On the Association between Outdoor $PM_{2.5}$ Concentration and the Seasonality of Tuberculosis for Beijing and Hong Kong

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ABSTRACT

Tuberculosis (TB) is still a serious public health problem in various countries. One of the long-elusive but critical questions about TB is what the risk factors are and how they contribute for its seasonality. An ecologic study was conducted to examine the association between the variation of outdoor $PM_{2.5}$ concentration and the TB seasonality based on the monthly TB notification and $PM_{2.5}$ concentration data of Hong Kong and Beijing. Both descriptive analysis and Poisson regression analysis suggested that the outdoor $PM_{2.5}$ concentration could be a potential risk factor for the seasonality of TB disease. The significant relationship between the number of TB cases and $PM_{2.5}$ concentration was not changed when regression models were adjusted by sunshine duration, a potential confounder. The regression analysis showed that a 10 μg/m^3 increase in $PM_{2.5}$ concentrations during winter is significantly associated with a 3% (i.e. 18 and 14 cases for Beijing and Hong Kong, respectively) increase in the number of TB cases notified during the coming spring or summer for both Beijing and Hong Kong. Three potential mechanisms were proposed to explain the significant relationship: (1) increased $PM_{2.5}$ exposure increases host's susceptibility to TB disease by impairing or modifying the immunology of the human respiratory system; (2) increased indoor activities during high outdoor $PM_{2.5}$ episodes leads to an increase in human contact and thus the risk of TB transmission; (3) the seasonal change of $PM_{2.5}$ concentration is correlated with the variation of other potential risk factors of TB seasonality. Preliminary evidence from the analysis of this work favors the first mechanism about the $PM_{2.5}$ exposure-induced immunity impairment. This work adds new horizons to the explanation of the TB seasonality and improves our understanding of the potential mechanisms affecting TB incidence, which benefits the prevention and control of TB disease.

Keywords: Tuberculosis; $PM_{2.5}$; Seasonality; Public health.

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Capsule

The TB seasonality is significantly related to the variation of outdoor $PM_{2.5}$ concentration, and three potential mechanisms are proposed to understand the significant relationship.

1. INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is one of the most pernicious diseases of global health concern. There were estimated 9.6 million new TB cases and 1.5 million deaths in 2014, making it a leading cause of death worldwide (WHO, 2015). The current TB control strategy mainly relies on the early case detection and effective treatment, and its impact has been found to be less than expected and preventive interventions based on social, economic, and environmental interventions are needed (Lönnroth et al., 2009). One of the critical steps in the preventive interventions is to identify high-risk groups in the population, which requires the understanding of the associations between various risk factors and TB. Existing studies have found that the occurrence of TB disease was associated with diabetes mellitus, alcohol, nutritional status, HIV infection, crowding, migration, aging, economic trends, smoking, and indoor air quality, etc. (Leung et al., 2005; Lin et al., 2008; Murray et al., 2011). The influences of these risk factors toward the occurrence of TB disease have been explained in terms of their ability to increase human exposure to the microorganism or decrease host's defense against TB disease. Another critical step in the preventive interventions is to recognize the potential temporal variation of TB incidence. The identification of high-risk groups informs us how to distribute preventive resources, while the information of the temporal variation of TB incidence advises when to distribute them. A combination of the two types of information would enhance the precision of preventive interventions toward TB control and management.

The temporal variation of TB disease is featured by its seasonality in various countries or regions, such as South Africa, India, Japan, Kuwait, Spain, UK, Ireland, etc., where the incidence of TB was found to peak during the spring and summer season (Fares, 2011). One common mechanistic hypothesis for the seasonality was that the transmission risk of *M. tuberculosis* should be the greatest during winter months, which was manifested a few months later due to delays in the diagnosis and treatment of TB (Kolappan and Subramani, 2009). The increased infection transmission risk during winter was further attributed to various risk factors like Vitamin D level variability (e.g., due to reduced sunlight exposure during winter), indoor activities, and seasonal change in immune system function, etc. (Chan, 2000; Fares, 2011; Koh et al., 2013; Korthals Altes et al., 2012; Nagayama and Ohmori, 2006; Naranbat et al., 2009; Rios et al., 2000; Thorpe et al., 2004; Willis et al., 2012; Wingfield et al., 2014). The existing explanations of TB seasonality are constantly challenged by emerging data, leading to significant controversy. For example, while some studies (Koh et al., 2013; Visser et al., 2013) conjectured that the reduced Vitamin D level due to sunshine dips during winter increased the transmission risk of TB disease, leading to the peaks of TB incidence a few months later, the study by Willis et al. (2012) suggested that reduced winter sunlight exposure may not always be a strong contributor to TB risk, based on the latitude-independence of TB seasonality in the US. Hence, the fundamental mechanisms underlying the seasonality of TB disease remain elusive and additional factors need to be explored.

To understand the seasonality of TB disease, it would be straightforward to explore the risk factors that are featured by seasonality. One of such risk factors is outdoor particulate matter (PM). Due to the influences of meteorological factors (e.g., precipitation, humidity, and wind speed), the outdoor PM concentrations generally peak during the winter or spring months while bottoms during the summer months in various megacities such as New Delhi of India and Beijing of China (Tiwari et al., 2013; Zheng et al., 2005). Existing studies have shown that the incidence of TB was related to outdoor $PM_{2.5}$ concentration. For example, a cohort study by Lai et al. (2015) revealed that exposure to $PM_{2.5}$ (particulate matter smaller than 2.5 μm in diameter) was associated with increased risk of TB disease. An ecologic study by Smith et al. (2014) also found that the incidence rate ratios of TB were higher for the cases of higher PM concentrations in North Carolina. The regression analysis of retrospective medical records showed that smear-positive TB was significantly associated with $PM_{2.5}$ concentration measurements from US Environmental Protection Agency (EPA)'s monitoring stations (Jassal et al., 2013). However, these studies were usually based on yearly data and thus could hardly be used to explain the seasonality of TB. Meanwhile, considering the seasonality of outdoor PM concentration and the association between PM concentration and TB, it is worth exploring whether ambient PM concentration is one of the risk factors for the TB seasonality.

In this work, an ecologic study is conducted to examine the association between the TB seasonality and outdoor $PM_{2.5}$ concentration. Both a descriptive method and a Poisson regression model are used to analyze the monthly $PM_{2.5}$ concentration and TB notification data of Beijing and Hong Kong. The regression analysis is also adjusted by including an existing environmental risk factor, sunshine duration. This work will shed light onto the seasonality of TB in terms of potential mechanisms.

2. METHODOLOGY

2.1 Data Source

The monthly data of Beijing and Hong Kong are utilized in the analysis. Beijing has a population of 21.52 million in 2014 and an area of 16,801 km^2. Beijing (39°55′N/116°23′E), located in the northern China, has a monsoon-influenced humid continental climate. During the past decade, haze has been one of the most serious environmental problems for Beijing, signified by the extremely high spikes of $PM_{2.5}$ concentration (Pui et al., 2014). The numbers of monthly TB cases from the year 2012 to 2014 were resourced from the Beijing monthly TB reports of Beijing Research Institute for Tuberculosis Control (BRITC) published online (BRITC, 2016). The monthly reports presented the total number of suspicious TB cases and the rates of confirmed TB cases (all forms of active TB) among the suspicious ones upon rechecking. The numbers of TB cases were estimated by multiplying the total number of suspicious TB cases and the rates. BRITC is responsible for the control and supervision of TB incidence in all 18 districts of Beijing and the diagnosis of TB is based on the criteria issued by the Ministry of Public Health (Jia et al., 2008). The geographic and demographic (e.g., age and sex) information about the TB cases was not available, but one existing study suggested that the most of TB cases notified were Beijing permanent residents and migrants (staying in Beijing for at least 1 month) (Jia et al., 2008). It is assumed that the $PM_{2.5}$ concentration (as an environmental measure) in Beijing is representative to the exposure for all TB cases notified. This type of assumption is indeed a typical limitation of ecologic studies characterized by missing individual level data, but it offers ecologic studies the advantage of being able to assess ecologic effects very straightforwardly and cost efficiently (Walter, 1991). $PM_{2.5}$ concentration data was from the Mission China (MC) air quality monitoring program by the U.S. Department of State (DoS., 2016). The measurements were taken at the rooftop of the U.S. embassy's building (39°95′N/116°47′E) located in Chaoyang district, Beijing, with a Met One BAM-1020 β attention monitor (Met One Instruments, USA) (Xie et al., 2014). The sampling interval is 1 hour. A recent study by Wang et al. (2013) showed that the $PM_{2.5}$ concentration data from the MC program reflected well the citywide $PM_{2.5}$ data measured at multiple sites. The $PM_{2.5}$ concentration data was also found to be in very good agreement with the ones reported by Beijing Municipal and Environmental Monitoring Center for 35 sites in Beijing (Jiang et al., 2015) and the study by Li et al. (2013) for a site about 10 km northwest of the U.S. embassy. Hence, the $PM_{2.5}$ concentration data from the U.S. embassy is assumed to be of representativeness to the $PM_{2.5}$ concentration exposure for the overall population. Actually, the $PM_{2.5}$ concentration data from Mission China (MC) air quality monitoring program has been used to analyze the relationship between particulate air pollution and ischaemic heart disease morbidity and mortality (Xie et al., 2014). The monthly $PM_{2.5}$ concentration data were calculated by averaging the hourly-based original data (hours without data records were excluded during the averaging). The monthly data of sunshine duration of Beijing was directly obtained from China Statistical Yearbook by the National Bureau of Statistics of China (NBSC, 2016). Considering the potential manifestation delay of risk factors on TB (Schaaf et al., 1996), the $PM_{2.5}$ concentration and sunshine duration data of the year 2011 (i.e. one year ahead of TB data) were also presented. Note that the $PM_{2.5}$ concentration and sunshine duration data were used in the following descriptive analysis, but they were only explored up to six months prior to TB data during the following regression analysis.

Hong Kong has a population of 7.24 million in 2014 and an area of 1,104 km^2. Hong Kong (22°18′N/114°12′E), located on the southern coast of China, has a humid subtropical climate. The numbers of monthly TB cases (all forms of active TB) were obtained from the yearly TB notification reports (from the year 2012 to 2015) by the Center for Health Protection under the Department of Health (CHP, 2016). Both clinical case definition (e.g., signs and symptoms compatible with active TB and supporting evidence from relevant and clinically indicated diagnostic evaluation such as abnormal and unstable chest radiographs) and laboratory criteria (e.g., isolation of *M. tuberculosis* complex from a clinical specimen and demonstration of *M. tuberculosis* from a clinical specimen by nucleic acid) have been adopted to identify TB cases to minimize variations in notification practices, and the TB incidence could be well approximated by the reported notification rate considering a good health care infrastructure, easy access to health care, and well developed reporting systems in Hong Kong (DoH, 2006). Although the geographic and demographic (e.g., age and sex) information about the TB cases was limited, the available data from the tuberculosis and chest service of the Department of Health suggested that the vast majority (>73%) of TB cases were permanent residents (DoH, 2013). Hence, the outdoor

$PM_{2.5}$ concentration data could also be assumed to be representative for the exposure of TB cases notified for the ecologic study. The monthly $PM_{2.5}$ concentration data from the year 2012 to 2015 were obtained from the statistics of Environment Protection Department (EPD) published online (EPD, 2016). The $PM_{2.5}$ concentration was measured using both automatic analysers based on oscillating microbalance (R&P TEOM Series 1400a and Thermo Scientific TEOM 1405) and beta attenuation (Met One BAM1020 and T-API 602 Beta Plus), and high volume samplers based on gravimetric methods (Thermo Scientific Partisol-Plus 2025) and the measurements generally had a precision of around 5% (EPD, 2014). The monthly $PM_{2.5}$ concentration data were calculated by averaging the measurements at 12 general monitoring stations (Central/Western, Sham Shui Po, Eastern, Shatin, Tsuen Wan, Kwai Chung, Tai Po, Tuen Mun, Kwun Tong, Tap Mun, Tung Chung, and Yuen Long) widely across Hong Kong. The locations of the monitoring stations have been carefully selected by considering the unique high-rise development of Hong Kong and following the guidelines of the U.S. Environmental Protection Agency (EPA) to ensure the representativeness of the data (EPD, 2014). The data in several months of some stations (May-2014 of Eastern, February-2012 of Shatin, July-2013 to December-2013 of Tai Po, and December-2015 of Tap Mun) are missing and not accounted for in the analysis. The $PM_{2.5}$ concentration data of the year 2011 is not available because $PM_{2.5}$ was included as a monitoring parameter by EPD since 2012. The overall coefficient of variation (COV=standard deviation/mean) of the measured $PM_{2.5}$ of each month from all the stations is 14.3%, suggesting a limited spatial variation of PM2.5 concentration. The sunshine duration data from the year 2011 to 2015 was obtained from the yearly summary by Hong Kong Observatory (HKO, 2016).

2.2 Statistical Analysis

The means and standard deviations of the monthly data of TB cases, $PM_{2.5}$ concentration, and sunshine duration were calculated for descriptive analysis. The incidence of TB diseases has been represented by Poisson distributions in existing studies (Baker et al., 2008; MacIntyre et al., 1997; Ostermann and Brauer, 2001; Sapkota et al., 2005), and Poisson regression analysis was used in this work. The number of TB cases per month, Y, is assumed to follow a Poisson distribution, i.e.

$$\Pr\{Y = y\} = (e^{-\mu}\mu^{y})/y! \quad (1)$$

μ is the mean of the distribution and denoted by the observed number of TB cases per month. The corresponding Poisson regression model is

$$\log(\mu) = \alpha + \boldsymbol{X}'\boldsymbol{\beta} \quad (2)$$

where α is the intercept, $\boldsymbol{X}'$ is a vector of covariates (predictors), and $\boldsymbol{\beta}$ is a vector of regression coefficients. The unit of analysis is the population of Beijing and Hong Kong and the response is the number of notified TB cases per month in Beijing and Hong Kong. The predictor is $PM_{2.5}$ concentration for simple analysis, while sunshine duration, as a potential confounder, is further introduced for multivariable analysis. The null hypothesis is that the increase in $PM_{2.5}$ concentration is not significantly associated with the increase in the number of TB cases. The regression analysis was performed using xlstat (version 2016.3; http://www.xlstat.com/).

There should be a time lag (or offset) between the effect of risk factors and the notification of TB cases due to the potential existence of reduced accessibility to health care services, and the time or delay of disease diagnosis and notification. In other words, if the increased $PM_{2.5}$ concentration does increase the risk of acquiring TB disease, it takes additional time for patients to develop a clinically observable immunological response, to be identified by a health care institution, and to be reported. The time lag considered in the regression analysis is the period when the increased TB cases under the effect of increased $PM_{2.5}$ concentration get notified. Note that this time lag might be different from the latent period of TB. The latent period of TB varies from few months to years: when susceptible individuals get infected, they may experience primary progression to an infectious state within a few years, or progress to active disease via endogenous reactivation a few years later, or simply do not develop the active disease for their whole life (Murray et al., 2011). The shortest time lag considered in this work corresponds to the case when infected individuals immediately progress to active disease under the effect of increased $PM_{2.5}$ exposure, but it still consists of the time for the development of clinically observable immunological response, and disease diagnosis and notification. The study of Naranbat et al. (2009) suggested this shortest time lag should be more than 2 months, considering that the median interval between being infected and developing an observable immunological response to *M. tuberculosis* was around 7 weeks (Poulsen, 1949). A recent retrospective cohort study (Paynter et al., 2004) showed that the median health care delay in the treatment of pulmonary TB is around 2 to 3 months, based on which the study of Leung et al.

(2005) suggested the overall lag time could be up to 6 months. Hence, four respective time lags (Δt), i.e. 3, 4, 5 and 6 months are imposed during the regression analysis between the number of TB cases and predictors; that is, the TB case data at time t corresponds to the data of predictors at time ($t - \Delta t$). For Hong Kong, the $PM_{2.5}$ data is only available from the year 2012 onwards, and the time lag is done by moving the data of TB cases backward (e.g., for the 3-month lag, the TB case data of April 2012 corresponds to the $PM_{2.5}$ data of January 2012).

3. RESULTS AND DISCUSSION

3.1 Descriptive Analysis

The monthly-based statistics (i.e. averages and standard deviations calculated based on the monthly data of each year) of each year for Beijing and Hong Kong is given in Table 1. The monthly number of TB cases per unit population density (population/area) for Beijing is around 7 times of that for Hong Kong during the year 2012-2014. The standard deviation of the number of TB cases for Beijing is about 2-3 times of that for Hong Kong, suggesting a greater seasonal fluctuation for the number of TB cases in Beijing. Coincidentally, the standard deviation of $PM_{2.5}$ concentration for Beijing is also about 2-3 times of that for Hong Kong. The $PM_{2.5}$ concentration of Beijing is about triple of that of Hong Kong, which may suggest that $PM_{2.5}$ exposure may affect people in Beijing and Hong Kong to a different extent. The $PM_{2.5}$ concentration data of Beijing is more than double the China Ministry of Environmental Protection (MEP) annual $PM_{2.5}$ standard of 35 μg/m^3. The $PM_{2.5}$ concentration data of Hong Kong meets the Hong Kong EPD annual $PM_{2.5}$ standard of 35 μg/m^3. Both the $PM_{2.5}$ data of Beijing and Hong Kong are higher than the US EPA annual standard of 12 μg/m^3. Due to higher latitude, the average monthly sunshine duration of Beijing of each year is generally 40 to 80 hours longer than that of Hong Kong. But the standard deviation of sunshine duration for Beijing is generally smaller than that of Hong Kong, suggesting a smaller seasonal fluctuation of sunshine duration in Beijing.

Figure 1 shows the monthly variation of the number of TB cases, $PM_{2.5}$ concentration, and sunshine duration for Beijing and Hong Kong. The time series curves of 3-month moving average are also shown in Figure 1 to illustrate the trends of variates. It is shown that the TB notification data of Beijing and Hong Kong exhibits similar seasonality. The peak levels of TB cases generally occur during March-May (spring) and July-August (late summer), while the trough levels generally occur during November to February (late fall to winter). The study of Leung et al. (2005) found similar TB seasonality for Hong Kong and suggested it should be related to some environmental factors which displayed similar seasonal variation. The $PM_{2.5}$ concentration generally peaks during winter, while troughs during summer, for both Beijing and Hong Kong. If we follow the logic similar to that used by existing studies to explain the seasonality of TB disease based on Vitamin D variability, the high levels of $PM_{2.5}$ concentration during winter may correspond to increased risk of acquiring TB disease, which is then manifested by the peak of TB cases during the coming spring or summer. Similarly, the low $PM_{2.5}$ concentration during summer is manifested by the low number of TB cases during late fall and winter. It is interesting to note that the number of TB cases generally drops during June for Beijing, which may also be related to the trough levels of $PM_{2.5}$ concentration two to four months ago. Therefore, $PM_{2.5}$ exposure appears to be a reasonable factor for explaining the TB seasonality. This is subject to further confirmation by the following regression analysis. The $PM_{2.5}$ concentration of Beijing exceeds the annual standard of 35 $\mu g/m^3$ for most of the months, while the $PM_{2.5}$ concentration of Hong Kong generally exceeds the standard during the winter months, suggesting the effect of $PM_{2.5}$ concentration may be more significant for Beijing. The sunshine duration of Beijing generally peaks during spring and fall and troughs during summer and winter. For Hong Kong, the sunshine duration generally peaks during late summer and early fall, and troughs during winter. The reduced sunshine during winter has been postulated to be able to increase the transmission risk of TB by reducing Vitamin D level in the human body (Koh et al., 2013; Visser et al., 2013). However, the variations of sunshine duration are different, while the TB seasonality is consistent between Beijing and Hong Kong. This suggests that the sunshine-modulated Vitamin D level may not always be the core risk factor for TB seasonality, and extra factors need to be explored.

3.2 Regression Analysis

The results of regression analysis between the number of TB cases and $PM_{2.5}$ concentration for Beijing and Hong Kong are listed in Table 2. For the purpose of illustration, the numbers of TB cases are plotted against $PM_{2.5}$ concentrations and sunshine duration in Figure 2 for Beijing (Figure 2 (a) and (c)) and Hong Kong (Figure 2 (b) and (d)) using the raw data and results of regression models. The regression models (simple and/or multivariable analysis) for the cases of 6-month time lag are applied in Figure 2. Note that there are only regression models based on the multivariable analysis for the number of TB cases vs. sunshine duration (Figure 2 (c) and (d)). The overall averages of sunshine duration calculated based on the data of each year in Table 1 are used for the models of multivariable analysis in Figure 2 (a) and (b), while the overall averages of $PM_{2.5}$ concentration based on Table 1 are used in Figure 2 (c) and (d).

For Beijing, the increase in the number of TB cases is significantly associated ($P<0.001$) with the increase in the $PM_{2.5}$ concentration for all time-lag cases in the simple analysis. The significance of the relationship is not changed when the regression analysis is adjusted by the sunshine duration that has been considered to be related to the seasonality of TB disease by the existing studies (Koh et al., 2013; Visser et al., 2013). The significant relationship found for the cases of 3- to 6-month time lags means that the notified TB cases in the current month are significantly associated with the $PM_{2.5}$ concentration levels back to 3 to 6 months ago. In other words, the peaks of TB cases during spring and summer are significantly related to the $PM_{2.5}$ concentration during the last winter when the haze episodes happen mostly frequently and the $PM_{2.5}$ concentration is high (Pui et al., 2014). Hence, the seasonality of TB disease in Beijing is significantly related to the variation of $PM_{2.5}$ concentration. The study of Paynter et al. (2004) found that median patient-related delay (onset of symptoms to first contact with health services) was between 34.5 to 54 days while median health care-related delay (first contact with health services to initiation of treatment) was 29.5 days. If we assume that the delay in the diagnosis and notification of TB disease is 2 months, the increased occurrence of active TB in the current month is significantly related to the $PM_{2.5}$ concentration increase back to 1 to 4 months ago. The regression coefficients of $PM_{2.5}$ concentration in all the cases are generally consistent with each other and are around 3×10^{-3}. Corresponding to the Poisson regression model, this suggests that a 10 $\mu g/m^3$ increase in the $PM_{2.5}$ concentration back to 3 to 6 months ago corresponds to a 3% increase in the number of TB cases notified in the current month, i.e. 18 cases in view of the monthly TB cases of around 600 in Beijing. In the multivariable analysis, there is a controversy in the relationships between the number of TB cases and sunshine duration for different time lag cases (3- and 4-month vs. 6-month). The increase in the number of TB cases is significantly associated with the increase of sunshine duration for the cases of 3- and 4-month time lags. This is contradicting with the existing studies (Koh et al., 2013; Visser et al., 2013) that conjectured that vitamin D deficiency caused by the dip of sunshine duration during the winter months was related to the increase in the number of TB cases during the coming spring or summer months. However, for the case of 6-month time lag, the significant relationship between sunshine duration and the number of TB cases is consistent with the conjecture of the existing studies. This controversy stems from the underlying correlation between the sunshine duration and $PM_{2.5}$ concentration and the fact that the periodicity of sunshine seasonality is around half of that of $PM_{2.5}$ concentration for Beijing (Figure 1). Specifically, peaks in the sunshine duration during the spring months correspond to the relatively low levels of $PM_{2.5}$ concentration while dips in sunshine duration during the summer months correspond to the relatively low levels of $PM_{2.5}$ concentration as well. Hence, the significant relationship between the number of TB cases and sunshine duration is probably the reflection of the relationship between the number of TB cases and $PM_{2.5}$ concentration.

For Hong Kong, the increase in the number of TB cases is significantly associated ($P<0.001$) with the increase in $PM_{2.5}$ concentration for the cases of 5- and 6-month time lags in the simple analysis. The significance of the relationship is not changed in the multivariable analysis where the sunshine duration is introduced. The significant relationship found for the cases of 5- to 6-month time lags means that the notified TB cases in the current month are associated with the $PM_{2.5}$ concentration levels back to 5 to 6 months ago. If we assume that the delay in the recognition and notification of TB disease is 2 months as well, the increased occurrence of active disease in the current month (spring) is significantly related to the $PM_{2.5}$ concentration increase back to 3 to 4 months ago (winter). Hence, the seasonality of TB disease in Hong Kong is also significantly related to the variation of $PM_{2.5}$ concentration. Interestingly, the regression coefficients in the cases of a significant relationship for Hong Kong are also around 3×10^{-3}, consistent with those in the case of Beijing. In view of the monthly TB case of 400 in Hong Kong, this means that a 10 $\mu g/m^3$ increase in the $PM_{2.5}$ concentration back to 5 to 6 months ago corresponds to 12 more TB cases notified in the current month. The multivariable analysis also shows that the increase in the number of TB cases is significantly associated with the decrease of sunshine duration for the cases of 4-, 5-, and 6-month time lags. The study of Leung et al. (2005) attributed the TB seasonality to the variation of vitamin level and solar radiation based on a qualitative analysis. Unlike the case of Beijing, there is no significant relationship between the sunshine duration and $PM_{2.5}$ concentration for the case of Hong Kong. Hence, the relationship between the number of TB cases and $PM_{2.5}$ concentration should not stem from the one between the number of TB cases and sunshine duration.

The difference in the time lags of the cases of a significant relationship between Beijing and Hong Kong emphasizes the importance of exploring the epidemiology of TB disease based on the specific environmental and climate background of a region or country. Although the TB seasonality featured by the peaks during spring and summer was observed for various countries or regions, it was not the case for some other places such as Russia and South Western Cameroon (Fares, 2011), which is potentially related to their unique environmental and climate backgrounds.

3.3 Potential Mechanisms

The regression analysis suggests that the seasonal variation of $PM_{2.5}$ concentration should be one potential risk factor for the seasonality of TB for both Beijing and Hong Kong. The $PM_{2.5}$-related TB seasonality could be explained in terms of several potential mechanisms, despite more future studies are needed for a definite conclusion.

First, increased $PM_{2.5}$ exposure could directly impair or modify the immunology of the human respiratory system which increases host's susceptibility to TB disease. The major infection site of *M. tuberculosis* is alveoli (Russell et al., 2010). Inside alveoli, alveolar macrophages work to ingest *M. tuberculosis* and inhibit their multiplication, forming a granuloma. If some of the organisms could not be controlled by the immune system and remain viable after the macrophages die, they may multiply intracellularly and spread through lymphatic channels or bloodstream to various areas of the body, leading to TB disease. It has been documented that $PM_{2.5}$ exposure could adversely impact lung immunology by inducing oxidative and nitrosative stressors (Kappos et al., 2004; Nel, 2005). Existing studies also showed that inhaled PM could weaken alveolar macrophage activity and mucociliary clearance function which are critical defense mechanisms against *M. tuberculosis* (D'amato et al., 2010; Smith et al., 2010). Indeed, the relationships between environmental tobacco smoke and indoor burning of solid fuel, and TB incidence have been relatively firmly established (Davies et al., 2006; Kolappan and Subramani, 2009; Lin et al., 2008; Murray et al., 2011), which were largely attributed to the down-regulation of macrophage (e.g., tumor necrosis factor (TNF)-α) in the lungs by inhaled particles increasing people's susceptibility to the progression or reactivation of TB to the active disease. Hence, it is possible that the increased $PM_{2.5}$ exposure during the winter months increases the risk of TB disease by increasing people's immune susceptibility, which is manifested a few months later (1-4 months for Beijing and 3-4 months for Hong Kong disregarding the delay in diagnosis and notification) followed by 2 months delay in diagnosis and notification. The similar regression coefficients of $PM_{2.5}$ concentration between the cases of Beijing and Hong Kong favor this mechanism, suggesting a consistent effect of inhaled $PM_{2.5}$ on the respiratory system between Beijing and Hong Kong. Furthermore, the regression results show that there is a difference in the time lags for the cases of a significant relationship (between the number of TB cases and $PM_{2.5}$ concentration) between Hong Kong and Beijing. This may be related to the difference in the magnitude of $PM_{2.5}$ concentrations between Hong Kong and Beijing (Table 1 and Figure 1). The $PM_{2.5}$ concentration of Beijing is about three times of that of Hong Kong. Existing studies (Choi et al., 2011; Karlsson et al., 2005; Ni et al., 2015; Zhang et al., 2015) have shown that the influence of $PM_{2.5}$ exposure towards the human respiratory system or immune systems was concentration dependent, with exposure to a higher concentration generally causing larger and acuter impairment. Hence, the much higher $PM_{2.5}$ concentration in Beijing could potentially have an acuter effect on the immune system and lead to the earlier manifestation of $PM_{2.5}$ exposure on TB disease. Last, it is worth noting that some studies (Douglas et al., 1998; Leung et al., 2005) showed seasonality heterogeneity among population groups of different ages: the seasonal fluctuation of TB is more significant among children and elderly. At the same time, other studies found that ambient PM has the strongest effect on the respiratory health of children and elderly (Halonen et al., 2008; Ko et al., 2007; Peel et al., 2005). This lays further support to the mechanistic hypothesis based on the immunity-impairing effect of $PM_{2.5}$ exposure.

Second, people are advised to stay indoors in the case of high outdoor $PM_{2.5}$ concentrations, which is one of the common protective measures against outdoor pollution events (Sapkota et al., 2005). The TB disease is widely recognized as solely airborne-mode-transmittable (Sehulster et al., 2003), and the increased indoor activities suggest an increase in human contact and thus the risk of TB transmission (Chen et al., 2011). Under a similar mechanism, household crowding has been long regarded as a risk factor for TB, and the relationship between increased TB incidence and higher levels of crowding has been well established (Baker et al., 2008; MacIntyre et al., 1997; Wanyeki et al., 2006; Wingfield et al., 2014). However, this mechanism may play a role for Beijing where there are frequent severe haze episodes while it may not be the case for Hong Kong where the protective measure is less necessary considering that the peak $PM_{2.5}$ concentration is much lower and may still be acceptable to the public. However, if the significant relationship found in this work is actually caused by the first or second mechanism, it would be expected that TB would continue to be a great burden to the developing countries like China and India which are suffering from both a high volume of TB cases and severe $PM_{2.5}$ pollution.

Third, the variation of $PM_{2.5}$ concentration may be related to the variation of other risk factors (confounders) of TB seasonality. Previously, the variation of vitamin D level with regards to the change of sunshine duration was proposed as a potential risk factor for the TB seasonality (Koh et al., 2013; Visser et al., 2013). However, in this work, the relationships between the number of TB cases and $PM_{2.5}$ concentration are not attributed to the ones between sunshine duration and the number of TB cases for both Beijing and Hong Kong. One possible alternative confounder is the direct seasonal change of immune system function (humoral and cellular immunity) (Fares, 2013), which is different from the one induced by seasonal environmental factors (e.g., PM2.5 concentration) as discussed with regards to the first mechanism. For example, experimental studies on rodents, birds and humans have suggested that the immune system is weakened during the winter (Altizer et al., 2006), while a down-regulation of interleukin (IL)-6, TNF-α, interferon (IFN)-γ, and interleukin (IL)-10 production happened during summer compared to winter as observed by other studies (Khoo et al., 2011). Obviously, more studies are needed to characterize the effect of the seasonal change of immune system function on the TB seasonality and the potential relationship between the variation of ambient $PM_{2.5}$ concentration and humoral and cellular immunity. Another possible alternative confounder is the existence of other air pollutants. Extensive studies have explored the health effects of various air pollutants, some of which pose similar health effects on the human respiratory system with $PM_{2.5}$ (Bascom et al., 1996; Fuertes et al., 2015; Sram et al., 2013). $PM_{2.5}$ was found to be significantly correlated to gaseous pollutants such as carbon monoxide, nitrogen oxides, and benzene for some urban environments

(Anttila et al., 2016), while no significant relationship was found in some others (Cadle et al., 1999). This kind of data with respect to Beijing and Hong Kong is still lacking. The existence of other pollutants in the air and significant positive (negative) relationship with $PM_{2.5}$ may suggest the obtained regression coefficients of $PM_{2.5}$ in this work are overestimates (underestimates) of the actual ones. Future studies examining the relationship between ambient $PM_{2.5}$ and other air pollutants would contribute to the understanding of this issue.

As an ecologic study, this work suffers from several limitations. First, it is impossible to account for the heterogeneity of exposure level and uniform exposure levels corresponding to outdoor $PM_{2.5}$ concentrations measured have been used. Considering large populations and samples (an advantage of an ecologic study) and a significant proportion of permanent residents in TB cases for both Beijing and Hong Kong, it is believed that the adverse effect from the uniform assumption could be mitigated. Second, temporal ambiguity exists in the analysis because of uncertainty in the actual time lag between $PM_{2.5}$ concentration and disease occurrence. This potential bias is mitigated by adopting multiple reasonable time lags in the analysis. Finally, although the effect of sunshine duration as a confounder has been examined, it is uncertain whether any other confounders or multicollinearity would also play a role. The existence of confounders and multicollinearity may lead to potential bias and affect the actual degree of association between the number of TB cases and $PM_{2.5}$ concentration, as inferred from the discussion on the second and third mechanisms. Hence, further studies such as cross-sectional, cohort and case-control ones are needed in the future to explore the fundamental mechanisms underlying the relationship between the seasonality of TB and $PM_{2.5}$ concentration.

4. CONCLUSIONS

In this work, an ecologic study was conducted to examine the association between the variation of ambient $PM_{2.5}$ concentration and the TB seasonality for Beijing and Hong Kong. The descriptive analysis and Poisson regression analysis showed that the increase in the number of TB cases notified in the current month was significantly related to the increased monthly $PM_{2.5}$ concentrations back to 3 to 6 months ago for Beijing and 5 to 6 months ago for Hong Kong. Specifically, the increased number of TB cases during spring and summer is significantly related to the increase in the $PM_{2.5}$ concentration during winter. For both Beijing and Hong Kong, a 10

$\mu g/m^3$ increase in $PM_{2.5}$ concentration months ago is significantly associated with a 3% increase in the number of TB cases. The time lags of the cases of a significant relationship are suggestive of manifestation delays and could be used as important surveillance information for the protection and control of TB infection, despite more information such as human susceptibility in terms of age, sex and races are needed. Three potential mechanisms were proposed to explain the significant relationship and preliminary evidence from the analysis of this work favors the mechanism based on the immunity-impairing effect of $PM_{2.5}$ exposure. The findings provide additional channels to understand the seasonality of TB disease which is long-elusive but critical for the prevention and control of TB disease. More studies exploring the fundamental mechanisms underlying the relationship between $PM_{2.5}$ concentration and TB infection are needed. Finally, it should be noted that other respiratory infectious diseases may differ significantly from TB disease in terms of epidemiology and pathogenesis and the obtained relationship for TB disease is not necessarily representative of other diseases.

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Table 1. Monthly-based statistics (averages and standard deviations) of TB cases, $PM_{2.5}$ concentration and meteorological factors for Beijing and Hong Kong for each year considered.

City		2011	2012	2013	2014	2015
Beijing	TB cases	-	585[a] (116)[b]	514 (118)	506 (141)	-
	$PM_{2.5}$ ($\mu g/m^3$)	86.87 (33.65)	85.18 (23.93)	97.81 (31.03)	94.82 (32.82)	-
	Sunshine duration (hrs)	207.14 (52.16)	204.18 (38.15)	197.59 (40.81)	195.34 (50.29)	-
Hong Kong	TB cases	-	405 (53)	389 (42)	399 (38)	401 (45)
	$PM_{2.5}$ ($\mu g/m^3$)	-	27.49 (8.66)	30.83 (16.12)	28.52 (12.22)	25.20 (10.82)
	Sunshine duration (hrs)	164.88 (36.84)	129.27 (52.19)	147.47 (52.56)	158.61 (56.40)	147.47 (52.30)

a: Values before the brackets denotes the averages calculated based on the monthly data of a year.

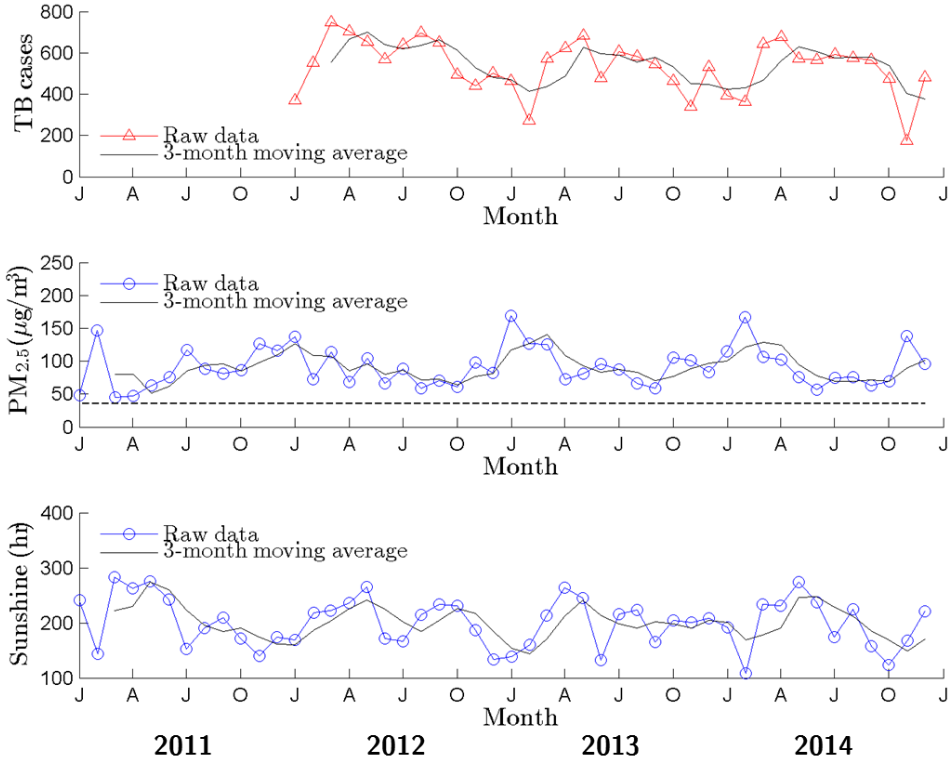
b: Values in the brackets denotes the standard deviations calculated based on the monthly data of a year.

Table 2. Results of regression analysis of TB cases and $PM_{2.5}$ concentration for Beijing and Hong Kong.

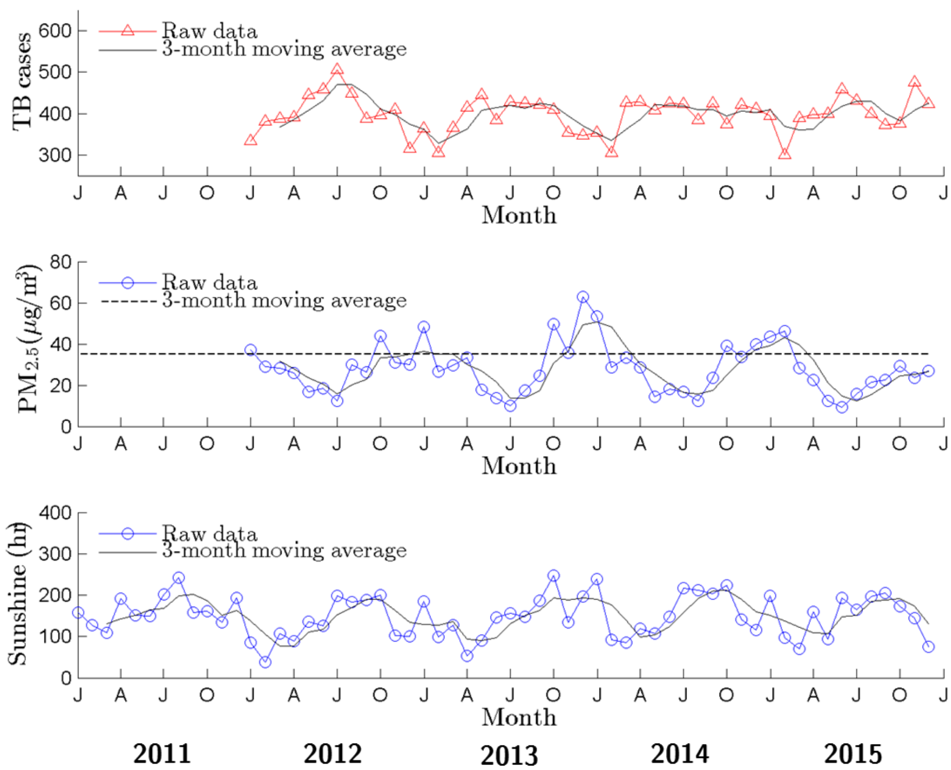
City	Variable	β_i							
		Simple analysis				Multivariable analysis			
		3 month	4 month	5 month	6 month	3 month	4 month	5 month	6 month
Beijing	Intercept	6.07 (6.02-6.12)[a]**	5.95 (5.90-5.99) **	5.99 (5.94-6.04) **	6.06 (6.00-6.11) **	5.92 (5.80-6.03) **	5.52 (5.40-5.65) **	5.95 (5.83-6.07) **	6.51 (6.39-6.63) **
	$PM_{2.5}$ concentration ($\times 10^{-3}$ $\mu g/m^3$)	2.26 (1.78-2.75) **	3.54 (3.06-4.02) **	3.08 (2.59-3.57) **	2.31 (1.82-2.81) **	2.66 (2.11-3.22) **	4.73 (4.15-5.31) **	3.19 (2.61-3.77) **	1.01 (0.42-1.59)**
	Sunshine duration ($\times 10^{-3}$ hrs)	-	-	-	-	0.576 (0.184-0.968) **	1.54 (1.13-1.95) **	0.155 (-0.254-0.564)	-1.65 (-2.06--1.24) **
Hong Kong	Intercept	6.01 (5.98-6.05) **	6.00 (5.96-6.03) **	5.93 (5.89-5.96) **	5.89 (5.85-5.92) **	6.07 (6.02-6.12) **	6.12 (6.07-6.18) **	6.03 (5.98-6.08) **	5.96 (5.90-6.01) **
	$PM_{2.5}$ concentration ($\times 10^{-3}$ $\mu g/m^3$)	-0.705 (-1.89-4.81)	-0.0921×10^{-5} (-1.28-1.09)	2.25 (1.07-3.43) **	3.47 (2.28-4.66) **	-0.489 (-1.69-0.71)	0.477 (-0.74-1.69)	2.84 (1.62-4.05) **	3.93 (2.70-5.15) **
	Sunshine duration ($\times 10^{-3}$ hrs)	-	-	-	-	-0.422 (-0.70--0.14)	-0.998 (-1 .28--0.71) **	-0.842 (-1 .13--0.55) **	-0.572 (-0.86--0.28) **

a: Values in brackets denotes 95% confidence intervals.

** denotes the regression coefficients with P<0.001.



(a)



(b)

Figure 1. The monthly variation of the number of TB cases, $PM_{2.5}$ concentration, and sunshine duration for (a) Beijing and (b) Hong Kong, respectively. The black solid lines are the curves of

3-month moving average. The horizontal black dash-lines in the sub-figures of $PM_{2.5}$ vs. Month indicate the China and Hong Kong $PM_{2.5}$ annual standard of 35 $\mu g/m^3$.

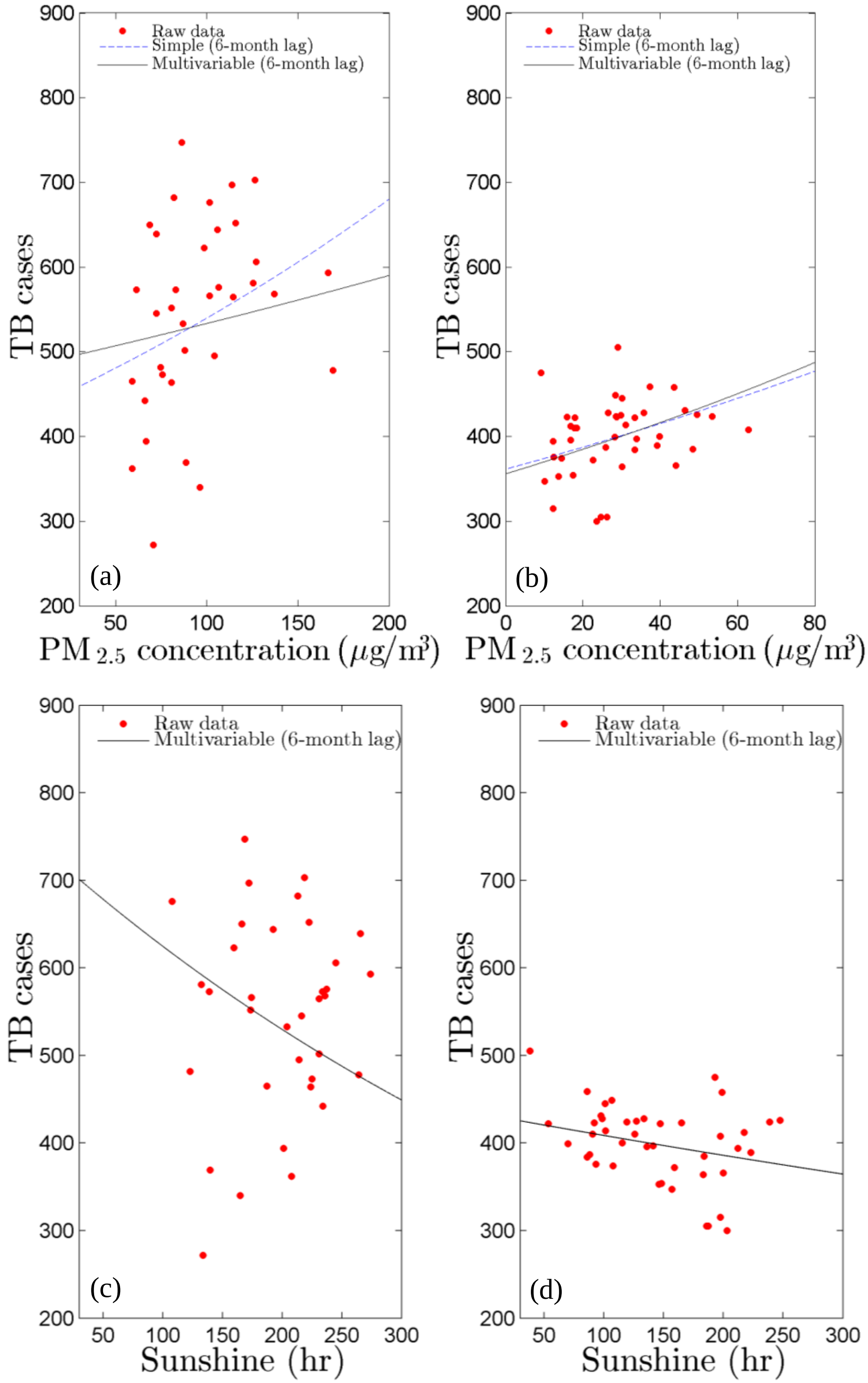


Figure 2. Number of TB cases plotted against $PM_{2.5}$ concentration and sunshine duration for Beijing ((a) and (c)) and Hong Kong ((b) and (d)). The red dots denote the raw data, while the

blue dash lines and solid lines denote the results from the regression models under the 6-month time lag in the simple analysis and multivariable analysis, respectively.

Figure

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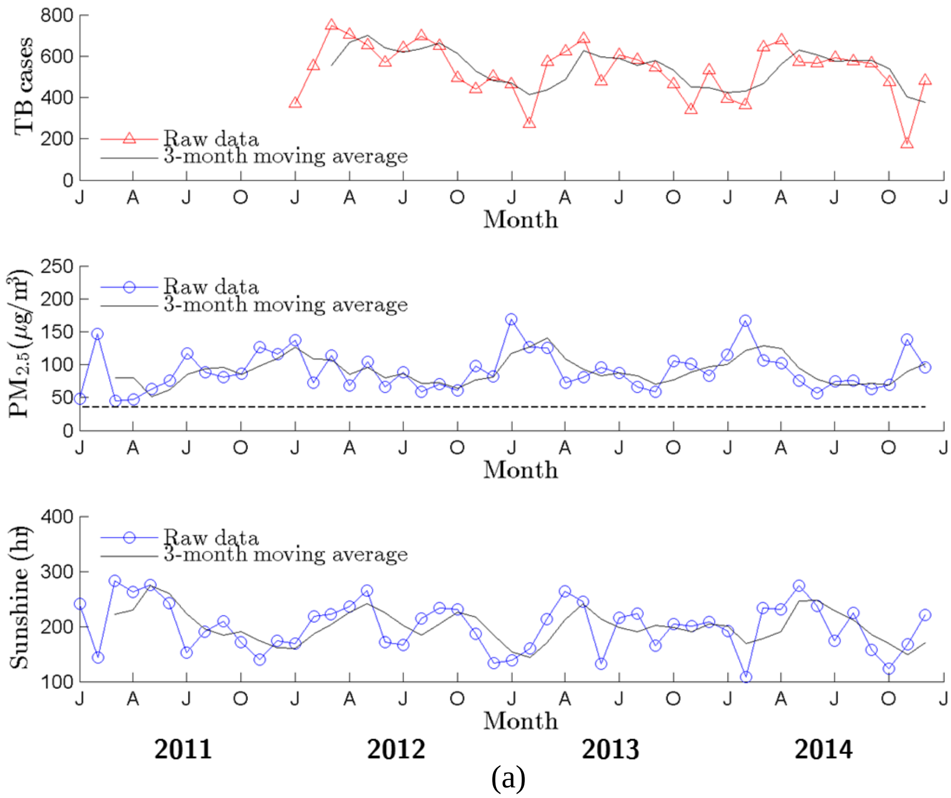

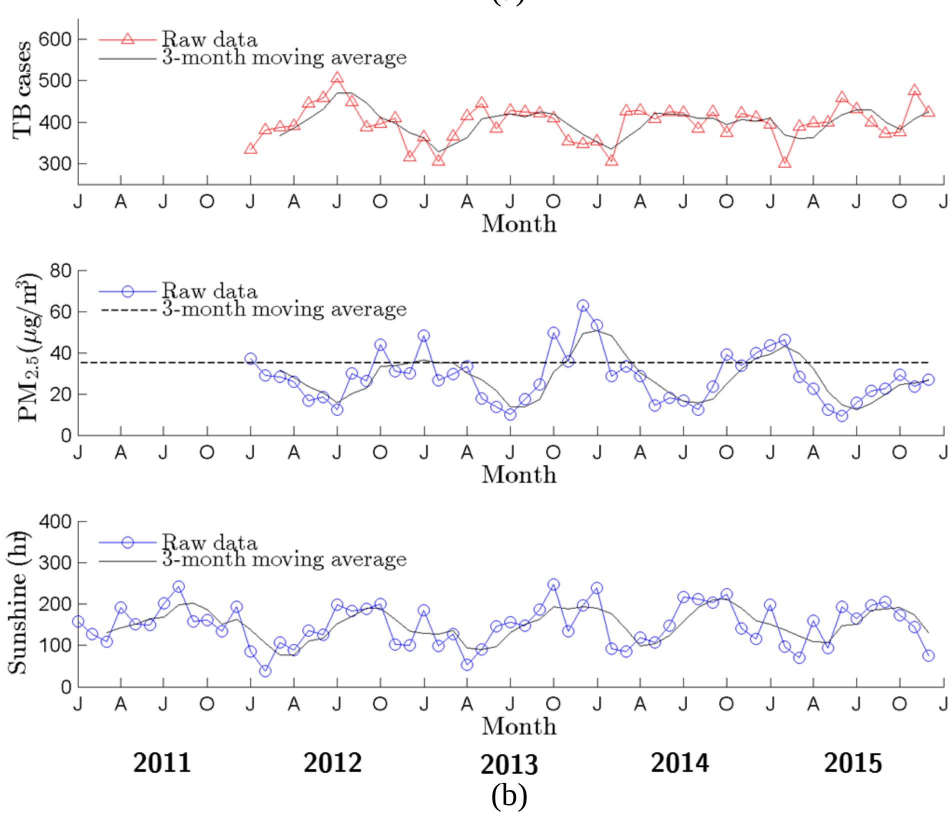

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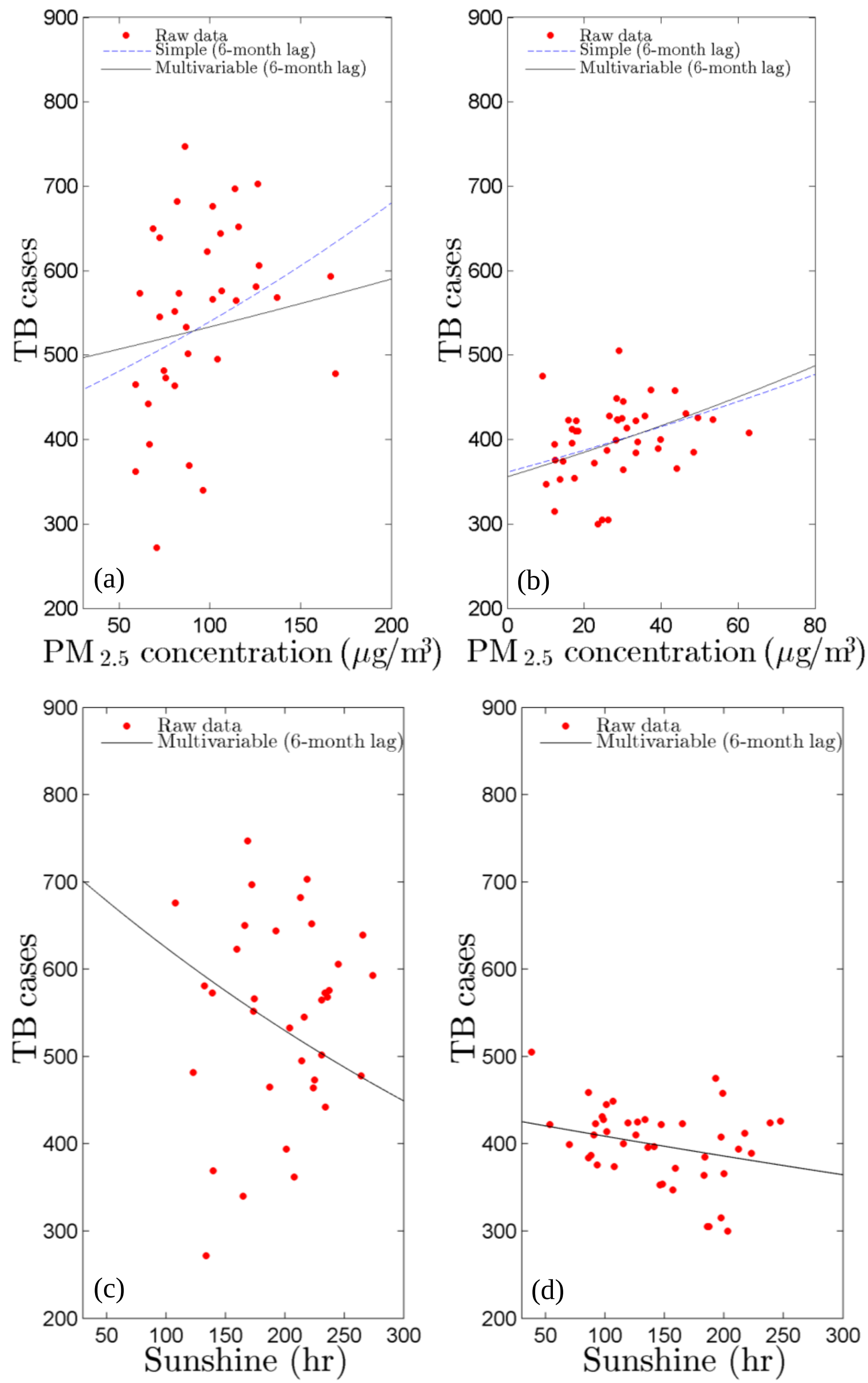

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Table

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Table 1. Monthly-based statistics (averages and standard deviations) of TB cases, $PM_{2.5}$ concentration and meteorological factors for Beijing and Hong Kong for each year considered.

City		2011	2012	2013	2014	2015
Beijing	TB cases	-	585[a] (116)[b]	514 (118)	506 (141)	-
	$PM_{2.5}$ ($\mu g/m^3$)	86.87 (33.65)	85.18 (23.93)	97.81 (31.03)	94.82 (32.82)	-
	Sunshine duration (hrs)	207.14 (52.16)	204.18 (38.15)	197.59 (40.81)	195.34 (50.29)	-
Hong Kong	TB cases	-	405 (53)	389 (42)	399 (38)	401 (45)
	$PM_{2.5}$ ($\mu g/m^3$)	-	27.49 (8.66)	30.83 (16.12)	28.52 (12.22)	25.20 (10.82)
	Sunshine duration (hrs)	164.88 (36.84)	129.27 (52.19)	147.47 (52.56)	158.61 (56.40)	147.47 (52.30)

a: Values before the brackets denotes the averages calculated based on the monthly data of a year.

b: Values in the brackets denotes the standard deviations calculated based on the monthly data of a year.

Table 2. Results of regression analysis of TB cases and $PM_{2.5}$ concentration for Beijing and Hong Kong.

City	Variable	β_i							
		Simple analysis				Multivariable analysis			
		3 month	4 month	5 month	6 month	3 month	4 month	5 month	6 month
Beijing	Intercept	6.07 (6.02-6.12)[a]**	5.95 (5.90-5.99) **	5.99 (5.94-6.04) **	6.06 (6.00-6.11) **	5.92 (5.80-6.03) **	5.52 (5.40-5.65) **	5.95 (5.83-6.07) **	6.51 (6.39-6.63) **
	$PM_{2.5}$ concentration ($\times 10^{-3}$ $\mu g/m^3$)	2.26 (1.78-2.75) **	3.54 (3.06-4.02) **	3.08 (2.59-3.57) **	2.31 (1.82-2.81) **	2.66 (2.11-3.22) **	4.73 (4.15-5.31) **	3.19 (2.61-3.77) **	1.01 (0.42-1.59)**
	Sunshine duration ($\times 10^{-3}$ hrs)	-	-	-	-	0.576 (0.184-0.968) **	1.54 (1.13-1.95) **	0.155 (-0.254-0.564)	-1.65 (-2.06--1.24) **
Hong Kong	Intercept	6.01 (5.98-6.05) **	6.00 (5.96-6.03) **	5.93 (5.89-5.96) **	5.89 (5.85-5.92) **	6.07 (6.02-6.12) **	6.12 (6.07-6.18) **	6.03 (5.98-6.08) **	5.96 (5.90-6.01) **
	$PM_{2.5}$ concentration ($\times 10^{-3}$ $\mu g/m^3$)	-0.705 (-1.89-4.81)	-0.0921×10^{-5} (-1.28-1.09)	2.25 (1.07-3.43) **	3.47 (2.28-4.66) **	-0.489 (-1.69-0.71)	0.477 (-0.74-1.69)	2.84 (1.62-4.05) **	3.93 (2.70-5.15) **
	Sunshine duration ($\times 10^{-3}$ hrs)	-	-	-	-	-0.422 (-0.70--0.14)	-0.998 (-1 .28--0.71) **	-0.842 (-1 .13--0.55) **	-0.572 (-0.86--0.28) **

a: Values in brackets denotes 95% confidence intervals.

** denotes the regression coefficients with P<0.001.