


Figure S3 Time series of 24-hour sampling for *NanoTracer* (UFP) (5-minute interval), *MicroPEM* (PM_{2.5}) (1-minute interval), *MicroAeth* (BC) (5-minute interval) and *PEK Lite* (NO₂ and CO) (1-minute interval) during the side-by-side tests at the middle of the trial.

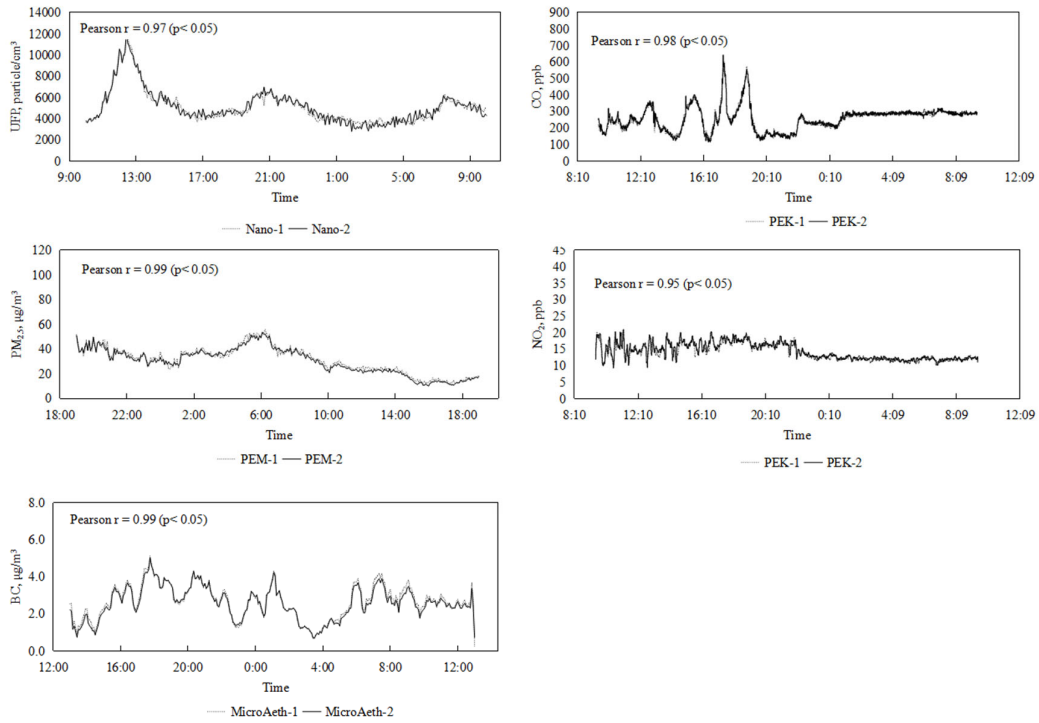


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