

DOCTORAL THESIS

Reactive oxygen species generation and gene expression linked to sources of atmospheric fine particulate matter (PM_{2.5}) in Hong Kong

Cheng, Yubo

Date of Award:
2019

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

Abstract

Fine particulate matter (PM_{2.5}) is the leading public health risk factor of global disease burden, which has caused 4.2 million deaths in 2015. This thesis aims to improve the scientific understanding on the sources and health impacts of PM_{2.5} in Hong Kong. Various chemical and biological analytical techniques were applied to characterize the chemical and toxicological properties of PM_{2.5} samples collected in Hong Kong during 2011-2012. Positive matrix factorization (PMF), together with the quantified chemical markers and water-soluble PM_{2.5}-induced reactive oxygen species (ROS) activity as the input matrix, was performed to apportion the source-specific contributions to ambient organic carbon (OC) and the oxidative potential of water-soluble PM_{2.5}. Zebrafish was applied as in-vivo model to evaluate the PM_{2.5}-induced differential expression genes (DEGs). An L₂-normalization integrated PMF was developed and applied to quantitatively assess the ability of PM_{2.5} to induced DEGs in relation to various sources and chemical compositions of PM_{2.5}. The main findings are summarized below:

- (1) Thirty nine primary organic aerosol (POA) and secondary organic aerosol (SOA) markers of various anthropogenic (i.e. biomass burning (BB)) and biogenic sources (i.e. isoprene, monoterpenes and β -caryophyllene) were identified and quantified. High levels of OC and SOA markers were observed on regional pollution days than long regional transport (LRT) pollution and local emissions days. A kinetic model (Kintecus) was applied to explore the major formation channels of isoprene SOA, and it was found that isoprene SOA was mainly

formed through the ring-opening reaction of isoprene epoxydiols (IEPOX) in Hong Kong.

(2) PMF analysis, together with the chemical markers measured in Chapter 1 &2, was performed to evaluate the sources of OA in Hong Kong. Sea salt, marine vessels, vehicle emissions, BB/SOA, SOA, and secondary sulfate (SS) were apportioned as the major sources of ambient OC in Hong Kong. Secondary formation, including SOA, BB aging and SS sources, was found to be the major contributor to OC (~51%) throughout the whole year. BB was the major anthropogenic contributor to OC on regional days (28.8%); while marine vessel was the dominated primary source of OC on local days (33.2%). SOC concentrations were estimated using a tracer-based method (SOC_{TBM}) and PMF (SOC_{PMF}). Both SOC_{TBM} and SOC_{PMF} showed highest concentrations on regional days (SOC_{TBM} : $0.74 \mu\text{g m}^{-3}$; SOC_{PMF} : $3.27 \mu\text{g m}^{-3}$). Among all SOA precursors, monoterpenes had the most abundant contribution (40.9%) to SOC_{TMB} during the whole year. Moreover, sulfate has significant impacts on SS-related SOC and SOA from monoterpenes and naphthalene. Particle acidity (H_{P^+}) showed correlation with SOC from BB aging. These results provide us a quantitative understanding on the SOA origins in the region, which lays a foundation for the source apportionment of $\text{PM}_{2.5}$ -induced toxicity in the following chapters.

(3) Cell-free dithiothreitol (DTT) and $\cdot\text{OH}$ generation assays were applied to measure the ROS activity induced by water-soluble $\text{PM}_{2.5}$ collected in Hong Kong during 2011-2012. Different levels of ROS activity were observed for different chemical

fractions of PM_{2.5} and PM_{2.5} from various sources. Six factors, i.e. SS, BB, SOA, vehicle emissions, marine vessels and metal factors were apportioned by PMF as the major sources of water-soluble PM_{2.5} induced ROS potential. Metal factors was found to be the major contributor to both DTT activity (39.1%) and ·OH generation ability (84.5%) throughout the year, especially on LRT (DTT: 54.8%; ·OH generation: 91.1%) and regional days (DTT: 53.9%; ·OH generation: 87.7%). On local days, contribution of marine vessels to DTT oxidation become more significant (48.7%), however its contribution to ·OH generation is negligible. Metal factors is by far the most significant contributor to ·OH generation, even on local days (73.1%). It is interesting to note that all six PMF-resolved sources are associated with DTT oxidation, however only three sources (i.e. metal factor, vehicle emissions and SOA) showed contributions to ·OH generation. Moreover, among these six sources, marine vessels exhibited the highest intrinsic DTT ability; while metal factor was the most effective source in ·OH generation.

(4) Zebrafish embryo (AB strain) was applied as the *in-vivo* model to assess PM_{2.5} toxicity in Hong Kong through genome-wide gene transcriptional analysis. The results showed that embryonic exposure to PM_{2.5} could induce remarkable changes in gene expression patterns in zebrafish. DEGs between PM_{2.5} extract treated and untreated zebrafish embryo samples were identified, and they were found mainly associated with responses to xenobiotic stimulus, and muscle and heart development and functions. The correlation analysis between co-expressed gene modules and chemical species of PM_{2.5} implied the different chemical

compositions and sources of PM_{2.5} have significant influences on the PM_{2.5}-induced biological responses.

(5) An L₂-normalization integrated PMF was developed to analyze the high throughput biological and chemical data simultaneously, which quantitatively evaluated the ability of PM_{2.5} to induce DEGs in relation to sources and compositions. In this chapter, nine sources associated with PM_{2.5}-induced DEGs were well apportioned, i.e. fresh sea salt, aged sea salt, SS, SOA, BB, coal combustion, vehicle emissions, marine vessels and metal factors. Among these sources, metal factors (annual mean: 26.5%, range: 17.6-39.3%) and vehicle emissions (annual mean: 16.3%, range: 0.0-25.3%) are the two leading contributors to PM_{2.5}-induced DEGs levels. PM_{2.5} from combustion related sources (e.g. vehicle emissions, metal factors, BB) and sea salt exhibited stronger ability to induce DEGs than those from secondary sources. Although secondary formation (including SOA and SS) has a significant contribution to ambient PM_{2.5} (12 µg m⁻³, 40%), its capacity of DEGs induction is quite low. Moreover, several biological functions and pathways influenced by PM_{2.5} from various sources have also been well evaluated.

In this study, large scales of biological and chemical data were analyzed for the first time by a L₂-normalization integrated PMF to apportion the PM_{2.5}-induced DEGs, and this thesis work firstly reported the major sources of water-soluble PM_{2.5}-induced ROS in Hong Kong. Results from this study provide a scientific basis for the prediction of PM_{2.5}-associated adverse health outcomes and can help the policy

makers to formulate cost-effective and targeted PM_{2.5} mitigation strategies to protect public health.

Table of Contents

DECLARATION	i
Abstract.....	ii
Acknowledgements.....	vii
Table of Contents	ix
List of Tables.....	xiii
List of Figures	xiv
List of Abbreviations	xvi
Chapter 1.....	1
General Introduction	1
1.1 Chemical composition and sources of organic aerosol in PM _{2.5}	1
1.2 PM _{2.5} -associated toxicity	4
1.3 Source apportionment methods to apportion the sources of PM _{2.5} -induced biological responses	6
Chapter 2 Characterization of SOA and other polar oxygenated organic compounds in ambient aerosols at a urban site in Hong Kong	9
2.1 Introduction.....	9
2.2 Experimental section.....	10
2.2.1 Aerosol sampling	10
2.2.2 Analysis of elemental and organic carbon	11
2.2.3 Analysis of major inorganic ions in aerosol phase (Cl ⁻ , NO ₃ ⁻ , SO ₄ ²⁻ , C ₂ O ₄ ²⁻ , Na ⁺ , Ca ²⁺ , Mg ²⁺ , K ⁺ , NH ₄ ⁺)	11
2.2.4 Analysis of OA species	122
2.2.5 Calculation of particle acidity and total liquid water content (LWC).....	17
2.2.6 Kinetic model of loss of isoprene intermediates.....	18
2.3 Result and discussion.....	19
2.3.1 Meteorological conditions, EC and OC	19
2.3.2 Characterization of SOA tracers and other polar oxygenated organic compounds	21

2.3.2.1 Isoprene, monoterpene and β -caryophellene SOA tracers.....	28
2.3.2.2 Saccharides and Dicarboxylic acids	33
2.4 Summary.....	35
Chapter 3 Tracer-based source apportioning of atmospheric organic carbon and the influence of anthropogenic emissions on SOA formation in Hong Kong	36
3.1 Introduction.....	36
3.2 Experimental section.....	39
3.2.1 Analysis of metals	39
3.2.2 PMF analysis.....	40
3.2.3 Analysis of hopanes	40
3.3 Result and discussion.....	42
3.3.1 Source apportionment of organic aerosol	42
3.3.2 Estimation of SOC origin	47
3.3.3 Effects of anthropogenic influences on secondary aerosol formation	51
3.4 Summary.....	56
Chapter 4 Oxidative potential of fine particulate matter (PM _{2.5}) in Hong Kong: Characterization and source apportionment	57
4.1 Introduction.....	57
4.2 Experimental section.....	60
4.2.1 Analysis of hopanes, water-soluble organic carbon (WSOC) and Humic-like substances (HULIS)	60
4.2.3 Preparation of the stock solutions for ROS assay.....	61
4.2.4 Sample pretreatment for ROS assay	61
4.2.5 Estimation of oxidative stress	61
4.2.6 Source apportionment.	63
4.3 Results and discussion	65
4.3.1 Characterization of ROS activity induced by water-soluble PM _{2.5}	65
4.3.1.1 Water-soluble PM _{2.5} -induced DTT consumption and 2-OH formation	67
4.3.1.2 Linear regression of DTT and 2-OHTA activity with PM _{2.5} chemical	

composition.....	70
4.3.2 Source apportionment of ROS activity induced by WS-PM _{2.5}	74
4.3.2.1 Source-specific contributions to DTT and 2-OHTA activity	75
4.3.2.2 Intrinsic oxidative stress inducing ability of PM _{2.5}	82
4.4 Summary.....	86
Chapter 5 Gene expression patterns in zebrafish model induced by PM _{2.5} in Hong Kong.....	87
5.1 Introduction.....	87
5.2 Experimental design	88
5.2.1 Exposure sample preparation.....	88
5.2.2 Zebrafish exposure experiment.....	88
5.2.3 Image acquisition on fixed larvae	89
5.2.4 RNA-seq library preparation and sequencing.....	89
5.2.5 Mapping and Processing of RNA-seq	90
5.2.6 Co-expressed gene module analysis	91
5.3 Results and discussion	92
5.3.1 Toxic effects of PM _{2.5} exposure on zebrafish embryonic development.....	92
5.3.2 RNA-seq data analysis.....	92
5.3.2.1 Significant differential expression genes (DEGs) induced by PM _{2.5}	95
5.3.2.2 Effect of PM _{2.5} on gene function	96
5.3.2.3 Cluster analysis of DEGs induced by PM _{2.5}	97
5.3.2.4 Gene expression networks associated with different sources of PM _{2.5}	99
5.4 Summary.....	103
Chapter 6 Integration analysis of high throughput biological and chemical data reveals the toxicity capacity of fine particulate matter (PM _{2.5}) from various sources	105
6.1 Introduction.....	105
6.2 Experiment section	106
6.2.1 Development of L ₂ -normalization integrated PMF	106
6.3 Results and discussion	111

6.3.1 Source apportionment of DEGs induced by PM _{2.5}	111
6.3.2 A quantitative assessment of toxicity capacity of PM _{2.5} from various sources .	116
6.3.3 Cluster analysis of DEGs induced by PM _{2.5} from various sources.....	119
6.3.4 Understanding of the biological functions and pathways influenced by PM _{2.5} from different source origins	1200
6.4 Summary.....	125
Chapter 7 Conclusions and future work	126
7.1 Conclusions.....	126
7.2 Future work.....	128
References.....	1300
List of publications.....	156
CURRICULUM VITAE.....	157