Characteristics of Airborne $PM_{2.5}$ from Nursery Pig House and its Mechanism of Inducing Inflammatory Response in Alveolar Macrophages

Chunmei Li

Nanjing Agricultural University

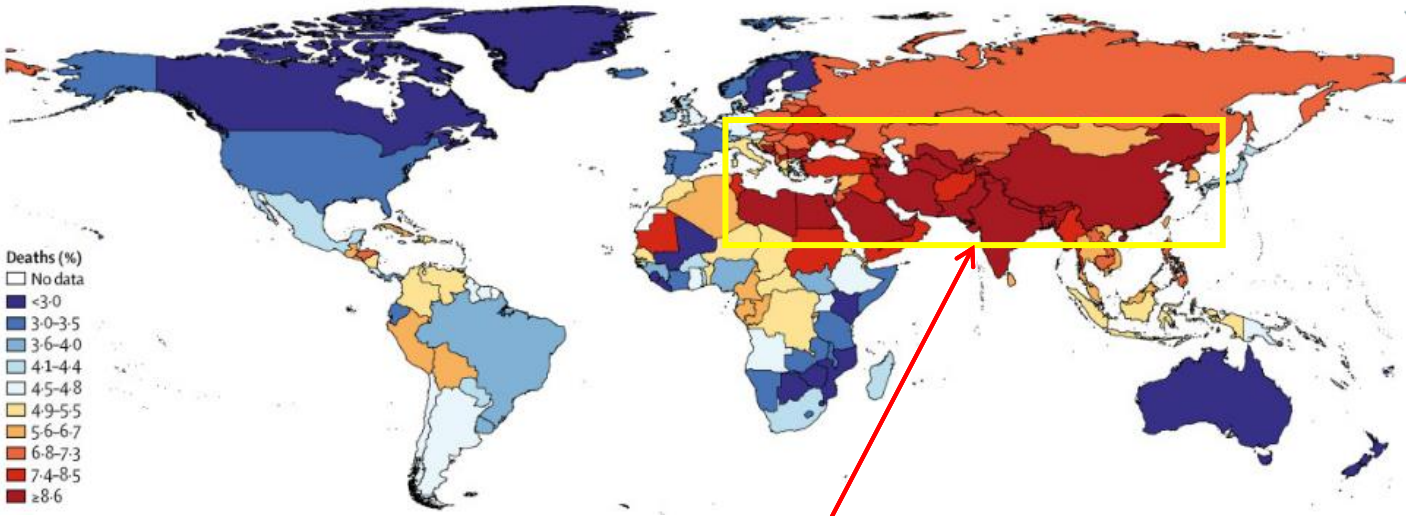
chunmeili@njau.edu.cn

2021.1.19

Session 2

Background

$PM_{2.5}$ pollution is a grave problem



Death

$PM_{2.5}$ concentration

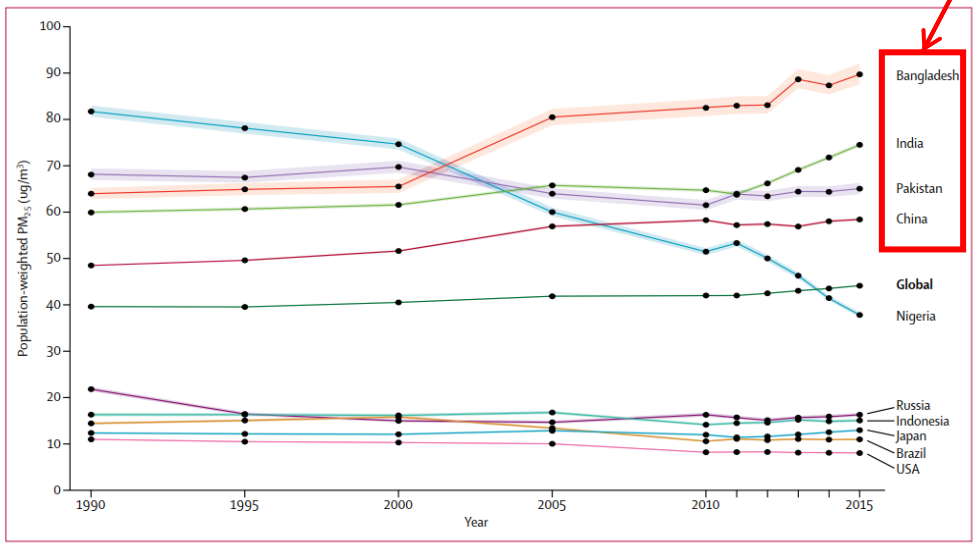


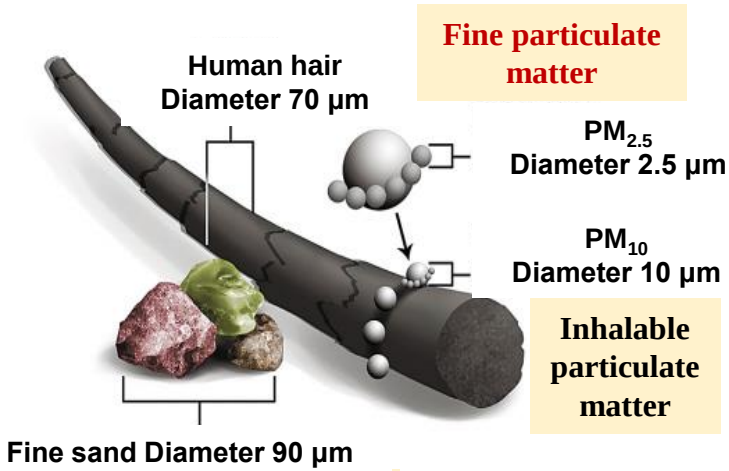
Figure 2: **Trends in population-weighted mean concentrations of particle mass with aerodynamic diameter less than 2·5 μm**
Global data and data from the ten most populous countries are shown. Shaded areas are 95% uncertainty intervals. $PM_{2.5}$=particle mass with aerodynamic diameter less than 2·5 μm.

$PM_{2.5}$ concentration is strongly correlated with death rate

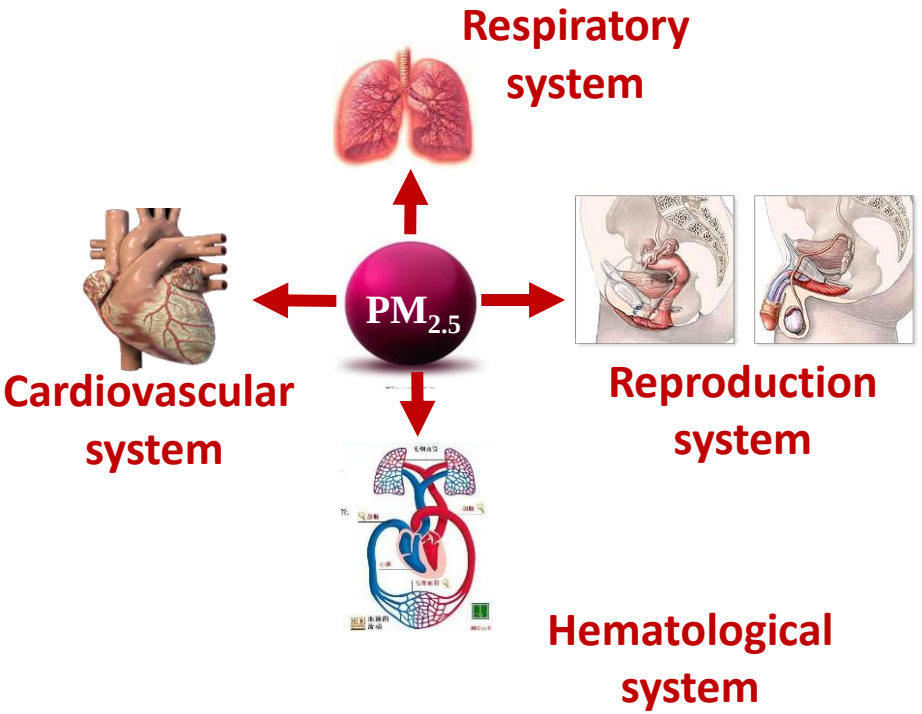
Lancet 2017; 389: 1907.

Background

Characteristic of $PM_{2.5}$



Hazard of $PM_{2.5}$



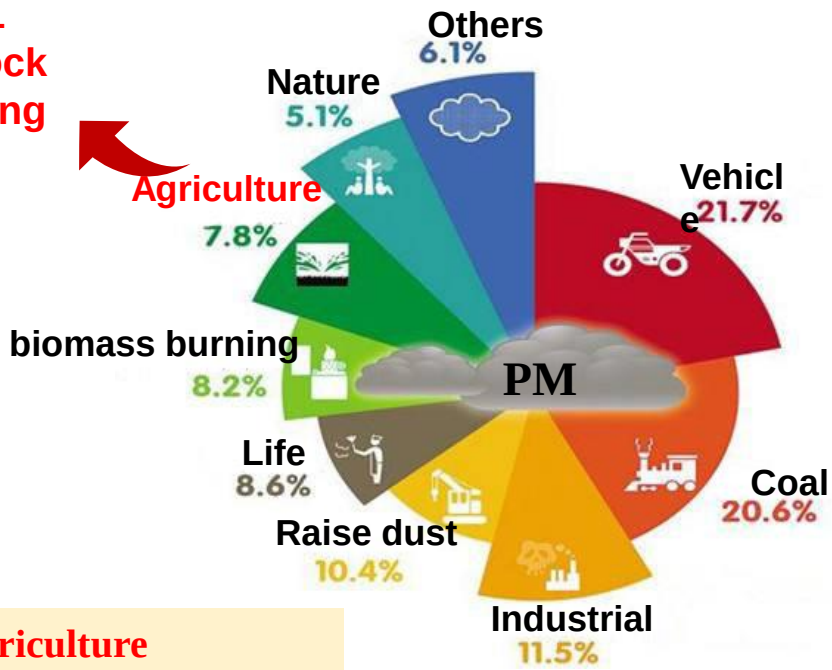
Small size, large specific surface area, stay in air for long time, transport for long distance, greater health threats (Mostafa，2016)

$PM_{2.5}$ has been listed in high risk inhalable particles by ISO 7708 (ISO 7708, 1995)

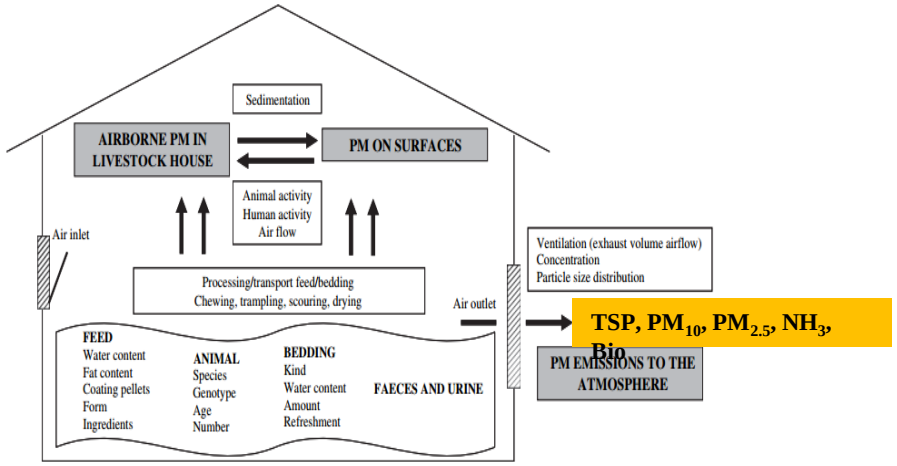
Background

PM emission

No.1 livestock breeding



Agriculture accounted for 7.8%



—— Cambra-Lo´pez, 2010

- In Europe, the **PM_{10}** and **$PM_{2.5}$** from livestock productions accounted for **8%** and **4%** of atmospheric PM10 and PM2.5
- **Intensive poultry** and **swine** houses contribute approximately **50%** and **30%** of total agricultural PM emissions, respectively

(Kaasik and Maasikmets, 2013)

$PM_{2.5}$ from pig house is different from the atmosphere

Main sources of PM

Feed

Hair

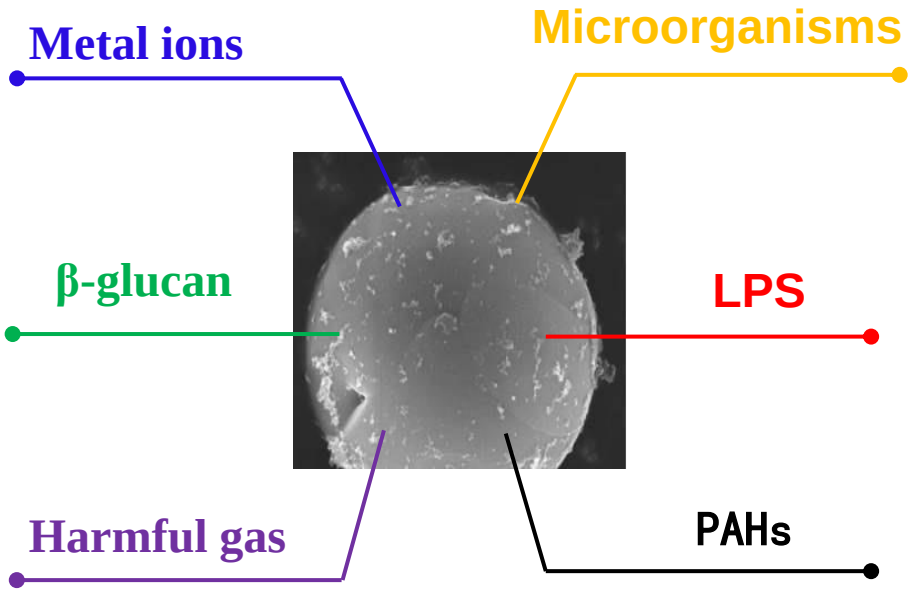
Manure

Skin

outside

padding

Main components of PM



Atmospheric Environment 2011, 45: 694-707

Hazard of PM from pig house

Markers of inflammatoin changed in farmers working in pig house

（bronchoalveolar lavage fluid and blood）

Inflammatory response

Table 5. Markers of inflammation in lavage fluids that have been altered after exposure to swine confinement house dust.

Markers in lavage	Reference
Total white blood cell count	(90,91,97,100,103)
Monocytes	(94)
Macrophages	(89–91,93,97)
Lymphocytes	(90,91,93,94,97,99)
Granulocytes	(93,94)
Eosinophils	(93,97)
Neutrophils	(90,91,93,97,99,100)
T-cell markers	(93)
IL-1α	(94)
IL-1β	(94,103)
IL-6	(94,103)
IL-8	(90,91,103)
Tumor necrosis factor-α	(94)
Albumin	(94,97,100)
Fibronectin	(100)
Hyaluronan	(100)

Table 6. Markers of inflammation in blood that have been altered after exposure to swine confinement house dust.

Markers in blood	Reference
Total white blood cell count	(90,92,95,97,103–105)
Monocytes	(90,92,99)
Lymphocytes	(93,103)
Granulocytes	(92,93,95)
Neutrophils	(90,99,103,104)
IL-1 receptor antagonist	(105)
IL-1β	(105)
IL-6	(92,94,95,103,105,106)
Tumor necrosis factor-α	(92,105)
Oroscomucoid	(97)
C-reactive protein	(97,104)
Fibrinogen	(106)

Comparing with atmosphere， PM from pig house can induce inflammatory response more easily

Environmental health perspectives 2000; 108: 685.

Mainly problems in pig houses

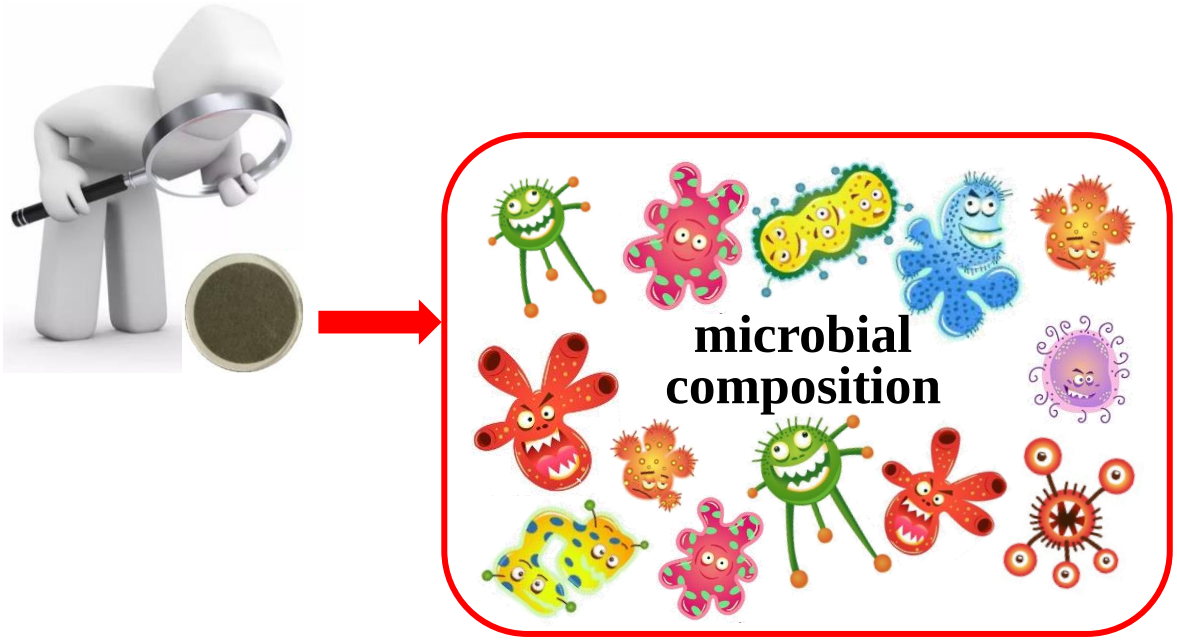
- $PM_{2.5}$ concentrations in livestock houses were higher than other inside house, the microorganism, metal elements adsorbed on the particles were both harmful to worker and animal health, as well as ambient environment.
- The shortage of ventilation in winter will cause the increase of particulates, which will affect the health of the animals in the house.
- However, few studies have focused on $PM_{2.5}$ from livestock houses.



Aims of the study

The objective of this study was to

- Analyze the bacterial and fungal assemblage contained in $PM_{2.5}$ from a nursery pig house across four seasons
- Investigate the ability of $PM_{2.5}$ from the nursery pig house to induce an inflammatory response in pigs



Q1：What are the kinds of microorganisms contained in $PM_{2.5}$ from a nursery pig house among different seasons？

Monitoring the environment inside the nursery pig house among different seasons

Spring May → **Summer July** → **Autumn October** → **Winter January**

7:00~03:00，every two hours, for continuous 7 days

7:00 9:00 11:00 13:00 15:00 17:00 19:00 03:00

Nursery pig house

R1

The microclimate variables measured in nursery pig house among different seasons

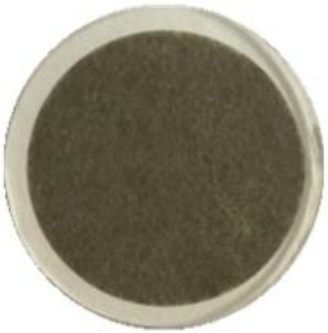
	Spring	Summer	Autumn	Winter
Temperature(° C)	23.84 ± 0.22^{a}	29.16 ± 0.26^{b}	26.35 ± 0.22^{c}	21.11 ± 0.73^{d}
RH(%)	74.69 ± 2.74^{a}	80.01 ± 1.28^{a}	78.80 ± 1.15^{a}	49.47 ± 1.07^{b}
THI	68.19 ± 0.10^{a}	72.47 ± 0.84^{b}	70.10 ± 0.26^{ab}	64.89 ± 0.65^{c}
Wind speed(m/s)	0.10 ± 0.01^{a}	0.23 ± 0.05^{b}	0.08 ± 0.01^{a}	0.10 ± 0.01^{a}
TSP (μg/m^3)	413.21 ± 39.82^{a}	49.57 ± 3.58^{b}	147.75 ± 18.27^{b}	983.2 4± 86.07^{c}
PM_{10} (μg/m^3)	183.02 ± 18.46^{a}	30.12 ± 1.91^{b}	94.76 ± 15.63^{c}	385.25 ± 16.61^{d}
$PM_{2.5}$ (μg/m^3)	58.07 ± 6.91^{ab}	15.80 ± 1.10^{a}	81.55 ± 15.11^{b}	144.18 ± 14.50^{c}
PM_1 (μg/m^3)	53.47 ± 6.3^{ab}	14.87 ± 1.18^{a}	75.56 ± 14.06^{b}	135.98 ± 12.85^{c}
NH_3 (mg/m^3)	10.07 ± 0.28^{a}	8.23 ± 0.04^{b}	8.42 ± 0.10^{b}	8.30 ± 0.09^{b}
CO_2 (mg/m^3)	1795.66 ± 88.31^{a}	908.26 ± 22.69^{b}	1848.46 ± 55.68^{a}	1903.98 ± 43.54^{a}

RH：relative humidity；THI： temperature-humidity index

$PM_{2.5}$ collection and microbial analysis

Spring May → Summer July → Autumn October → Winter January

$PM_{2.5}$ samples collected in the middle of the nursery pig hous

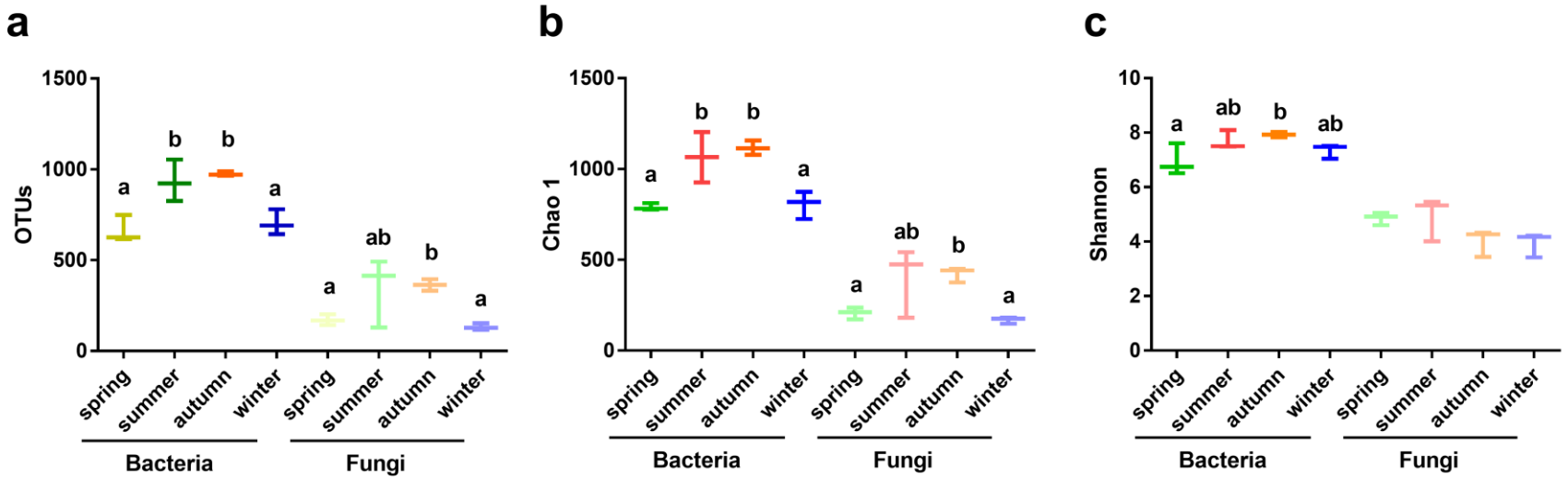


$PM_{2.5}$ samples

For bacteria： 16S rRNA;
For fungi ： ITS1 rRNA;

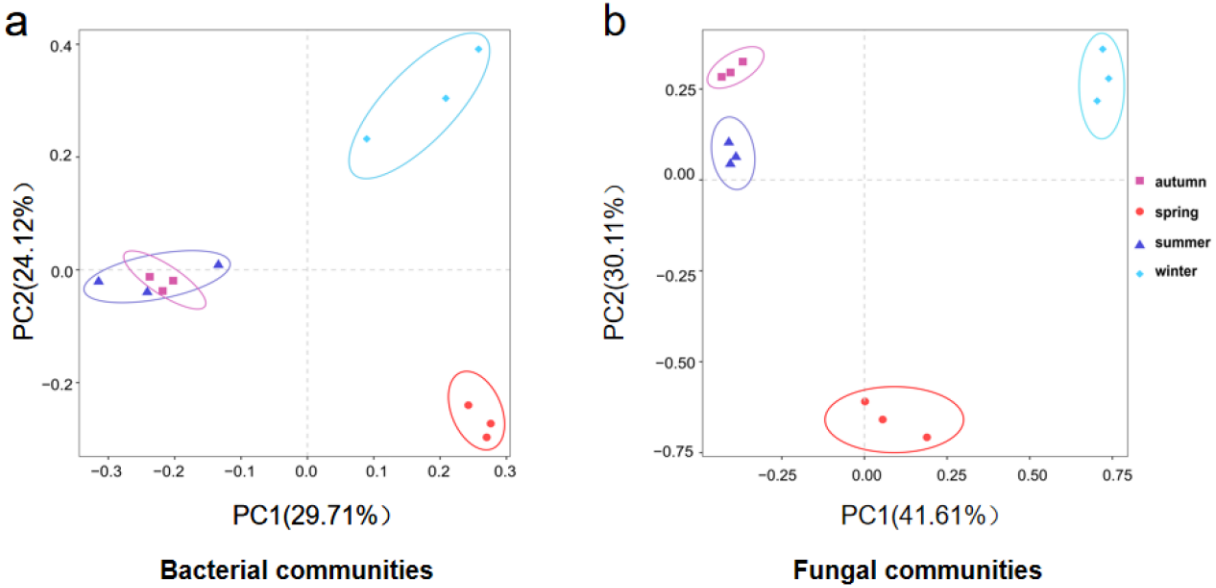
R2

Alpha- and Beta- diversity comparison among different seasons



Summer and Autumn higher than Spring and Winter

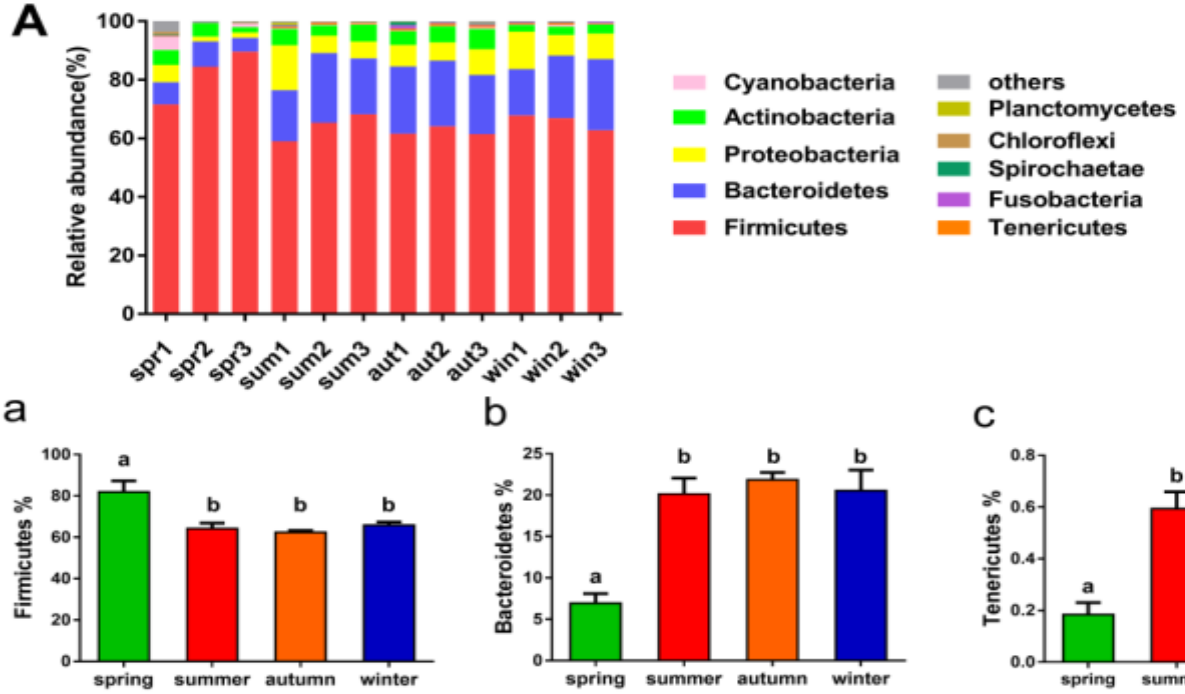
For bacteria, summer and autumn clustered together;
For fungi, samples clustered by season.



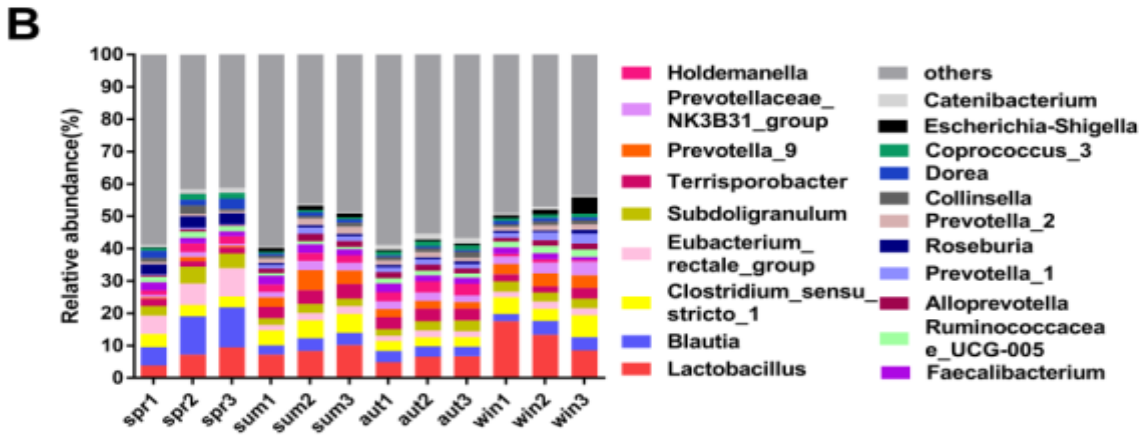
R3

Bacterial assemblage composition among different seasons

phylum



genus

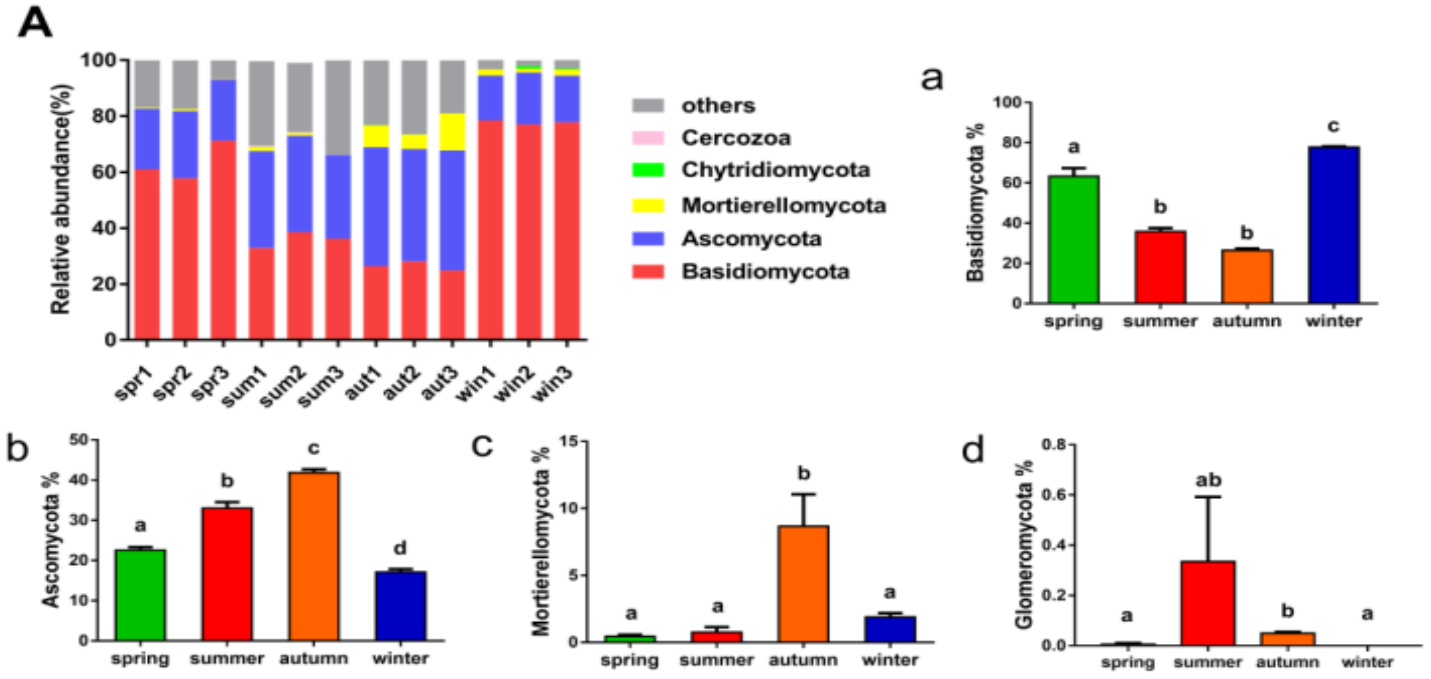


Among Top 20 bacterial genera, 16 kinds were significantly different

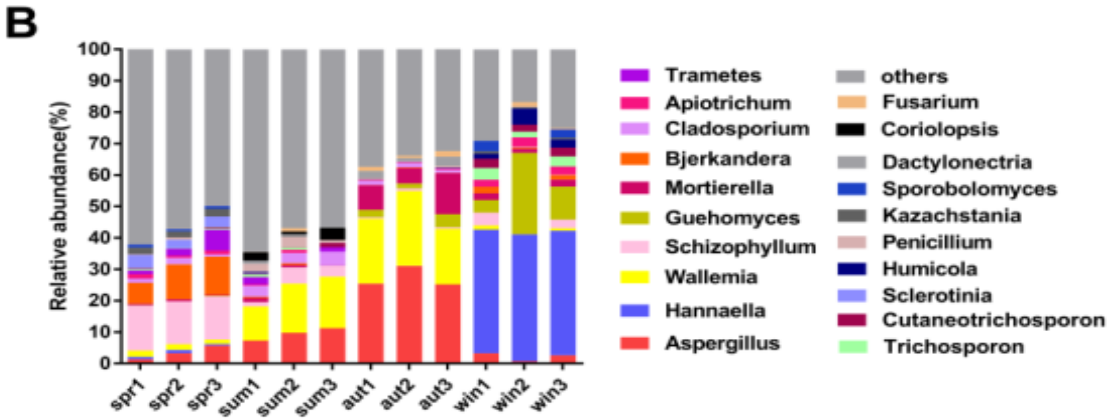
R4

Fungal assemblage composition among different seasons

phylum



genus



R5

The relative abundance of potential bacterial pathogen genera (%)

	Spring	Summer	Autumn	Winter
Total bacterial pathogen	0.99 ± 0.30^{a}	5.33 ± 0.73^{b}	3.10 ± 0.09^{b}	4.91 ± 2.04^{ab}
Pseudomonas	0.08 ± 0.03	0.53 ± 0.21	0.16 ± 0.02	0.60 ± 0.21
Escherichia-Shigella	0.09 ± 0.05	1.24 ± 0.04	0.53 ± 0.02	2.47 ± 1.29
Acinetobacter	0.10 ± 0.06	1.05 ± 0.41	0.39 ± 0.04	0.73 ± 0.23
Streptococcus	0.53 ± 0.05^{a}	1.03 ± 0.11^{b}	1.36 ± 0.04^{b}	0.99 ± 0.15^{b}
Staphylococcus	0.19 ± 0.17^{ab}	1.47 ± 0.14^{c}	0.67 ± 0.06^{bc}	0.13 ± 0.02^{a}

five pathogenic bacteria genera were identified

(Summer>Winter>Autumn>Spring)

R6

The relative abundance of fungal allergen genera (%)

	Spring	Summer	Autumn	Winter
Total fungal allergen	22.32 ± 1.01^{ab}	30.93 ± 3.29^{a}	49.36 ± 3.58^{c}	14.44 ± 2.73^{b}
Aspergillus	3.45 ± 1.27^{ab}	9.49 ± 1.15^{b}	27.23 ± 1.91^{c}	2.23 ± 0.75^{a}
Wallemia	1.77 ± 0.18^{a}	14.27 ± 1.73^{b}	20.74 ± 1.80^{c}	0.71 ± 0.37^{a}
Schizophyllum	13.89 ± 0.22^{a}	3.33 ± 1.10^{b}	0.53 ± 0.12^{b}	2.32 ± 1.21^{b}
Trichosporon	0.36 ± 0.11^{a}	0.51 ± 0.26^{a}	0.01 ± 0^{a}	2.89 ± 0.58^{b}
Humicola	0 ± 0^{a}	0.13 ± 0.13^{a}	0.12 ± 0.04^{a}	3.06 ± 1.06^{b}
Penicillium	0.34 ± 0.22^{a}	2.27 ± 0.71^{b}	0.43 ± 0.1^{a}	0 ± 0^{a}
Sporobolomyces	0.82 ± 0.08	0.01 ± 0.01	0 ± 0	1.99 ± 1.00
Trichoderma	0.02 ± 0.01^{a}	0.28 ± 0.13^{ab}	0.06 ± 0.02^{a}	0.53 ± 0.02^{b}

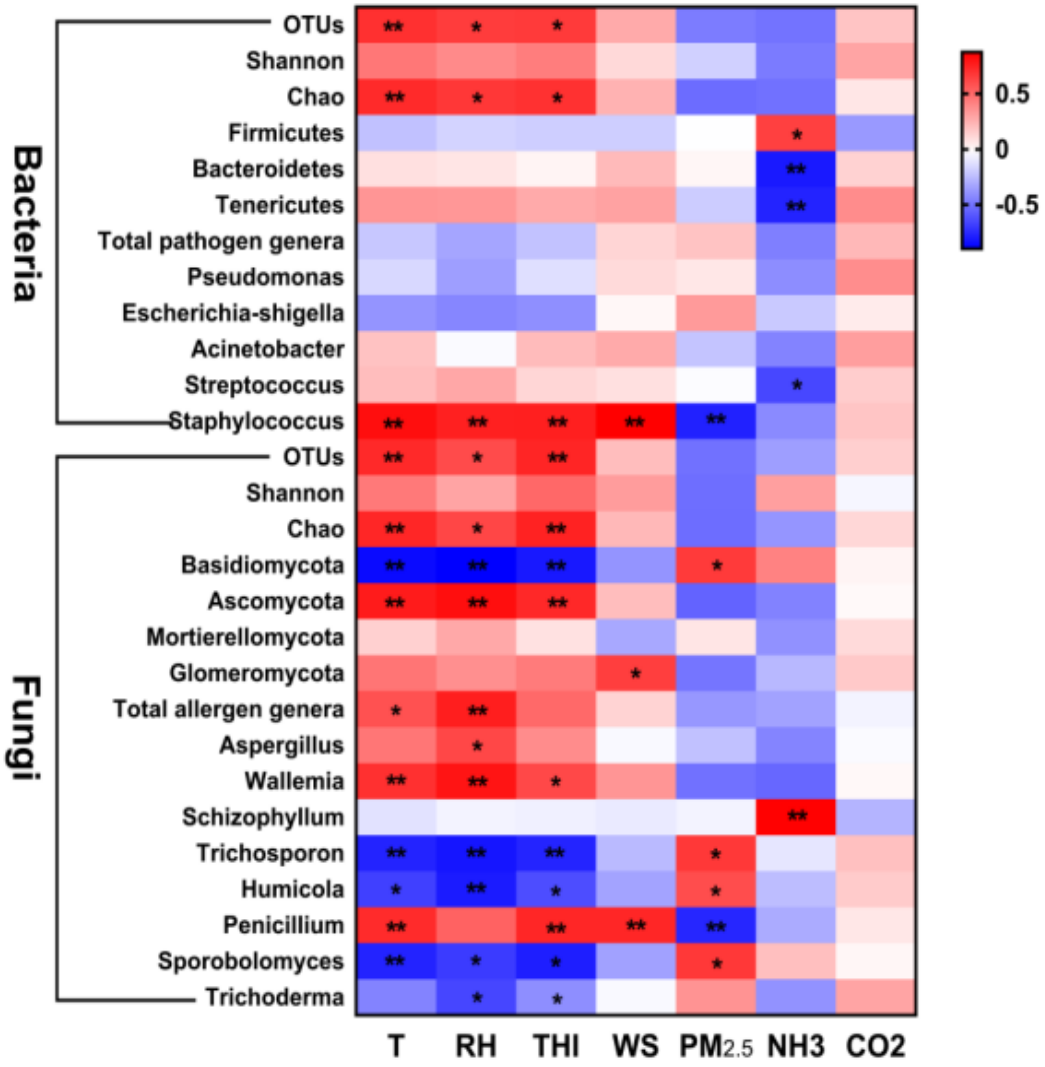
The genera with relative abundance > 0.01% were listed

20 fungal allergen genera were identified

(Autumn>Summer>Spring>Winter)

R7

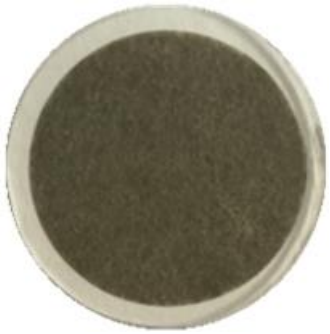
Correlation analysis between microbial indexes and microclimate variables



Whether bacteria or fungi, OTUs and Chao index had ***significantly positive correlations*** *with temperature, RH, and THI*

Note：T: tempetature;
RH: relative humidity;
THI: humidity-temperature index;
WS: wind speed

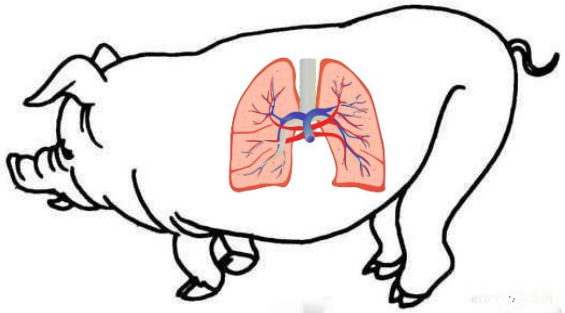
$PM_{2.5}$ samples

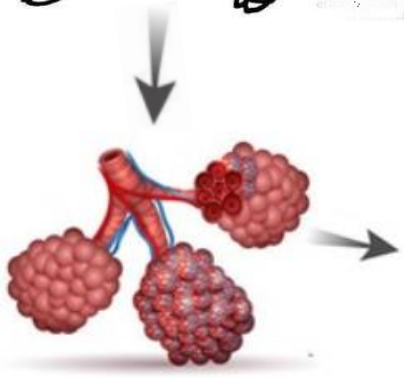


- High concentration
- Complex component
- Pathogenic microbiota
- Long exposure time

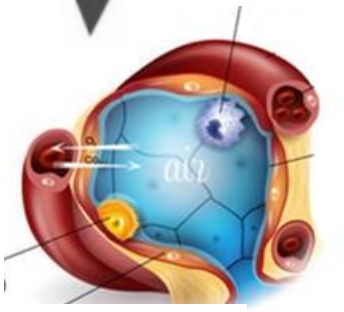
Q2：What is the mechanism of $PM_{2.5}$ induced inflammatory response in lung ？

Role of alveolar macrophages in lung





Alveolar macrophage



alveolar type II epithelial cells

alveolar type I epithelial cells

PAMPs/DAMPs

Pathogen/Damage-associated molecular patterns

phagocytosis

Over stimulate

Anti-inflammation — **balance** — **Pro-inflammation**

（Pro-inflammatory cytokines，Chemokines）

Alveolar epithelial cells

Pulmonary inflammatory environment

Cell selection and $PM_{2.5}$ preparation

Preparation of $PM_{2.5}$ sample stock solution:

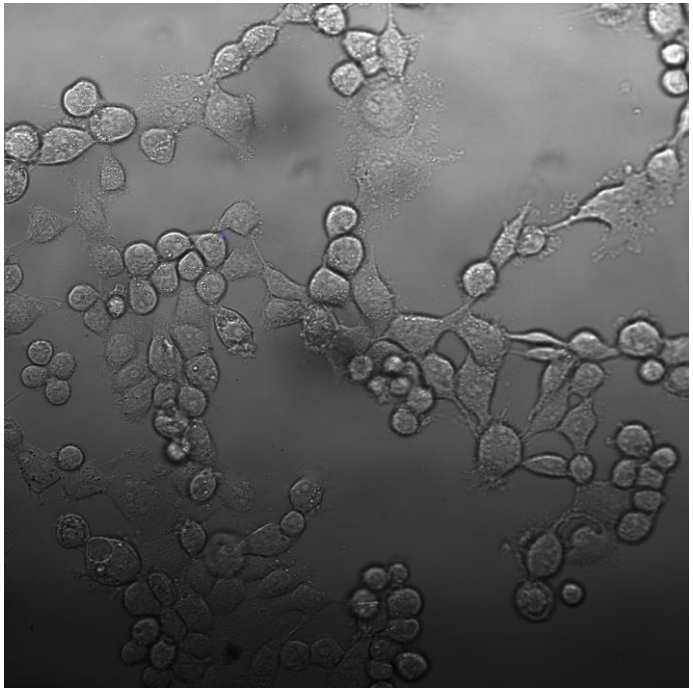
1 mg freeze-dried $PM_{2.5}$ dissolved into 1 ml DMEM medium to make sure the final concentration is 1 mg/ml for stock solution

Selection of cell line:

Procine alveolar macrophage 3D4/21

treatment — **Concentrations of $PM_{2.5}$**

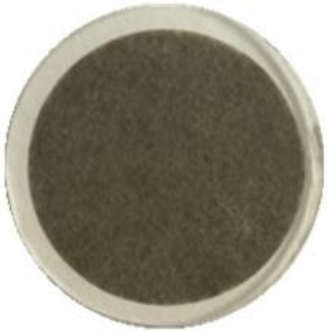
0 μg/ml | 12.5 μg/ml | 25 μg/ml | 50 μg/ml

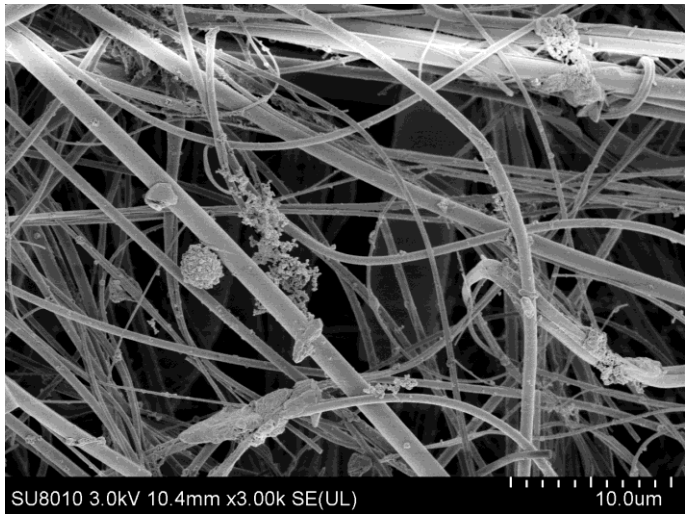


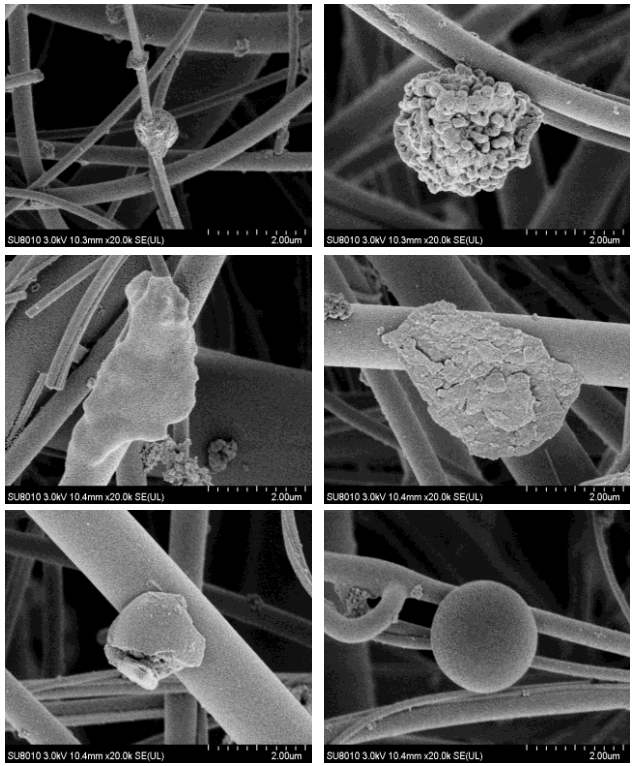
Procine alveolar macrophage 3D4/21

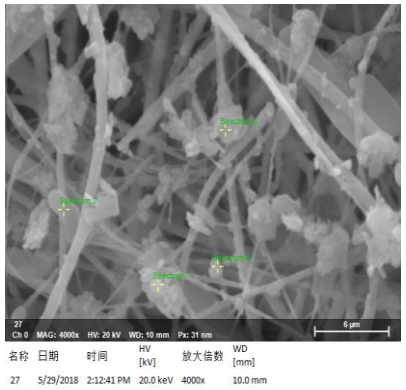
R8

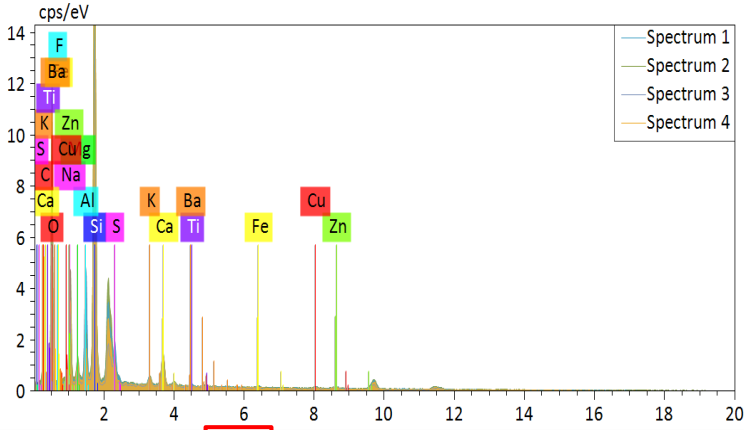
Morphology and chemical characterization of $PM_{2.5}$











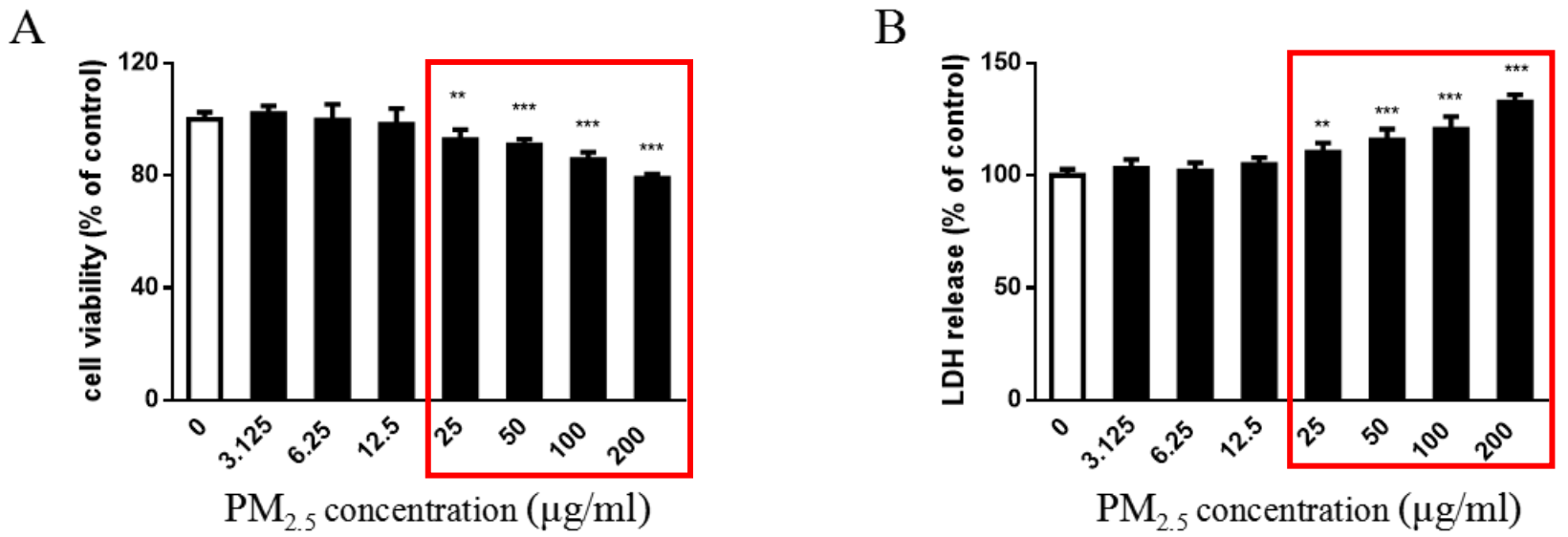
Spectrum	C	O	F	Na	Mg	Al	Si	S	K	Ca	Ti	Fe	Cu	Zn	Ba
Mean	11.61	41.72	1.21	8.03	1.06	3.78	23.71	3.27	0.92	4.40	0.59	0.49	0.70	1.61	1.41
Sigma	2.16	4.42	0.00	1.31	0.32	2.25	4.05	0.66	0.23	1.21	0.00	0.38	0.09	0.23	0.61

The concentration of **LPS** in $PM_{2.5}$:

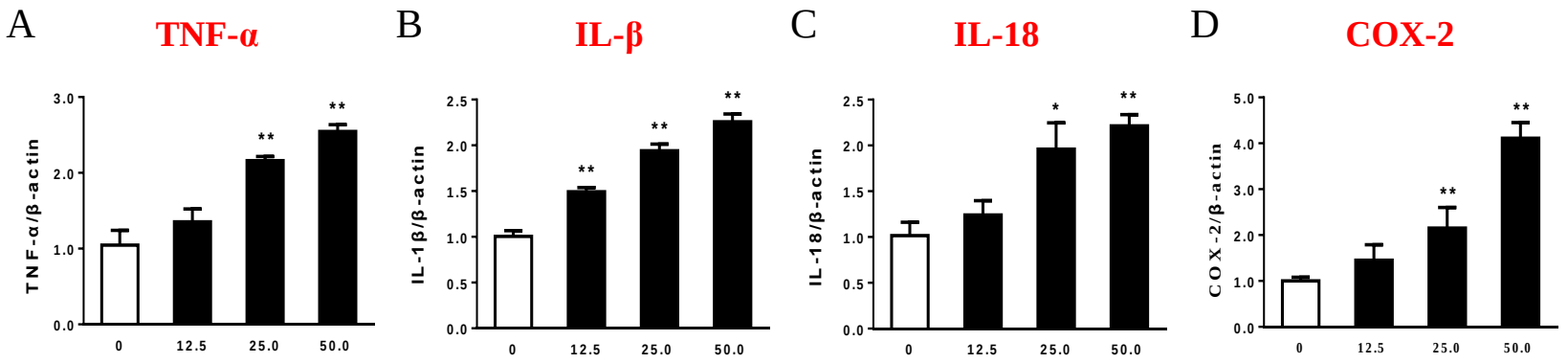
681.80 ±19.47 EU/mg

R9

$PM_{2.5}$ induced cytotoxicity and inflammatory cytokine release



mRNA expression levels of inflammatory cytokines



$PM_{2.5}$

ROS

TLR4

1、Recognize LPS (Cell wall components of Gram-negative bacteria)

2、Higher expression in alveolar macrophages (relative to other Toll-like receptors)

Induce pro-inflammatory cytokines

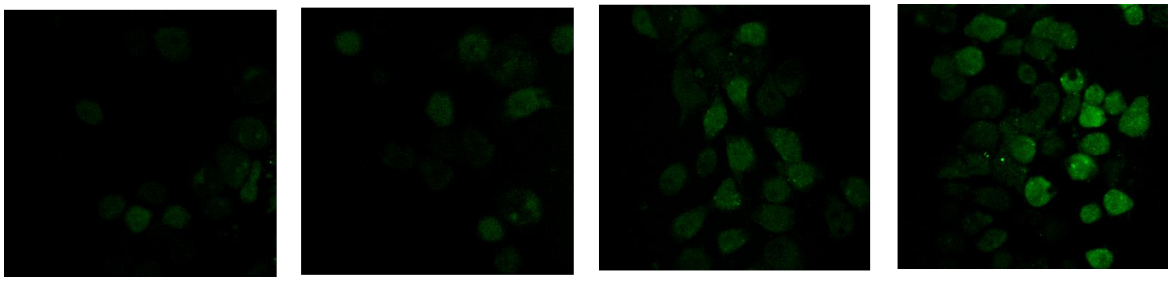
Increase cell apoptosis

Decrease cell viability

R10

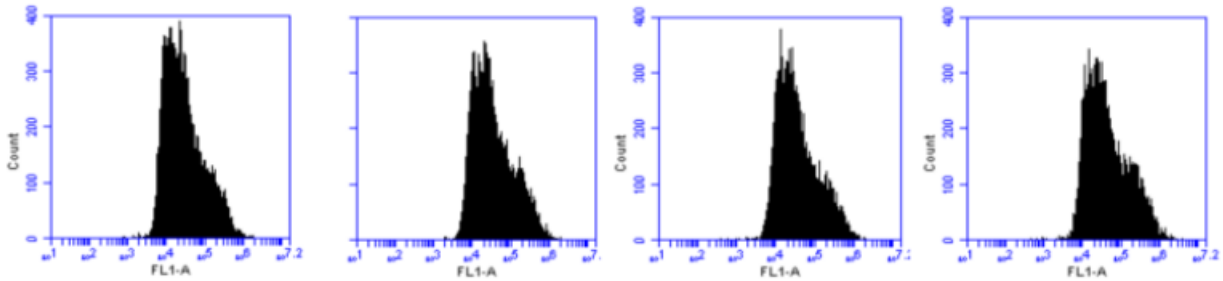
$PM_{2.5}$ increase ROS and Ca^{2+} level

A

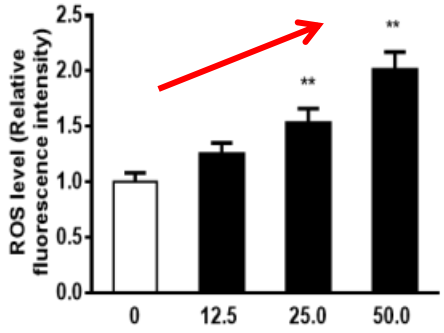


0 μg/ml 12.5 μg/ml 25 μg/ml 50 μg/ml

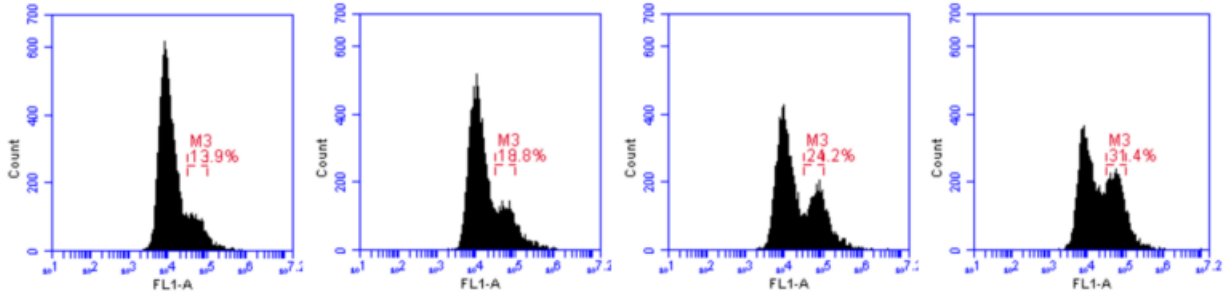
B



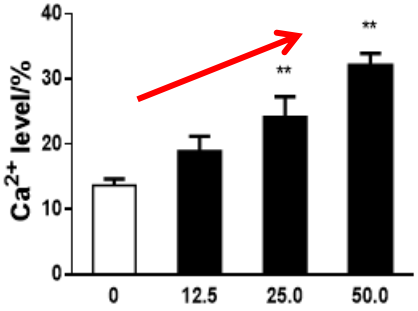
0 μg/ml 12.5 μg/ml 25 μg/ml 50 μg/ml



C

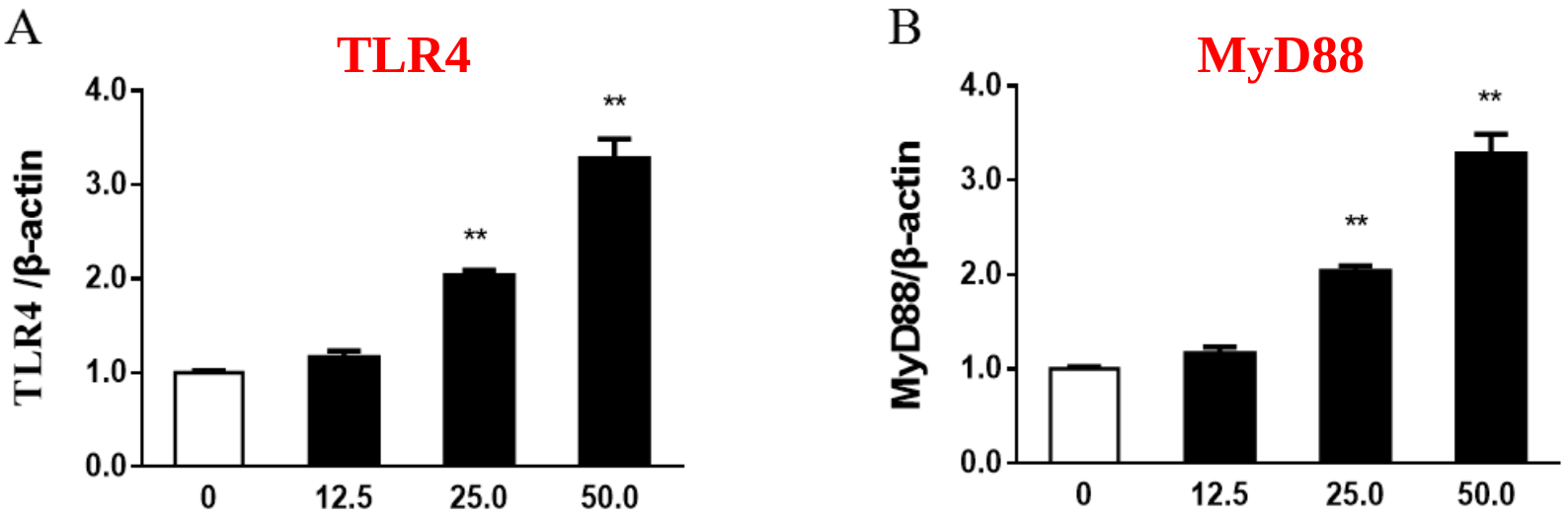


0 μg/ml 12.5 μg/ml 25 μg/ml 50 μg/ml



R11

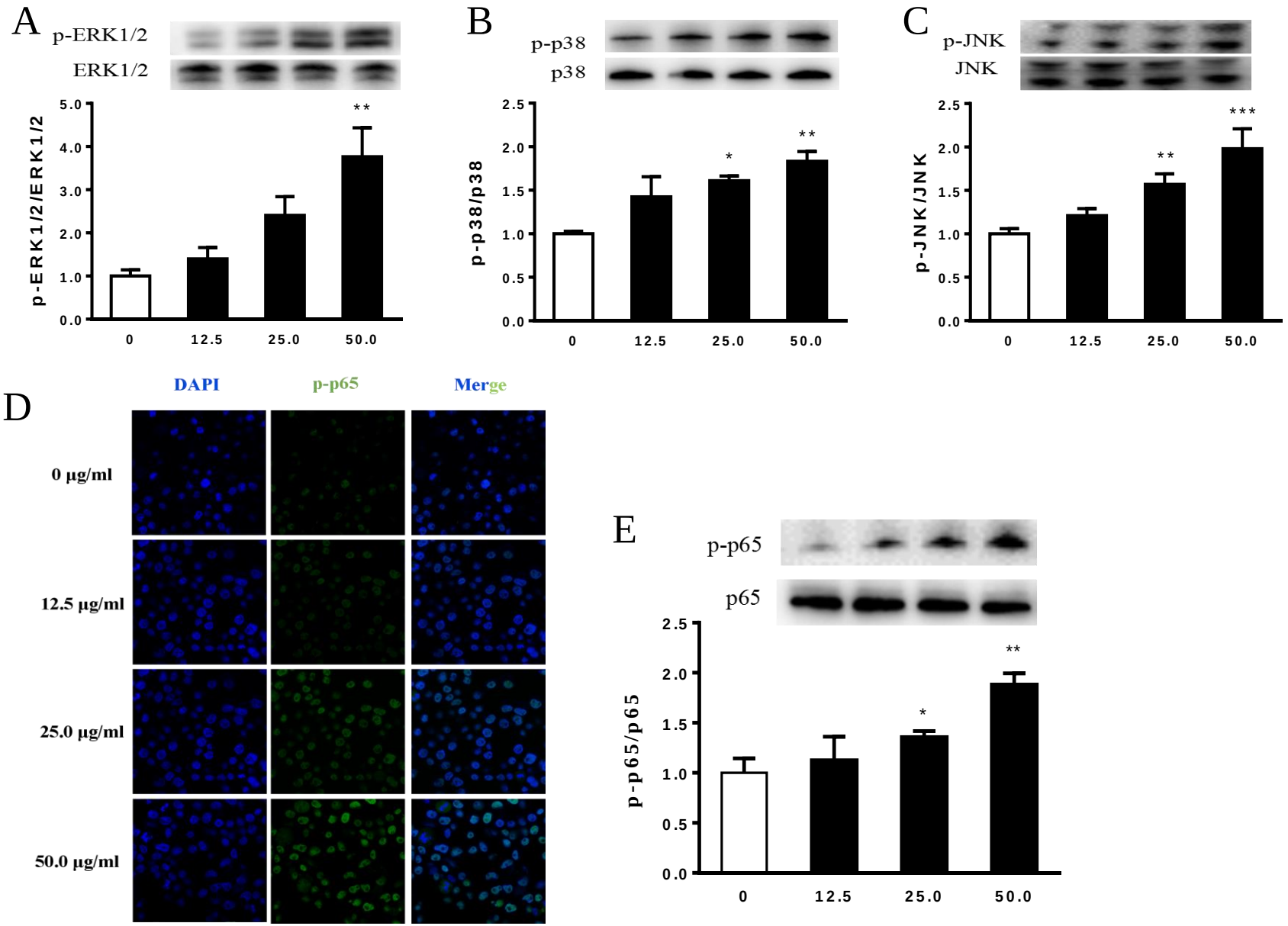
$PM_{2.5}$ activated TLR4/MyD88 pathway



Whether $PM_{2.5}$ could activate expression of downstream proteins（MAPKs and NF-κB） which are related with pro-inflammatory cytokines production?

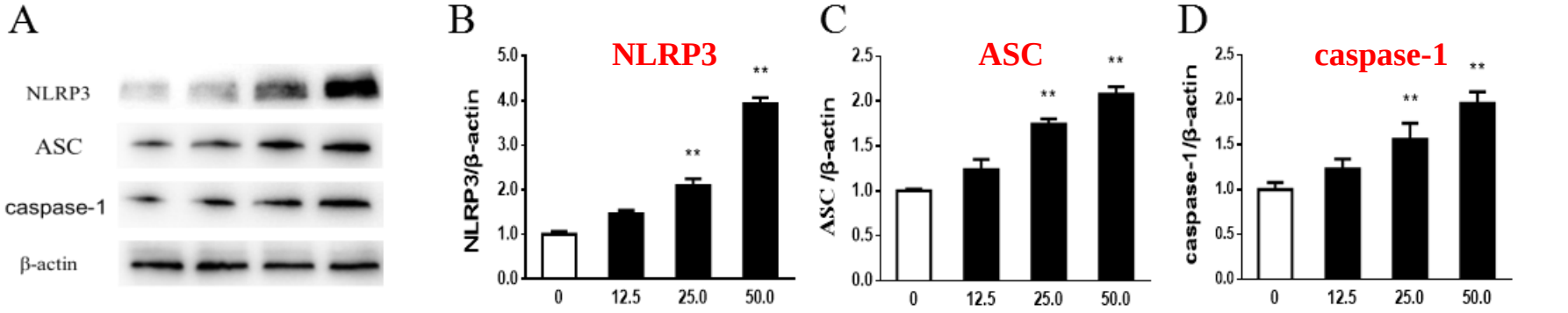
R12

$PM_{2.5}$ activated MAPK and NF-κB signaling pathways



R13

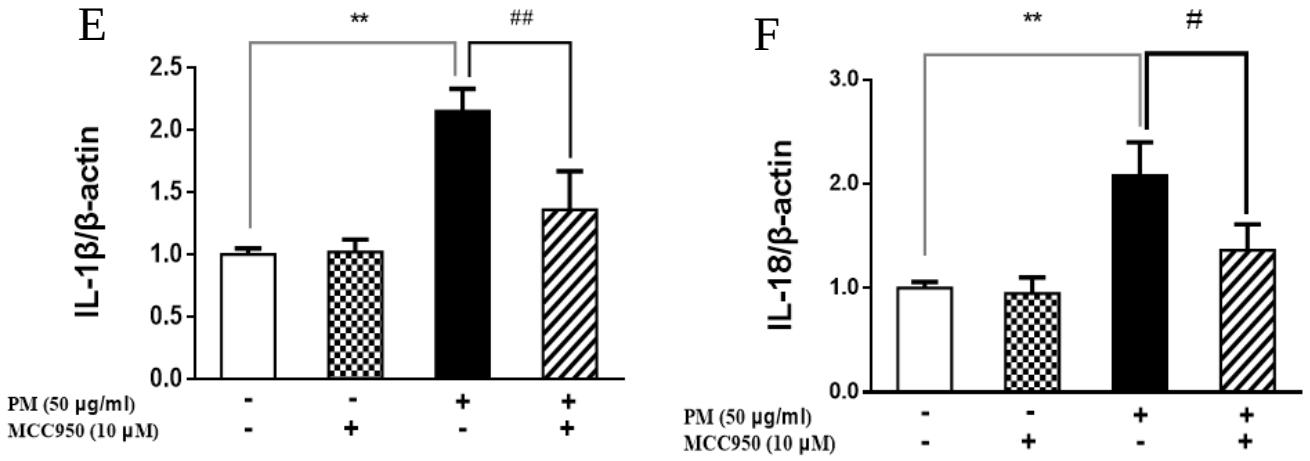
NLRP3 inflammasome involved in $PM_{2.5}$ induced inflammatory response



NLRP3

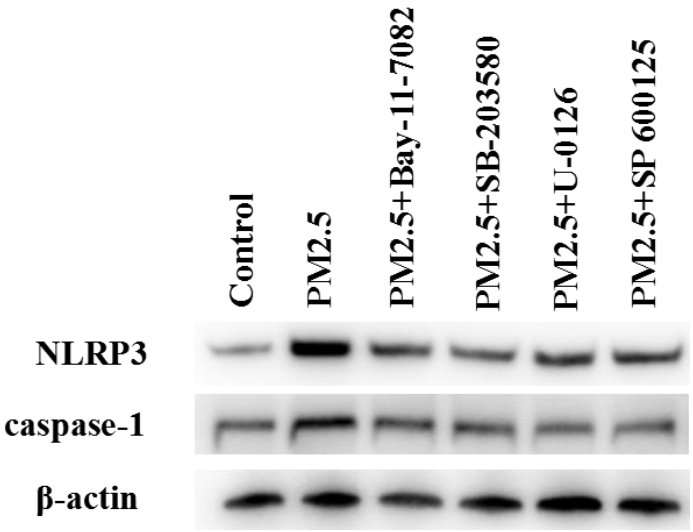
MCC950 | **inhibit**

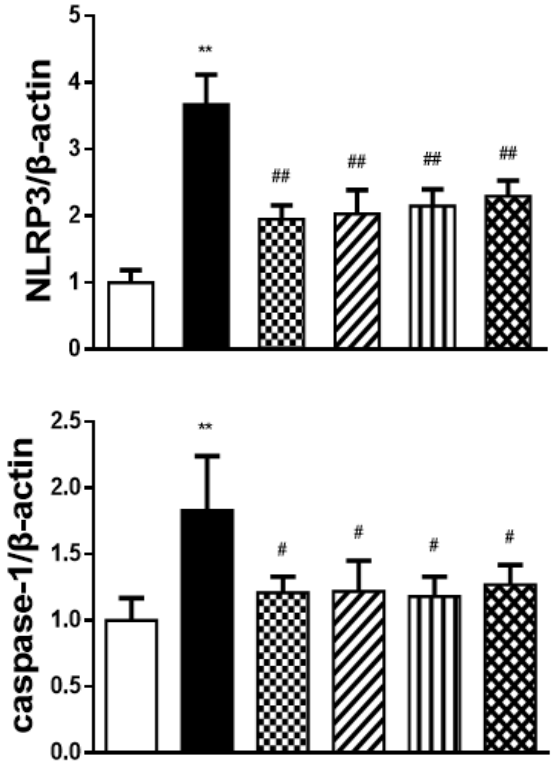
pro-inflammation：
IL-1β、IL-18



R14

The effect of NF-κB and MAPK on activating NLRP3 inflammasome





NF-κB inhibitor: Bay-11-7082

p38 inhibitor: SB 203580

ERK1/2 inhibitor: U-0126

JNK inhibitor: SP 600125

NF-κB and MAPK signaling pathway involved in NLPR3 inflammasome activation

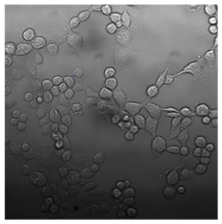
Summary

Nursery pig house

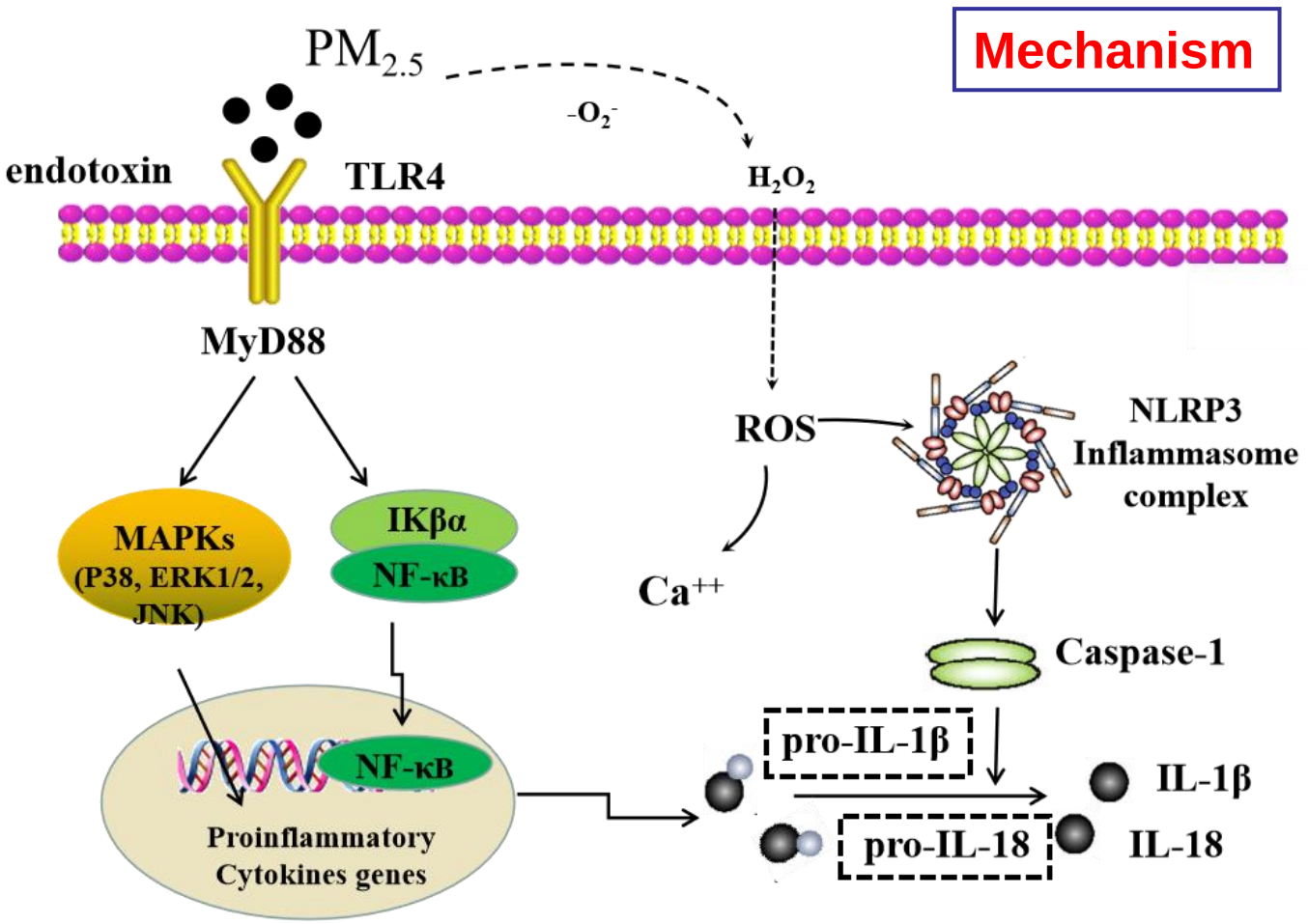
collect

$PM_{2.5}$ sample

treat



Alveolar macrophage



$PM_{2.5}$ can induce inflammatory response in alveolar macrophages by activating TLR4/MyD88 pathway and NLRP3 inflammasome

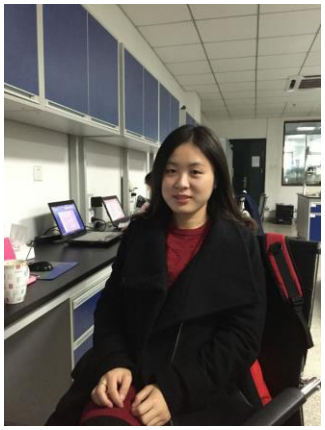
Conclusion

- Seasonal variation of bacterial and fungal assemblages contained in $PM_{2.5}$ from a nursery pig house.
- At the genus level, a total of 5 potential bacterial pathogen and 20 potential fungal allergen genera were identified across the samples, which indicated that $PM_{2.5}$ inside the nursery pig house has certain potential hazards.
- $PM_{2.5}$ can induce inflammatory response in alveolar macrophages by activating TLR4/MyD88 pathway and NLRP3 inflammasome.
- Endotoxin and ROS played important roles in $PM_{2.5}$-induced immune response.
- Results suggest that $PM_{2.5}$ from livestock house is a significant risk for immune response in alveolar macrophages.

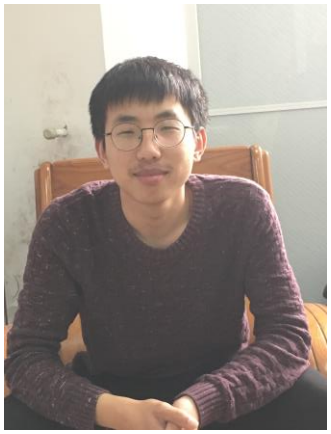
Acknowledgements

Team of Environmental Physiology and Animal Nutrition Research Laboratory

Tang Qian



Huang Kai



Grant Support

- **the National Key Research and Development Program of China (2016YFD0500505)**
- **the National Natural Science Foundation of China (31772648)**


ご清聴ありがとうございました!
Thanks for Your Attention!