Chapter 4

Atmospheric Pollution and Microecology of Respiratory Tract

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Additional information is available at the end of the chapter

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Abstract

This chapter elaborates the source and ingredients of atmospheric pollutants, the microecology of respiratory tract in animals and humans and the effect of atmospheric pollution on it and thus clarifies the relationship between air pollution and microecology of the respiratory tract based on the experiments.

Keywords: atmospheric pollution, microecology, respiratory tract, health

1. Introduction

This chapter includes five aspects such as atmospheric pollution, the hazards of atmospheric pollution to the body, microecology of respiratory tract, atmospheric pollution and microecology of respiratory tract in humans and atmospheric pollution and microecology of respiratory tract in rats; this chapter describes the source of atmospheric pollution, the classification of atmospheric pollutants (particulates, sulfur oxides, nitrogen oxides, pollution by polycyclic aromatic hydrocarbons (PAHs)), acute and chronic influence of atmospheric pollution to the body, the composition and physiological function of microecology of respiratory tract and focuses on the effect of air pollution on the microecology of respiratory tract. The results of oropharyngeal flora detection in children and rats have shown that atmospheric pollution can cause increase in the detection rates and flora density of both normal flora and pathogenic bacteriatherefore resulting in microecology imbalance. Exposure to air pollutants for different length of time can lead to corresponding acute or chronic injury of the respiratory system. Compared with total suspended particles (TSP) and PM_{10}, $PM_{2.5}$ can stay for long time in atmosphere and has long conveying distance, so that it has more influence on the quality of atmospheric environment and human health.

2. Atmospheric pollution

2.1. Source of atmospheric pollution

The most principal hazardous substances in atmospheric pollution include sulfur oxides, nitrogen oxides, dust, hydrocarbons and carbon oxides. Sulfur oxides mainly come from the combustion of fossil fuels, such as petroleum, coal and natural gas. Nitrogen oxides mainly come from vehicle exhaust, including multiple substances, such as NO and NO_2. Dust is the particulate whose diameter is generally 0.002–500 μm and mainly comes from the fuel combustion, solid crushing, sand storm and secondary hazardous substances, such as ozone and sulfuric acid mist, belonging to photochemical smog. The poisonous gases in industry, such as toluene and gasoline as well as heavily metals entering atmosphere such as mercury, chromium and zinc, would cause atmospheric pollution [1, 2].

Atmospheric pollution is caused by one or multiple kinds of pollutants in gaseousness, gas dispersoid, or particulate that exist in the atmosphere and it can cause harmful or abnormal effects to mankind and other organisms. The principal hazardous substances in atmospheric pollution include sulfur oxides, nitrogen oxides, dust, hydrocarbons and carbon oxides, etc. and atmospheric pollution has become a worldwide problem because of its wide source and large scope of influence.

The sources of atmospheric pollution mainly include fuel combustion, industrial production and transportation. The pollutants discharged by fuel combustion approximately account for 70% of total pollutants in atmosphere of China, 20% by industrial production and 10% by transportation. Among them, over 95% of pollutants discharged by fuel combustion come from coal burning; therefore, the main source of atmospheric pollution in China is the flue dust discharged from direct coal burning.

2.2. Classification of atmospheric pollution

According to their attributes, atmospheric pollution can be divided into three categories, i.e., chemical pollution, biological pollution and physical pollution. Among them, chemical pollution is the most diversified and it is the key point of atmospheric pollutants because of its wide scope of pollution. Chemical pollutants are discharged into atmosphere mainly in the form of waste gas. According to their physical states in atmosphere, they can be divided into gaseous and particulate states. Common gaseous atmospheric pollutants include SO_2, O_3, CO and NO_2. The particulates closely related to hygiene include total suspended particles (TSP) and inhalable particulate (IP). TSP is the generic term of various particulates dispersed in the atmosphere in the state of gas dispersoid. The particle size of TSP is 0.1–100 μm and the toxic substances contained in them, such as As, Cd, Pb, Ni and polycyclic aromatic hydrocarbon, can change the activity and metabolic status of cell enzyme. IP or PM (10) means the particulates whose aerodynamic equivalent diameter (AD) are less than 10 μm and it is then generally divided into coarse particulates (2.5 μm < AD < 10 μm), fine particulates or PM2.5(AD ≤ 2.5 μm) and ultra-fine particulates (UFPs) (AD ≤ 0.1 μm) by the particle size (**Figure 1**). Coarse particulate is mainly produced by mechanical actions, but the latter two kinds of particulates mainly come from fuel combustion, including direct discharge after combustion and generation from gaseous pollutants through chemical transformation [1–4].

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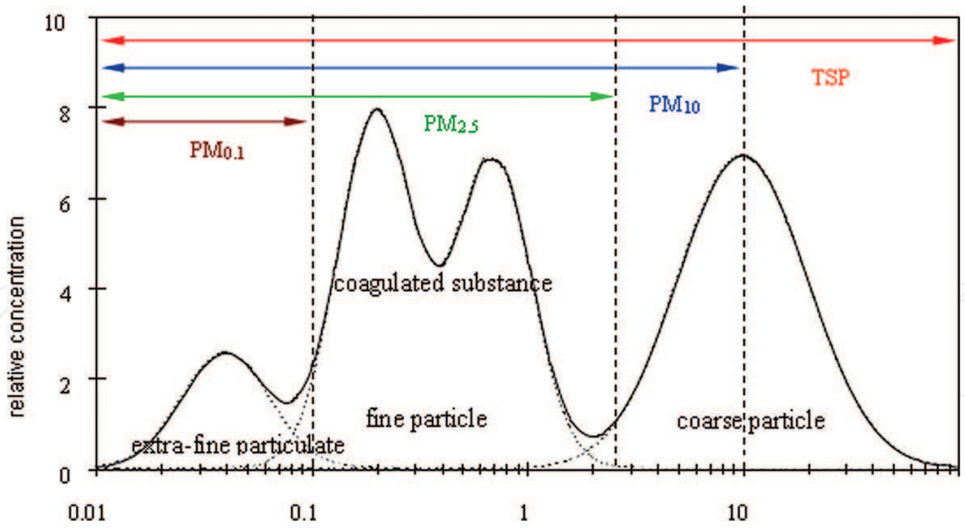

Figure 1. Particle aerodynamic diameter.

Atmospheric pollutants can be classified by different division methods, such as source, chemical component, particle size and indoor or outdoor environment. The pollutants directly discharged into the atmosphere are called primary pollutants and the pollutants produced after chemical reactions with other environmental substances are secondary pollutants. There are obvious correlations between them, but that is not always unchanged, since the lowered level of primary pollutants as precursor would not always automatically make the level of secondary pollutant correspondingly lower. For example, ozone (O_3) is mainly generated by nitrogen oxides (NO_X). When the discharge of NO_X is decreased, the level of O_3 in the atmosphere sometimes conversely rises. The particulates in atmospheric pollutants (PM) are commonly divided into three categories according to the aerodynamic diameter. Coarse particles (2.5–10 μm, PM_{10}) come from the aggregation of road dust, building scrap, or tiny combustion particles; fine particulates (<2.5 μm, $PM_{2.5}$) and extra-fine particulates (<0.1 μm, UFPs) are mainly formed in the combustion process of fossil fuel. US EPA has already limited the environmental control standard for $PM_{2.5}$ and PM_{10}. $PM_{2.5}$ is the subcomponent of PM_{10} and there is a standard specially stipulated for $PM_{2.5}$, so as to guarantee that $PM_{2.5}$ with smaller volume and greater permeability through respiratory tract can be effectively controlled because it is more influential. At present, little is known about the potential risks of UFPs. There is evidence to show its greater quantity, more contents of transition metals and oxidation-reduction chemical components, which can more easily penetrate blood circulation. Therefore, it has greater toxicity than $PM_{2.5}$ and PM_{10}. However, the corresponding control standard has not yet been promulgated [1–4].

2.2.1. Particulates

In the generally acknowledged atmospheric pollutants, the epidemiological links between particulates and population health effect endpoint is the closest. The quantitative hazard assessment of particulates on health has become one of the hot spots that received attention from international organizations in recent years, such as WHO and EU. It is stipulated by America that the mean daily value and mean annual value of IP (PM_{10}) shall

respectively be 0.15 and 0.05 mg/m^3. It is stipulated in GB3095–1996 promulgated by China in 1996 that the secondary standard for the mean daily value of PM_{10} shall be 0.15 mg/m^3 and the mean annual value shall be 0.10 mg/m^3. In 1997, Environmental Protection Agency of the United States (US EPA) was the first to promulgate the $PM_{2.5}$ standard and its mean daily value was strictly stipulated to be 0.065 mg/m^3 and its mean annual value to be 0.015 mg/m^3 [1–4].

The toxicity of particulates, both PM_{10} and $PM_{2.5}$ can increase the risk of lung cancer. It is shown by the American research that sulfate, nitrates, hydrogen ion, carbon element, secondary organic compounds and transition metals are all concentrated on the fine particulates, but Ca, Al, Mg and Fe elements are mainly concentrated on coarse particulates and they have different impacts on the human body. The hazard of $PM_{2.5}$ on the human body is greater than PM_{10}, which has become a new target of policy for environmental air control. With the development of transportation and the increase of motor vehicles as well as the increasing disruption of environment, the pollution of $PM_{2.5}$ gets more and more serious. It is found by researches that the ratio of atmospheric $PM_{2.5}$ in TSP is increased year by year and 96% of the particulates that deposit in the lower respiratory tract are $PM_{2.5}$. The $PM_{2.5}$ in the urban atmosphere mainly comes from the exhaust of traffic waste gas (18–54%) and the secondary pollution of gas dispersoid (30–41%) [1–4].

2.2.2. Sulfur oxides

The toxicity of SO_2: Sulfur dioxide is one of the main atmospheric pollutants. It is stipulated by the second class of national standard for atmosphere that the content of SO_2 in air shall be <0.06 mg/m^3. SO_2 can be adsorbed on the surface of $PM_{2.5}$ to enter deep into the respiratory tract, increasing its toxicity by three to four folds. SO_2 would be oxidized into SO_3 through metallic particle catalysis, whose risk is four times that of individual effects of SO_2. The carcinogenic action of benzo(a)pyrene, i.e., BaP can be increased under joint action of SO_2 and B[a]P.

Researches on the relations between SO_2 and the incidence risk of lung cancer. It is shown by epidemiological researches that SO_2 is closely related to the incidence of lung cancer. It is shown by the analysis of the mortality rate of lung cancer in Qingdao by Hu Yan [5]. that SO_2 in the atmosphere of downtown Qingdao has certain correlations with the increase in the mortality rate of lung cancer. Chen Shijie et al. [6] utilized grey relativity model to calculate the mortality rate data for lung cancer of mass and the mean annual concentration data of SO_2. It is shown by the results that the grey relativity between the mortality rate of lung cancer and the SO_2 8 years ago is the greatest, thus the latent period of SO_2 to cause lung cancer is 7 years. It is shown by the cohort research of 16,209 individuals in Norway that long-term exposure to SO_2 is related to the risk of lung cancer. Through the data collected by American Cancer Society for 16 years and the data of risk factor for the cause of death of 500, 000 Americans, Turner et al. [7]. found that SO_2 in air is related to the total morbidity rate and mortality rate of lung cancer. Through the cohort research of 57,613 workers exposed to SO_2 in pulp and papermaking industry in 12 countries, Moon et al. [8] found that the relative risk of lung cancer of the workers in the nonexposed group compared with those in the exposed group is 1.49 and its 95% *CI* is 1.14–1.96 [5, 6].

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2.2.3. Nitrogen oxides (NO_X)

The toxicity of nitrogen oxides: The nitrogen oxides in atmosphere mainly come from the waste gas exhausted from combustion of automobile, coal and petroleum. It is stipulated by the national atmosphere secondary standard that the content of NO_X in air shall be <0.10 mg/m^3. Photosynthesis shall occur at hydrocarbons under the effect of ultraviolet ray. Photochemical smog shall be produced and it might induce canceration.

Researches on the population epidemiology of nitrogen oxides: Through spatial geographical distribution map and Spearman rank correlation analysis to the death data of lung cancer in Jiangsu Province and to the air data of corresponding period, Lu et al. [9] found the positive correlation between the concentration of NO_X and the standardized mortality rate of lung cancer. It is shown by the cohort research of 16,209 individuals in Norway: long-term exposure to NO_X is related to the risk of lung cancer. When the concentration of NO_X is increased by 10 μg/m^3, the relative risk of lung cancer would be 1.11 and its 95% *CI* is 1.03–1.19. It is shown by the case-control study by Nyberg et al. [10]. in Sweden that with the NO_X pollution caused by exposure to traffic pollution for over 30 years, the risk of lung cancer is increased by one to two folds.

2.2.4. Pollution of polycyclic aromatic hydrocarbon

The toxicity of PAHs: Most PAHs in atmosphere are generated by the incomplete combustion of fuel in life and production activities of mankind. Multiple kinds of PAHs have been assessed to have carcinogenesis, especially among 16 kinds of PAHs under priority control publicized by US EPA, some of which are strongly carcinogenic compounds. PAHs themselves have no toxicity, but they present carcinogenic actions through metabolizing activation after entering the body. PAHs can also react with O_3, NO_X, and HNO_3, etc. and they might be converted into the compounds with stronger carcinogenic actions or mutagenic actions. At present, the PAHs proved to have relatively strong carcinogenesis by animal experiments which include benzo(a) pyrene, benz(a)anthracene, benzo(b)fluoranthene, dibenzo(a,h)pyrene and dibenz(a,h)anthracene, etc., among which the carcinogenic action is the strongest.

3. The hazard of atmospheric pollution to the body

With the increase of population density and the development of modern industry in the city, the influence of atmospheric pollution on mankind gets more and more serious. It is shown by many epidemiological evidence that the diseases of respiratory system are related to the low quality of air. At present, about 600 million tons of atmospheric pollutants are discharged annually into air worldwide, which is the direct cause for the increased number of hospitalized patients and outpatient patients, the decreased attendance rate of students and the increased morbidity rate of allergy. Life and environment shall be a unity and the life existence of living bodies that must adapt to the internal and external environment, since the maladjustment between them would merely cause diseases or death. Life and environment must be a unity of opposites and they are inseparable. All organisms have the limit of adapting to the

environment and anyone beyond the limit would lose life. Mankind is incessantly adapted to the environment and ceaselessly transforms the environment. Through the long-term repeated actions of low concentration of toxic and harmful pollutants in the environment on the body, chronic hazards would be incurred, which is caused by the sedimentation of poison itself in the human body or gradual accumulation of the tiny injuries to the body. At present, the world is confronted with three major environmental problems, i.e., destruction of ozone layer, acid precipitation and greenhouse effects, which are all related to the atmospheric pollution. The reason for the destruction of ozone layer is the impact of certain compounds, mainly including Freon. Under the photochemical action of ultraviolet ray, the harmful compounds in atmosphere might produce the active compounds to destruct the ozone layer, such as NO, NO_2, HO_2, Cl^- and ClO^-. The hole would be formed at the ozone layer after destruction and the function of ozone layer to block and absorb ultraviolet ray would be weakened. Excessive contacts of population with ultraviolet ray might cause corresponding diseases [11–14].

The combustion of large quantities of fuels would produce large quantities of CO, CO_2, SO_2, and NO_X, etc., which are discharged into the atmosphere and shroud in the air above the ground surface. As a result, the pH value of precipitation is lower than 5.6 and acid precipitation is thus formed. CO_2 can absorb long wave radiation, such as infrared ray and greenhouse effects can be formed by warmer weather aggravating the multiplying of pathogenic agents. Since varied kinds of atmospheric pollution are complicated, the categories of pollutants are multitudinous and their influences on human health are complicated (**Figure 2**).

Human and environment are closely related and the atmospheric pollution incurred by the progress of industrial civilization has substantial impacts on mankind. It is shown by researches

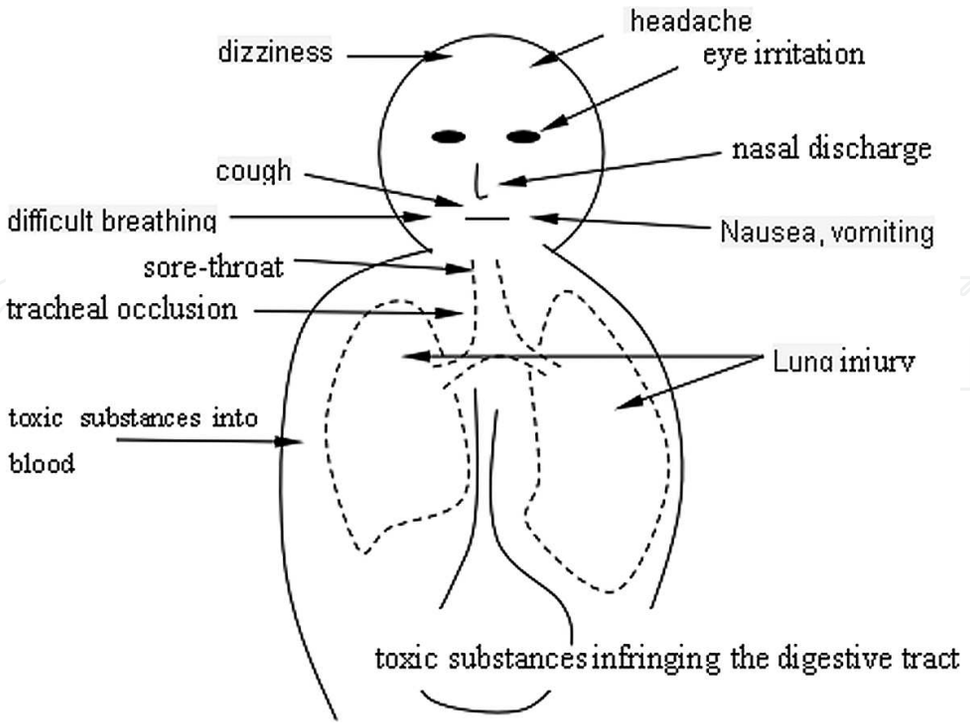

Figure 2. Polluted air is harmful to health.

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of environmental epidemiology that from slight symptom of respiratory system to the increase in outpatient number of cardiopulmonary diseases and mortality rate, all are closely related to atmospheric pollution. As estimated by World Health Organization (WHO), annual death of 800 thousand people and 4.6 million disability adjusted life expectancy (DALY) worldwide are related to urban atmospheric pollution. In 1775, British doctor Pott posed the hypothesis of flue gas pollution to be carcinogenic and in the mid-twentieth century, the occurrence of London smog incidents made people extensively pay attention to the health hazard of atmospheric pollution. However, its influence on health has not yet been fully recognized by mankind. The health hazard caused by atmospheric pollution has become a problem worldwide and it has caught the attention of multitudinous domestic and foreign scholars. Since the health effect is complicated, it is usually the results of multiple factors through comprehensive actions. Thus, it is very difficult to draw the explicit conclusion of a certain factor that causes a certain result. The influence of atmospheric pollution on health is mainly divided into acute influence and chronic influence [12–15].

3.1. Acute influence

The acute influence of atmospheric pollution on humans includes direct actions and indirect actions. For example, acute intoxication of certain poison belongs to direct actions; indirect actions can aggravate the deterioration of respiratory system or heart diseases, etc. and thus accelerate death of patients. There are a few domestic reports on the acute influence of atmospheric pollution on human body and it is pointed by some scholars that when the mean daily concentration of each index of atmospheric pollutants does not exceed the health standard but instantaneous discharge surpasses the maximum allowable concentration for single exposure, the importance of its influence on health might exceed the average day concentration. Therefore, the acute hazard of atmospheric pollution could be more concealed and its severity might be easily neglected by people [16–18].

3.2. Chronic influence

The chronic influence of atmospheric pollution on human body is a kind of composite action, which is manifested in multiple aspects. The early hazard of low concentration atmospheric pollution to human body is usually not fully manifested in the form of disease. Instead, it is mostly manifested as the preliminary effect of diseases. It is shown in the immunologic dysfunction of body and the change in blood and circulation system, which might induce and promote the incidence of allergic diseases, respiration system diseases and other diseases in human body. At the time of performing the epidemiological research, in order to reduce the influence of smoking and occupational factors, etc., children are preferably selected as the investigation objectives [13, 19–21].

From the metabolic process of body, it can be seen that human body and environment are closely related. Human body performs substance exchange with the surrounding environment through metabolism and dynamic equilibrium is normally kept between the substances in environment and human body. In case of abnormal changes in environment, normal physiological functions of human body would be affected with varying degrees and human body is

capable to adjust own physiological functions, so as to adapt to the incessantly changing environment. That normal physiological adjustment function of adapting to the environmental changes is formed by mankind in the long-term development process. If the abnormal changes in environment are within a certain limit, human body can adapt to them. If the abnormal changes of environment surpass the limit of normal physiological adjustment of human body, an anomaly might incur in certain functions and structures of the human body and even in pathological changes. That kind of environmental factor that might incur pathological changes in human body is called environmental pathogenic factor. The diseases of mankind are mostly caused by biological, physical and chemical pathogenic factors. The substances that cause environment pollution include chemical factors, biological factors and physical factors, etc. and they might become pathogenic factors when they reach a certain extent [19–21].

The contents of pollutants in environment are usually very few under many circumstances, after mankind lives in that kind of low concentration polluted environment for several months, several years and even tens of years, chronic hazards to the body might be gradually incurred to cause diseases. Disease is a process of pathological changes in the function, metabolism and morphology of body under the actions of pathogenic factors. Human body has certain compensatory ability against the functional hazard caused by disease factors. Some changes are compensatory and some changes are traumatic in the developing process of diseases and both changes coexist. When the compensatory action is stronger, body can still keep relative stability and the clinical symptoms of diseases would temporarily not emerge. When the pathogenic factors stop acting, body shall develop to restore health. But the compensatory ability is restricted. If the pathogenic factors continue to act, the compensatory functions would gradually be obstructed and the body will manifest unique clinical symptoms and signs of various diseases. The reacting process of human body to environmental pathogenic factors is shown in **Figure 3**.

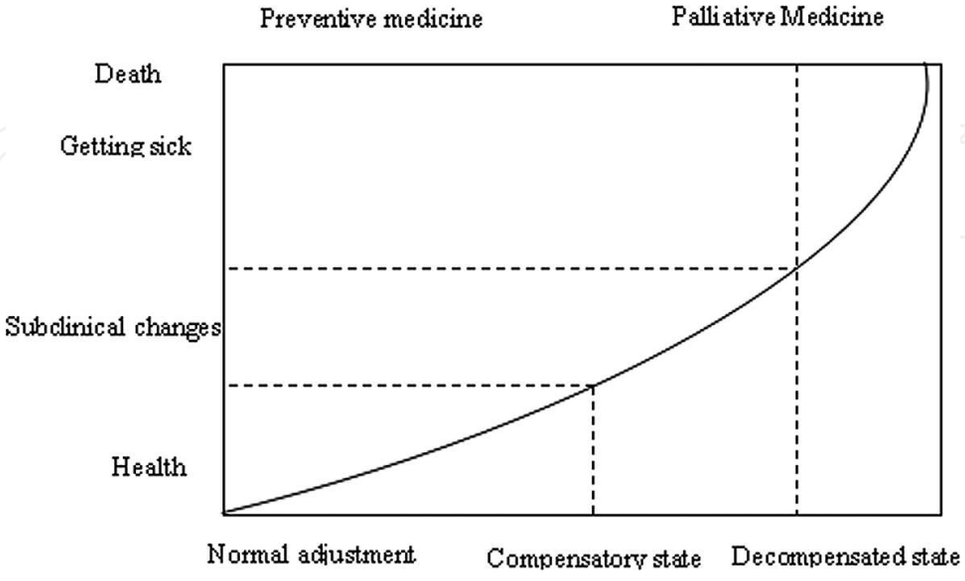

Figure 3. The process that human body react to environmental pathogenic factors.

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The incidence and development of disease is generally divided into the latent period (without clinical manifestation), prodromal period (with slight general discomfort), obvious clinical symptom period (when typical symptoms of the certain disease emerge) and prognostic period (restoring health or deterioration to death). Under the circumstance of acute intoxication, the first two periods of disease might be very short and obvious clinical symptoms and signs might appear very soon. Under long-term micro action of pathogenic factors (such as certain chemical substances), the first two periods of disease can be considerably long. Patients might have no obvious clinical symptoms and signs and instead, they might look healthy. But under continued actions of pathogenic factors, obvious clinical symptoms and signs might emerge. In addition, its resistance to other pathogenic factors (such as bacteria and virus) would decrease and therefore, that kind of person is in the latent period or compensatory state. The stage during which injuries have already occurred by a certain hazard but the clinical symptoms have temporarily not emerged, shall be deemed as the early period of disease (clinical prophase or subclinical state) [19–21].

It is shown by researches that 80–90% of mankind cancer is related to environmental factors, among which 90% are caused by chemical factors and 5% are caused by radioactive factor. But the chemical substances and radioactive substances within the contact scope of mankind mainly come from environmental pollution.

4. Microecology of respiratory tract

The respiratory system of mankind is composed of the following organs: Nose, pharynx, throat, trachea, bronchus and lung. Nose, pharynx and throat are the upper respiratory tract and trachea and bronchus are the lower respiratory tract. Lung is the place where gas exchange is performed. Other organs are the passages of gas and are generically called respiratory tract. Like the permanent planting of flora in other systems or parts of the body, that in the respiratory tract observes a certain regular pattern. For the microorganisms that exist at each part of human respiratory tract, their category and quantity are relatively stable under normal circumstances. Bacteria that are often separable from healthy human body mainly include alpha streptococcus, Neisseria Trevisan, Hemophilia, oral myxococcus, staphylococcus, corynebacteria and a certain anaerobe. Relatively single population of alpha streptococcus mainly lives on the surface of epithelial cells at oral pharynx and oropharynx mucosa. Saliva contains such immunological materials as immune globulin and lysozyme. As a result, as the long-term permanently planted bacteria at oral pharynx, alpha streptococcus has stronger immunity than the crossing bacteria. Alpha streptococcus normally accounts for over 90% of bacteria at oral pharynx [22–24].

The microenvironment of each part of respiratory tract is very complicated. Besides the ecological space of body and the process of its gas exchange with outside environment, which constitute important components of the microscopic environment of respiratory tract, microbiocoenosis (including bacteria, virus, mycoplasma and actinomycete, etc.) inevitably intervenes soon after the birth of individuals, since the passage is open to the exterior. They shall parasitize in different ecological niches of respiratory tract and they shall not be

pathogenic under health. Some can be combined with the receptors on the cell surface, so as to obstruct the invasion of the pathogenic microorganisms that enter later, playing the role of a barrier. The microbiocoenosis at ecological niches of human body, such as the upper respiratory tract and trachea, has relationship of mutual synergia and normal commensal flora can help host to resist the invasion of pathogenic microbes. That protective mechanism includes: (i) aiming at the competition of the same nutrient, disturbing the nutrition of attacking pathogenic bacteria; (ii) aiming at the affinity of the same category of cells, i.e., the commensal bacterium that has relatively big binding force to the receptors on the cells of the host, disturbing the attachment effect of attacking pathogenic bacteria; (iii) through irritating the host to produce immune factor, aiming at the attacking bacteria with "cross" antigen, exerting adverse influences to make the pathogenic bacteria not be permanently planted without difficulty; (iv) through producing bacteriocin or antibiotic, specifically or not disturbing the colonization action of identical or relevant sensitive microorganisms [22–24].

Oral pharynx is the passage to link oral cavity and nasopharynx with the lower respiratory tract and esophagus. Since it is connected with the external environment, it is a physiological part where many bacteria infringe the lower respiratory tract and lung to cause infection. About the general regular patterns for the cognition to microecology of human body, the core population combination that parasitizes at the oral pharynx of a healthy body shall fluctuate within harmonious physiological scope and they shall compose an important microbial barrier for oral pharynx, so as to resist the change in the external environment. The pathogenic bacteria would firstly be permanently planted at the mucosa part represented by oral pharynx, such as mouth and nasopharynx. Then it would reach the lower respiratory tract and lung and would further cause corresponding pathological reaction of the body. Oral pharynx has a high content of organic matter and rich nutrition and is exposed in very complicated microbial environment. Thus it has many opportunities to contact various bacteria. But the categories of bacteria that live at oral pharynx is actually very limited and the total biomass of bacteria is only moderate.

5. Research examples: atmospheric pollution and microecology of respiratory tract in human

In order to explore the early harmful effects of the atmospheric pollution on human health and screen sensible markers, children of school-age who are the susceptible population to the atmospheric pollution are selected in our studies. And the researches were performed in different polluted zones with the different extent of the atmospheric pollution of three cities in the northeast of China, including Benxi, Shenyang and Dalian. Measurement and multiple-analysis were performed to analyze the relationship between the atmospheric pollution and the microecology of the upper respiratory tract.

5.1. The influence of atmospheric pollution on the symptoms and diseases of children's respiratory system

Since the circumstances of air pollution in the three cities are difficult, contrast analysis was performed on the health status of the respiratory system of children in the three cities and the

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children in the contrast regions, so as to have an in-depth understanding of the influence that atmospheric pollution might produce to the body. By comparison of the three cities, children from Benxi and Shenyang are high in the positive rate of the general inflammatory symptoms and diseases of the respiratory system (such as cough, expectoration, sustained cough, sustained cough with expectoration, pneumonia, etc.), but the rate is relatively low in Dalian due to the more serious atmospheric pollution in Benxi and Shenyang (**Table 1**). Significant differences are found in the disease and incidence circumstance of the respiratory system among children in three cities ($P < 0.01$).

Through the investigations to the symptoms and diseases of the respiratory system of 18,000 children in different polluted zones of different cities, it is shown by the analysis of the investigation that the extent of atmospheric pollution is positively related to various symptoms and diseases of respiratory system. In the analysis to each kind of symptoms and diseases, it is all selected into the equation under the condition of $P < 0.05$ for atmospheric pollution and the OR value is all >1, which means atmospheric pollution can increase the risk of symptoms and diseases of children's respiratory system. Thus it is a hazardous factor to cause symptoms and diseases of the respiratory system.

	Benxi region	Shenyang region	Dalian region	Control
Symptom				
Cough	58.6 (3169)	59.5 (3500)	43.6 (1719)	44.1 (947)
Persistent cough	4.0 (216)	4.2 (246)	2.2 (87)	1.9 (40)
Expectoration	36.0 (1947)	37.4 (2198)	28.1 (1108)	26.8 (575)
Persistent cough and expectoration	2.4 (127)	2.6 (151)	1.1 (43)	1.2 (25)
Wheezing	7.3 (396)	1.4 (82)	0	1.4 (29)
Respite	4.5 (241)	2.2 (132)	2.5 (100)	1.4 (29)
Disease				
Bronchitis	10.2 (550)	17.3 (1019)	22. 5 (887)	9.9 (211)
Asthmatic bronchitis inflammation	1.7 (94)	3.3 (195)	5.3 (210)	2.0 (43)
Pneumonia	9.1 (494)	22.1 (1297)	17.5 (692)	11.6 (246)
Eczema	8.9 (479)	12.1 (710)	7.2 (282)	7.5 (159)
Asthma	2.2 (121)	6.1 (359)	10.3 (407)	5.0 (106)
Urticaria	5.8 (314)	7.8 (457)	5.0 (196)	5.5 (116)
Allergic rhinitis	2.0 (106)	3.7 (217)	2.6 (103)	2.2 (46)

**$P < 0.01$.

Table 1. The prevalence rate and the positive rate of children' respiratory system symptoms and diseases in different city.

5.2. The influence of atmospheric pollution on flora of the respiratory tract

According to the atmospheric monitoring data and urban function zoning of Shenyang, heavily polluted regions and light polluted regions are selected (**Table 2**). By means of the

	TSP $\overline{X}$ ± SD	SO_2 $\overline{X}$ ± SD	NO_x $\overline{X}$ ± SD
Heavily polluted region	0.432 ± 0.097	0.169 ± 0.075	0.089 ± 0.021
Light polluted region	0.190 ± 0.041*	0.012 ± 0.041*	0.019 ± 0.058*

*$P < 0.05$.

Table 2. The yearly mean value of the main substance that pollute air in light and heavy polluted regions of Shenyang city (mg/m^3).

cluster sampling, samples of 40 and 50 children (half boys and half girls) aged 6–8 from different regions were selected respectively in 2000, while samples of 40 and 40 children were selected respectively in 2002. They were required not to use antibiotic drugs in the past 3 months. The qualitative location and quantitative analysis was performed to the flora status at the upper respiratory tract of children in the heavily polluted regions and contrast regions, so as to explore the influence of atmospheric pollution on microecology of the respiratory tract.

Normal flora at oral pharynx is the natural barrier for the body. If such barrier is destructed by harmful factors, the flora would be imbalanced in quantity and category. As a result, the ability of the defense barrier would decline, exogenous pathogenic bacteria would intrude, or endogenous conditions would cause massive multiplication of pathogenic bacteria to incur infection. It is shown by our research that the density of the aerobic and anaerobic bacteria at oral pharynx of children in the seriously polluted air regions is higher than that of the light polluted regions and the abnormal flora also emerged, showing status of the excessive growth of flora at pharynx of children in the heavily polluted regions (**Table 3**). It is also shown that the detection rate of the conditional pathogenic bacteria of children in the heavily polluted regions was higher than that of the contrast regions. Permanent colonization of the pathogenic bacteria also emerged at oral pharynx of children in the heavily polluted regions, such as beta streptococcus, Klebsiella pneumonia, Serratia liquefaciens and Pseudomonas maltophilia (**Table 4**). The mutual antagonism and mutual restriction objectively exist in the microbes and this antagonism mainly comes from the microbial community. If the community is destructed, it

	Aerobic		**Anaerobic**		**Total number**	**Amount (lgX ± SD)**
	Detectable number	**Amount**	**Detectable number**	**Amount**		
Heavily polluted region (40)	763	2.883 ± 0.245*	837	2.923 ± 0.255*	1600	3.147 ± 0.260*
Light polluted region (50)	546	2.737 ± 0.926**	321	2.507 ± 0.050**	867	2.899 ± 0.194**

*$P < 0.05$.
**$P < 0.01$.

Table 3. The distribution of bacteria cluster in children' pharynx in different polluted regions (CFU/ml).

Bacterial species	Heavily polluted region (N = 40)		Control (N = 50)			
	Detection number	Detection rate (%)	Detection number	Detection rate (%)		
Aerobic						
Neisseria	33	82.5	39	78.0	0.82	>0.05
Streptococcus pneumoniae	21	52.5	17	34.0	3.12	>0.05
Streptococcus	11	27.5	24	48.0	3.93	<0.05
Group D streptococci	6	15.0	5	10.0		
Streptococci	2	5.0	0	0.0		
Liquefied Serratia	1	2.5	0	0.0		
Pseudomonas maltophilia	1	2.5	0	0.0		
Diphtheroids	1	2.5	2	4.0		
Klebsiella pneumoniae	1	2.5	0	0.0		
Staphylococcus aureus	0	0.0	1	2.0		
Micrococcus	0	0.0	1	2.0		
Anaerobes						
Peptostreptococcus	19	47.5	18	36.0	1.21	>0.05
Veillonellaceae	16	40.0	29	58.0	2.88	>0.05
Bacteroides	9	22.5	12	24.0	0.03	>0.05
Excellent bacillus	8	20.0	6	12.0	1.08	>0.05
Fusobacterium nucleatum	4	10.0	9	18.0	1.15	>0.05
Propionic acid bacteria	2	5.0	2	4.0		
Lactobacillus	1	2.5	2	4.0		
Iraq Actinomyces	1	2.5	0	0.0		
Clostridium perfringens	1	2.5	2	4.0		
Bifidobacterium	0	0.0	1	2.0		

Table 4. The comparison of the bacteria examined from children's pharynx in light and heavy polluted regions.

would be favorable for the translocation of alien microbes and the multiplication of the pathogenic bacteria. It is shown by the research studies that the atmospheric pollution can cause the changes in the microbial community at oral pharynx of the body. Therefore, the atmospheric pollutants as exogenous factors have caused the destruction to the microecology balance of the upper respiratory tract. It is shown by the research that the incidence rate of the chronic inflammation at the upper respiratory tract and the incidence rate of chronic bronchitis of pupils in the heavily polluted regions are both higher than that of the light polluted regions (**Tables 5** and **6**), which can be attributed to the microecology imbalance at pharynx of the upper respiratory tract caused by the atmospheric pollution.

	Cough	Persistent cough	Expectoration	Cough and expectoration	Persistent cough and expectoration	Wheezing	Respite
Light polluted region							
First grade	59.7 (597)	3.9(39)	37.5(375)	33.3(333)	2.2(22)	1.8(18)	2.5(25)
Sixth grade	54.8 (544)	4.2(42)	34.1(338)	29.9(297)	1.9(19)	0.6(6)	2.6(26)
Total	57.3 (1141)	4.1(81)	35.8(713)	31.6(630)	2.1(41)	1.2(24)	2.6(51)
Heavily polluted region							
First grade	64.5 (632)	4.2(41)	42.4(416)	38.4(376)	3.1(30)**	2.0(20)	2.2(22)
Sixth grade	61.7 (586)**	5.9(56)**	38.7(367) **	34.5(327)**	3.9(37)*	1.3(12)	2.1(20)
Total	63.1 (1218)**	5.0(97)	40.6(783)**	36.4(7037)**	3.5(67)**	1.7(32)	2.2(42)

*$P < 0.05$.
**$P < 0.01$.

Table 5. The positive rate of children' respiratory system symptom in different polluted regions in Shenyang City (%).

	Bronchitis	Asthmatic bronchitis	Pneumonia	Asthma	Eczema	Urticaria	Allergic rhinitis
Light polluted region							
First grade	20.5(205)	3.4(34)	21.5(215)	6.3(63)	15.3(153)	7.4(74)	2.3(23)
Sixth grade	13.0(129)	3.1(31)	21.1(209)	5.6(56)	12.8(127)	10.1(100)	4.5(45)
Total	16.8(334)	3.3(65)	21.3(424)	6.0(119)	14.1(280)	8.73(174)	3.4(68)
Heavily polluted region							
First grade	23.0(225)	4.1(40)	25.9(254)	8.8(86)**	12.8(125)	5.5(54)*	1.9(19)
Sixth grade	14.2(135)	2.6(25)	22.4(213)	5.8(55)	8.7(83)*	9.8(93)	5.1(48)
Total	18.7(360)	3.4(65)	24.2(467)*	7.3(141)*	10.8(208)**	7.6(147)	3.5(67)

*$P < 0.05$.
**$P < 0.01$.

Table 6. The children' prevalence rate of respiratory disease in different polluted regions of Shenyang City (%).

It is shown by the dynamic analysis for 2 years that the detectable rate of alpha streptococcus at oral pharynx of children in the heavily polluted regions is lower than that of the contrast regions and the difference is significant (**Table 4**). Alpha streptococcus is the normal flora at the pharynx mucosa and it can be antagonistic against the abnormally permanent planting of the certain pathogenic bacteria, playing the role of a barrier. The reduction in the alpha streptococcus of children in the heavily polluted regions would certainly decrease the protective effects of the pharynx barrier and would increase the susceptibility to infection. It is

considered by some scholars that alpha streptococcus can be taken as the probiotics for the upper respiratory tract. It can play a role in preventing and treating the infection of the upper respiratory tract by means of biological oxygen capture, flora adjustment and biological antagonism, which is consistent with our research results. It is also shown by this research that atmospheric pollution can cause the reduction of alpha streptococcus and further cause the increase of other abnormal flora. As a result, the infection of the respiratory tract might be increased. Therefore, we consider that alpha streptococcus can be used as one of the probiotics for the upper respiratory tract and it needs to be further developed as a microbial preparation, so as to prevent and treat infection of the upper respiratory tract and maintain the microecology balance.

6. Atmospheric pollution and microecology of respiratory tract in rats

To explore the regulation mechanisms of air pollution-related molecules and demonstrate the effects of air pollutants on the microecology of the respiratory tract, Wistar rats were used to expose to simulated real atmospheric pollution. It is beneficial to provide theoretical basis for the influence of air pollutants to the health of human body and the prevention and control of atmospheric pollution [25].

6.1. The influence of total suspended particulate (TSP) to the respiratory tract flora of rats

The relationships between normal microbiota and the host include ecosystems, biomes, species and individuals. Normal microbiota are divided into origin flora and foreign flora. The origin flora has more obvious physiological function if they come in contact with the host tissue cells closely, therefore providing more protection to the host and vice versa. Microecological balance is the ecological balance at the cellular level and molecular level, including microorganisms and host aspects. Normal microbiota of local ecological balance have obvious biological antagonist action to the pathogenic bacteria. Microecological imbalance will occur when this balance is damaged or influenced by external environmental factors.

From the ecological perspective, the normal flora of pharynx is the natural protective barrier. If the bacterial flora imbalance in quantity and types occur when the barrier suffers from the damage of harmful factors, the defensive barrier will break; therefore, the invading exogenous pathogen or some kind of endogenous bacteria will multiply and cause infection.

6.1.1. The influence of TSP to the bacterial species of respiratory tract of rats

TSP was collected continuously at a flow rate of 1000 l/min from areas of coal burning, traffic congestion in Shenyang city (China) during heating season and made into suspension by ultrasonic oscillation. The aerobic and anaerobic bacteria on the oropharynx of Wistar rats were analyzed before or after exposure to TSP. The results are shown in **Table 7**.

Klebsiella pneumoniae and *Staphylococcus aureus* were not detected on the oropharynx of Wistar rats before exposure to TSP, however, *K. pneumoniae* were detected on the oropharynx of six rats ($P < 0.01$) and *S. aureus* were detected on the oropharynx of two rats after they exposure to

Bacterial species	No TSP exposure(n = 78)		TSP exposure(n = 67)	
	Rats number	Detection rate	Rats number	Detection rate
Escherichia coli	20	25.6	16	23.9
Diphtheroid bacillus	13	16.7	9	13.4
Group A *Streptococcus*	31	39.7	33	49.3
Neisseria	20	25.6	11	16.4
Streptococcus oralis	13	16.7	6	9.0
Klebsiella pneumoniae	0	0.0	6	9.0*
Staphylococcus aureus	0	0.0	2	3.0
Streptococcus pneumoniae	7	9.0	6	9.0
Staphylococcus epidermidis	11	14.1	14	20.9
Moraxella lacunata	29	37.2	26	38.8
Micrococcus	6	7.7	5	7.5
Veillonella	17	21.8	13	19.4
Lactobacillus	6	7.7	10	14.9
Peptostreptococcus	38	48.7	24	35.8
Bacteroides	21	26.9	21	31.3
Clostridium perfringens	6	7.7	4	6.0
Eubacterium	17	21.8	16	23.9
Peptococcus	6	7.7	5	7.5

*Compared with no TSP exposure, $P < 0.01$.

Table 7. The comparison of bacterial detection rates before or after TSP exposure (%).

TSP (**Table 7**). The detection rate of *Escherichia coli* on the oropharynx of rats was higher after low, medium and high concentration of TSP exposure, furthermore, the detection rate of peptostreptococcus was proportional to the concentration of TSP (**Table 9**). *E. coli* and peptostreptococcus are the normal flora of intestinal tract; therefore, the detection results show that the bacterial species on the oropharynx of Wistar rats changed after exposure to TSP, resulting in the colonization of pathogenic bacteria and intestinal bacteria.

Normal flora plays an important role for maintaining ecological balance and stable internal environment. So many microaerobic, aerobic and anaerobic bacteria colonize in the upper respiratory tract of healthy people including 21 genera and 200 species of bacteria. The normal flora of respiratory tract are affected by the normal flora on the oropharynx because of the anatomic relationship. Compared with the control group, the detection rates of aerobic and anaerobic bacteria had no significant difference after low, medium and high concentration of TSP exposure because of the acute toxicity experiment with short time but large dose of TSP exposure. The changes of microbiocenosis in respiratory tract affected by air pollution

are gradual, there will be more apparent changes after long-time exposure to pollutants eventually.

6.1.2. The influence of TSP to the density of bacteria in the respiratory tract of rats

The first step of bacterial colonization is adhesion. Similar to other biological cells, the bacterial proteins can be ionized into positively-charged amino ($-NH^+$) and negatively charged carboxyl ($-COO^-$) in solution and the degree of ionization is related to the pH of the environment. Negatively charged bacteria mainly exist in the alkaline solution and vice versa. The isoelectric point of gram-positive bacteria is pH 2–3 and the isoelectric point of gram-negative bacteria is pH 4–5; therefore, all the bacteria are negatively charged in neutral or weak alkaline medium with pH 7.2–7.6 and the gram-positive bacteria contain much more negative charges than gram-negative bacteria. For charge dependence, the bacterial adhesion increases with the pH of the environment. Moreover, the damage of host immune defense (such as malnutrition, basic disease, etc.) also can provide conditions for bacterial adhesion. However, there is mutual constraint relationship among bacterial flora such as space competition and nutrient competition.

The density of bacteria on the oropharynx of rats before or after TSP exposure was determined and the results are shown in **Tables 8** and **9** and **Figure 4**. There was no significant difference in the bacterial density of aerobe flora, however, the density of anaerobic bacteria flora was markedly reduced ($P < 0.05$) on the oropharynx of rats after TSP exposure compared with the results before TSP exposure (**Tables 10** and **11**). The density of bacteroides and eubacterium of anaerobic bacteria were markedly reduced ($P < 0.05$) on the oropharynx of rats after TSP exposure compared with the results before TSP exposure. There was no significant difference

Group	*n*	Aerobic bacteria	Anaerobic bacteria	Average bacterial density
No TSP exposure	78	2.4111 ± 0.5868	2.5983 ± 0.4850	2.5047 ± 0.5447
TSP exposure	67	2.3353 ± 0.6566	2.4039 ± 0.6178*	2.3696 ± 0.6361

*Compared with no TSP exposure, $P < 0.05$.

Table 8. The comparison of bacterial density before or after TSP exposure.

Time	*n*	Aerobic bacteria	Anaerobic bacteria**	Average bacterial density*
No TSP exposure	78	2.4111 ± 0.5868	2.5983 ± 0.4850*	2.5047 ± 0.5447**
1st day after TSP exposure	31	2.4223 ± 0.7594	2.5503 ± 0.6115*	2.4863 ± 0.6868*
8th day after TSP exposure	36	2.2605 ± 0.5534	2.2778 ± 0.6033	2.2691 ± 0.5749

*Compared with 1st day, $P < 0.05$.
**Compared with 8th day, $P < 0.05$.

Table 9. The comparison of bacterial density in different time after TSP exposure.

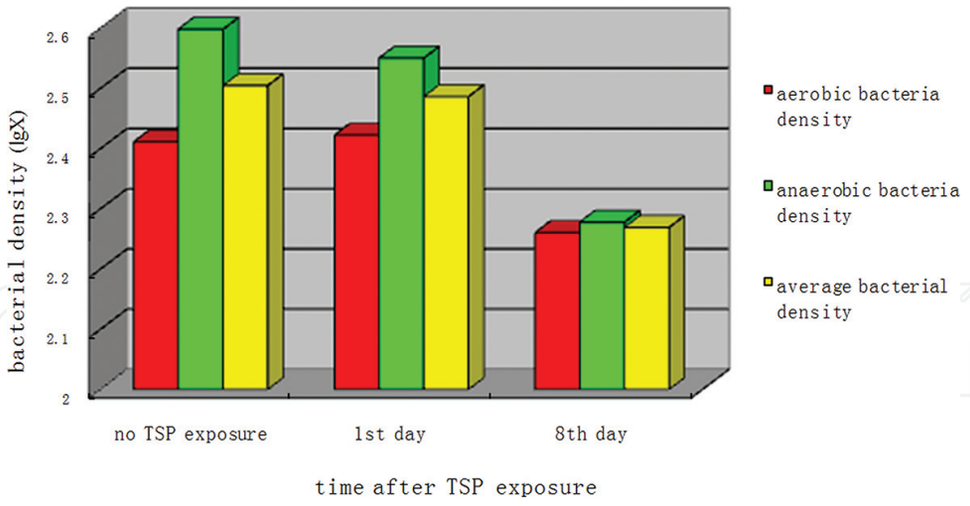

Figure 4. The bacterial density in different time after TSP exposure.

in the bacterial density of aerobe flora before TSP exposure and on the first day after TSP exposure, however, the density of aerobic bacteria was downturn on the 8th day after TSP exposure and the density of anaerobic bacteria flora was obvious decline with the passage of time ($P < 0.05$) on the oropharynx of rats after TSP exposure compared with the results before TSP exposure (**Tables 7** and **9**). The average density of bacteria were significantly decreased

Group	*n*	Aerobic bacteria	Anaerobic bacteria	Average bacterial density
Control	18	2.2531 ± 0.7952	2.2922 ± 0.6948	2.2531 ± 0.7837
Low concentration	16	2.4139 ± 0.4964	2.4936 ± 0.4958	2.4537 ± 0.4706
Middle concentration	18	2.3190 ± 0.7240	2.5295 ± 0.7411	2.4243 ± 0.7299
High concentration	15	2.3698 ± 0.5890	2.2915 ± 0.5143	2.3306 ± 0.5448

Table 10. The comparison of bacterial density in different TSP concentrations.

Aerobic bacterial species	No TSP exposure		TSP exposure
	N	$\overline{X}_{lgX}-S_{lgX}$	$\overline{X}_{lgX}-S_{lgX}$
Escherichia coli	8	1.1899 ± 0.8867	0.8677 ± 0.8393
Diphtheroid bacillus	2	2.6276 ± 0.2129	1.8891 ± 0.5826
Group A *Streptococcus*	15	2.2335 ± 0.3932	2.3379 ± 0.4941
Neisseria	2	2.2516 ± 0.6799	1.6901 ± 0.3012
Streptococcus pneumoniae	2	2.8037 ± 0.2129	1.6901 ± 0.5502
Moraxella lacunata	9	1.9036 ± 0.7001	2.0308 ± 0.2370

Table 11. The comparison of aerobic bacterial density before or after TSP exposure.

($P < 0.05$). There was no apparent change in the bacterial density of aerobe flora because of the short interval time of TSP exposure. The density of peptostreptococcus, bacteroides and eubacterium of anaerobic bacteria were markedly reduced ($P < 0.01$ or $P < 0.05$) on the oropharynx of rats over time after TSP exposure. The results showed that the flora on the oropharynx of rats changed and there were apparent changes in the normal flora especially the obvious reduction of anaerobic bacteria as the extension of the TSP exposure time. The normal flora in upper respiratory tract plays an important role in maintaining immune defense of respiratory tract. They can serve as antigens to stimulate the host to produce antibodies, at the same time, stimulate macrophages, enhanced the activity of interferon, release toxic terminal metabolites such as bacteriocin, bacteriocidin and fatty acid to constitute a biological barrier to prevent the invasion of invading bacteria. Studies have shown that anaerobic bacteria, especially the obligate anaerobic bacteria are the vast majority in number in the respiratory tract and they are indigenous microflora and physiological microbes. Obligate anaerobic bacteria have a strong biological antagonism effect, they integrate with mucosal epithelial cells closely forming a layer of biofilm to provide the space-occupying protective effects on the host cells. If the barrier is destroyed, the obligate anaerobic bacteria cannot survive because of the destruction of the anaerobic environment, foreign bacteria will invade. In summary, the normal flora on the oropharynx can be damaged by air pollution, thus it is easy for pathogenic bacteria to colonize.

6.1.3. The influence of TSP concentration to the flora of respiratory tract of rats

Normal flora is influenced by various aspects especially the physiological functions and the pathological features of the host, so that, different groups of rats are used to explore the relationship between the density of aerobic and anaerobic bacterial flora and the concentration exposed to TSP and the results are shown in **Table 10** and **Figure 5**.

Compared with control group, there was no significant difference in the bacterial quantity (**Table 16**), however, the quantity of aerobic bacteria fluctuated up and down with different dose of TSP exposure, the quantity of anaerobic bacteria was increased related to the dose of

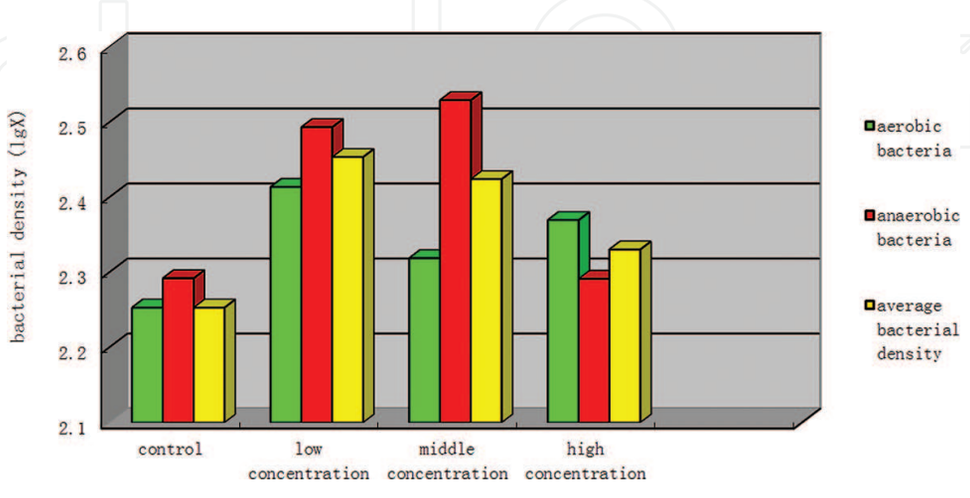

Figure 5. The bacterial density in different TSP concentration.

TSP exposure, but decreased in high concentration group ($P > 0.05$). The average density of bacteria had no significant difference too. In the aerobic bacteria, the density of *E. coli* increased obviously after TSP exposure, the density of Neisseria of high concentration group was obviously higher than that of control group and low concentration group ($P < 0.05$) on the oropharynx of rats. In the anaerobic bacteria, the density of Veillonella in the high concentration group was significantly decreased ($P < 0.05$) on the oropharynx of rats.

The colonization of bacteria is influenced by the host factors and the normal microbiota. Tension of the mucous membrane of the host, cilia movement and various discharge are important factors affecting bacteria to colonize. Normal microbiota members mainly come from anaerobic bacteria, especially the obligate anaerobe. Normal flora is an important factor in protecting human health. The results confirmed that air pollution can cause changes in the microecological condition of respiratory tract. Anaerobic bacteria on the oropharynx are the important biological barrier to resist external environmental change. Therefore, when the concentration of air pollutants is not very high, the quantity of anaerobic bacteria increases to maintain the ecological balance and prevent invading bacteria from colonizing. However, when the concentration of air pollutants is very high, the quantity of anaerobic bacteria decreases to result to the microecological imbalance in respiratory tract. Thus, it is easy for pathogenic bacteria and intestinal bacteria to colonize.

6.1.4. The changes of flora in the upper respiratory tract of rats before or after TSP exposure

The results of aerobic bacteria and anaerobic bacteria on the oropharynx of rats before or after TSP exposure are shown in **Tables 11** and **12**.

Compared with no TSP exposure, there was no significant difference in the bacterial density of aerobe flora after TSP exposure (**Table 11**). In the aerobic bacteria, the detection rate of group A Streptococcus increased after TSP exposure ($P > 0.05$), which is different from the results of our research on crowd. Maybe the resident flora of rats and humans are different and the results only come from the acute toxicity experiment with short time but large dose of TSP exposure. In the anaerobic bacteria, the density of bacteroides and eubacterium were obviously reduced ($P < 0.05$) on the oropharynx of rats after TSP exposure (**Table 12**).

	No TSP exposure		**TSP exposure**
Anaerobic bacterial species	***N***	$\overline{X}_{lgX}$–S_{lgX}	$\overline{X}_{lgX}$–S_{lgX}
Peptostreptococcus	14	2.2253 ± 0.5418	2.1307 ± 0.6799
Veillonella	5	1.6403 ± 0.3261	1.4897 ± 0.2718
Bacteroides	8	2.3978 ± 0.4495	1.7658 ± 0.6459*
Eubacterium	5	2.6384 ± 0.4277	2.1574 ± 0.4548**

*Compared with no TSP exposure, $P < 0.05$.
**Compared with no TSP exposure $P < 0.01$.

Table 12. The comparison of anaerobic bacterial density before or after TSP exposure.

6.2. The influence of PM_{10} to the respiratory tract flora of rats

Particulate matter less than 10 μm in diameter (PM_{10}) was collected using low flow of PM10 air sampling instrument and glass fiber filter film with 55 mm diameter from areas of coal burning in winter heating season, traffic and life pollution area in Shenyang city (China). Wistar rats were used as a model to analyze the changes of flora on the oropharynx before or after exposure to PM_{10}. The results are shown in **Tables 13–15**.

Bacterial species	No PM10 exposure		PM10 exposure %		$\chi 2$	*p*
	n	%	*n*	%		
Bacillus subtilis	8	17.0	3	6.4	1.647	0.198
Staphylococcus aureus	6	12.8	16	34.0*	4.807	0.027
Staphylococcus epidermidis	22	46.8	20	42.6	0.043	0.836
Acinetobacter baumannii	8	17.0	9	19.1	0.000	1.000
Neisseria	6	12.8	2	4.2	1.230	0.267
Group A *Streptococcus*	18	38.3	15	31.9	0.187	0.666
Micrococcus luteus	9	19.1	2	4.2*	3.706	0.050
Staphylococcus saccharolylicus	11	23.4	3	6.4*	4.113	0.040
Peptostreptococcus	13	27.6	12	25.5	0.000	1.000
Eubacterium aerofaciens	4	8.5	5	10.6	0.000	1.000
Veillonella parvula	20	42.6	9	19.1*	4.987	0.025
Clostridium harmless spore	3	6.4	6	12.8	0.492	0.486
Actinomyces israelii	2	4.2	4	8.5	0.178	0.677
Clostridium difficile	1	2.1	5	10.6	—	—.
Streptococcus anginosus	0	0.0	3	6.4	—	—
Clostridium death	0	0.0	4	8.5	—	—
Fusobacterium nucleatum	0	0.0	6	12.8	—	—

*Compared with no PM10 exposure, $P < 0.05$.

Table 13. The comparison of bacterial detection rates before or after PM_{10} exposure ($n = 47$).

Group	Aerobic bacteria ($n = 44$)	Anaerobic bacteria ($n = 47$)	Total bacteria ($n = 47$)
No TSP exposure	331.2 ± 3.5	177.3 ± 2.1	601.9 ± 1.9
TSP exposure	452.3 ± 2.9	251.8 ± 2.2*	796.5 ± 1.8
t(paired)	−1.769	−2.407	−2.620
P	0.084	0.020	0.012

*Compared with no PM10 exposure, $P < 0.05$.

Table 14. The comparison of the bacterial density before or after PM_{10} exposure (CFU/ml, G ± S).

Group	*n*	Aerobic bacteria	Anaerobic bacteria	Total bacteria
Control	12	241.3 ± 8.1	177.0 ± 2.9	630.2 ± 2.6
1st day	12	854.9 ± 1.3*	288.9 ± 1.9	1011.8 ± 1.5
7th day	11	164.6 ± 4.0	334.0 ± 2.3	627.3 ± 1.8
30th day	12	581.7 ± 1.8*	222.1 ± 1.9	865.2 ± 1.5
F		3.700	1.414	1.622
P		0.019	0.252	0.198

*Compared with control group, $P < 0.05$.

Table 15. The comparison of bacterial density in different time after PM_{10} exposure (CFU/ml, G ± S).

6.2.1. The influence of PM_{10} to the detection rate of respiratory tract flora of rats

Compared with no PM_{10} exposure, the detection rate of *S. aureus* increased significantly ($P < 0.05$), the detection rate of *Micrococcus luteus, Staphylococcus saccharolylicus* and *Veillonella parvula* decreased obviously ($P < 0.05$). It was interesting that three strains of *Streptococcus anginosus*, four strains of Clostridium death and six strains of *Fusobacterium nucleatum* were detected on 30th day after exposure to PM_{10} (**Table 14**).

6.2.2. The influence of PM_{10} to the density of respiratory tract flora of rats

After PM_{10} exposure, the total bacterial content and the density of anaerobic flora on the oropharynx of rats were significantly higher than that before PM_{10} exposure ($P < 0.05$) (**Table 14**, **Figure 6**). Compared with no PM_{10} exposure, the detection rate of *V. parvula*

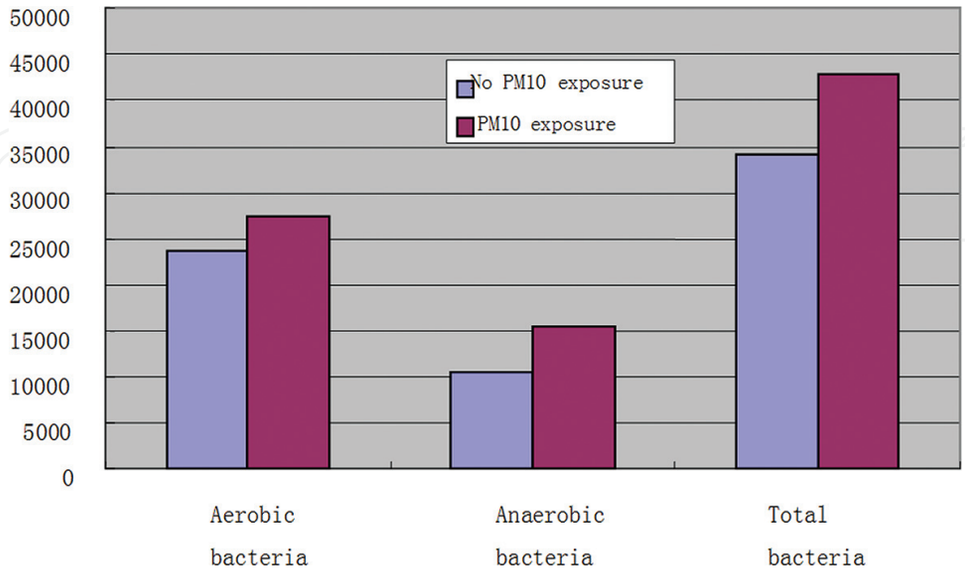

Figure 6. The comparison of the bacterial number before or after PM_{10} exposure.

decreased obviously, however, the density of it increased significantly after PM_{10} exposure ($P < 0.05$). Moreover, the density of *Actinomyces israelii* increased significantly after PM_{10} exposure ($P < 0.05$).

6.2.3. The changes of flora density in the upper respiratory tract of rats before or after PM_{10} exposure

Compared with no PM_{10} exposure, the density of peptostreptococcus and *V. parvula* increased significantly in the same rats ($P < 0.05$).

6.2.4. The changes of flora density in the upper respiratory tract of rats at different time after PM_{10} exposure

The density of aerobic flora on the oropharynx of rats on the first day after PM_{10} exposure was significantly higher than that in control group ($P < 0.05$), while it decreased on 7th day but increased obviously on 30th day after PM_{10} exposure($P < 0.05$). The total bacterial content and the density of anaerobic flora on the oropharynx of rats were increased volatility after PM_{10} exposure, but no significant difference (**Table 15**, **Figure 7**).

The results of present study showed that if the microecological balance was affected by some abnormal interference and destruction, there would be quantitative, qualitative or positioning changes in microbial population; therefore, the function of microbial biological barrier was weakened, so that some physiologic microbes became pathological bacteria. The results of this study showed that the detection rates of *S. aureus, Clostridium difficile, S. anginosus, Clostridium death* and *F. nucleatum* were much higher after PM_{10} exposure than that before PM10 exposure. The density of aerobic and anaerobic flora on the oropharynx of rats were significantly higher, furthermore, the density of *V. parvula* and *A. israelii* increased significantly than that before PM_{10} exposure ($P < 0.05$) (**Table 14**).

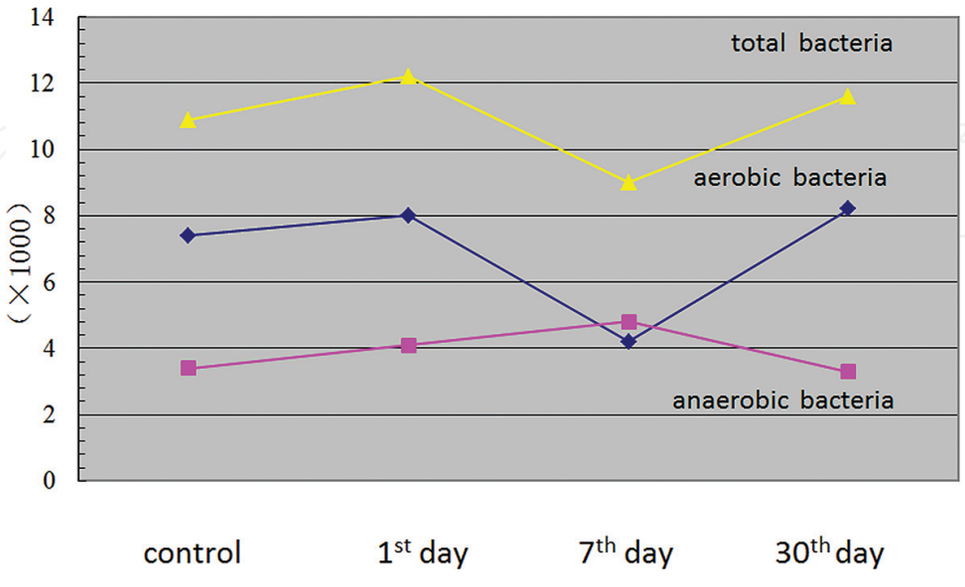

Figure 7. The changes of bacterial number in different time after PM_{10} exposure.

The results of Wistar rat animal model of air pollution showed that after PM_{10} exposure, the density of anaerobic flora was significantly higher than that before PM_{10} exposure in the same rats ($P < 0.05$) and the total bacterial content had increased significantly ($P < 0.05$). In the anaerobic flora, the density of peptostreptococcus and *V. parvula* increased significantly ($P < 0.05$). All of these suggest that the air pollutants can lead to the increase of the bacterial intensity including both normal flora and pathogenic bacteria, therefore resulting in microecological imbalance.

The results of **Figure 8** showed that the density of aerobic flora on the oropharynx of rats on the first day after PM_{10} exposure was significantly higher than that in control group, while it decreased on 7th day but increased obviously on 30th day after PM10 exposure ($P < 0.05$). Which suggested that large amount of bacteria bred and resulted to microecological imbalance after 1 day PM_{10} exposure. With the extension of time to PM_{10} exposure, there was a compensatory state after 7 days PM_{10} exposure, however, the microecological balance was broken again after 30 days PM_{10} exposure and led to a significant increase in bacterial number and density ($P < 0.05$).

C. difficile is the normal flora in human intestine, which can lead to false membranous enteritis. It can be detected on the oropharynx of rats on 30th day after PM_{10} exposure, which suggests that ectopic metastases occur in the flora at that time. In recent years, the infection of the lungs and pleura caused by anaerobic bacteria especially peptostreptococcus and *F. nucleatum* is increasing and the proportion is as high as 50–80%. The present results showed that the density of anaerobic flora especially *F. nucleatum, V. parvula* and peptostreptococcus was significantly higher than that before PM_{10} exposure which provide further proof that the exposure to the air pollutants for long time can lead to respiratory infections.

Infection is a process in which microbe, host and environment interact with each other. Under the conditions of microecological balance, normal flora is harmless and beneficial to the host, however, the quantitative, qualitative, or positioning changes of normal flora will lead to infection under the condition of microecological imbalance. The results of our present experiments indicated that the density change of bacterial flora could act as a sensitive indicator of

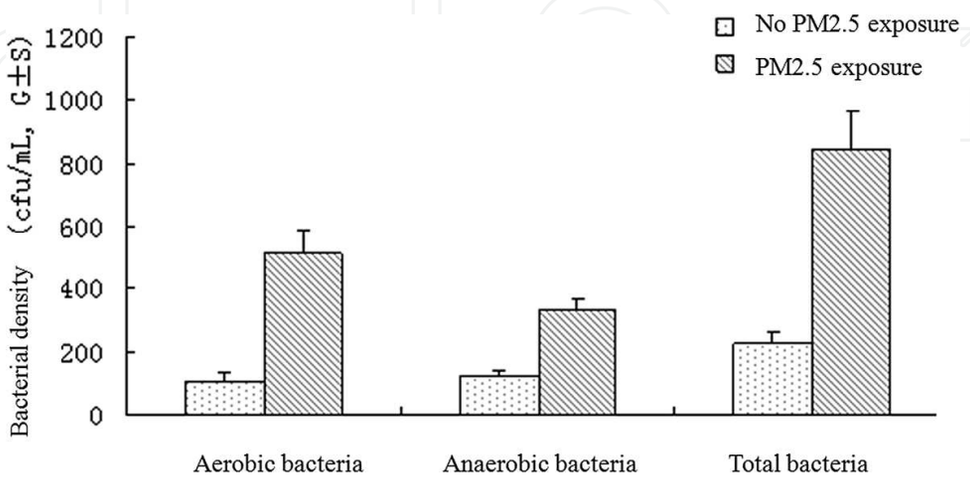

Figure 8. The comparison of bacterial density before or after $PM_{2.5}$ exposure ($^{\Delta}P < 0.01$).

air pollution effecting on human body. Air pollution could cause microecological imbalance in the respiratory tract of human body, furthermore, lead to the infection of respiratory tract and other respiratory diseases.

6.3. The influence of $PM_{2.5}$ to the respiratory tract flora of rats

Particulate matter less than 2.5 μm in diameter ($PM_{2.5}$) was collected using portable air sampling instrument and glass fiber filter film with 47 mm diameter from areas of coal burning in winter heating season and the traffic downtown area in Shenyang city (China). Wistar rats were used as model to analyze the changes of the respiratory tract flora on the oropharynx before or after exposure to $PM_{2.5}$. The results are shown in **Figures 8** and **9**.

6.3.1. The influence of $PM_{2.5}$ to the detection rate of respiratory tract flora on the oropharynx of rats

Compared with no $PM_{2.5}$ exposure, the detection rates of Cellulomonas/Mycobacterium, *Actinomyces naeslundii, Bacteroides ureolyticus* and *Gemella morbillorum* increased significantly ($P < 0.05$). The detection rates of *E. coli* and *Staphylococcus sciuri* increased significantly too ($P < 0.01$). The detection rates of *K. pneumoniae, Staphylococcus gallinarum* and *Staphylococcus lentus* increased but had no statistical significance ($P > 0.05$). *Streptococcus pneumoniae, S. anginosus, S. aureus, Mannheimia haemolytica, Plesimonas shigelloides* and *Pasteurella pneumotropica* were detected after $PM_{2.5}$ exposure which did not exist on the oropharynx of rats before $PM_{2.5}$ exposure (**Table 16**).

6.3.2. The influence of $PM_{2.5}$ to the density of respiratory tract flora on the oropharynx of rats

Compared with the results before $PM_{2.5}$ exposure, the density of aerobic and anaerobic bacteria as well as the total population of bacteria significantly increased ($P < 0.01$) (**Table 17**). In the aerobic bacteria, the density of *S. sciuri, Staphylococcus xylosus* and so on increased obviously. The density of some bacteria such as *S. anginosus, S. aureus* and *S. pneumoniae* which were

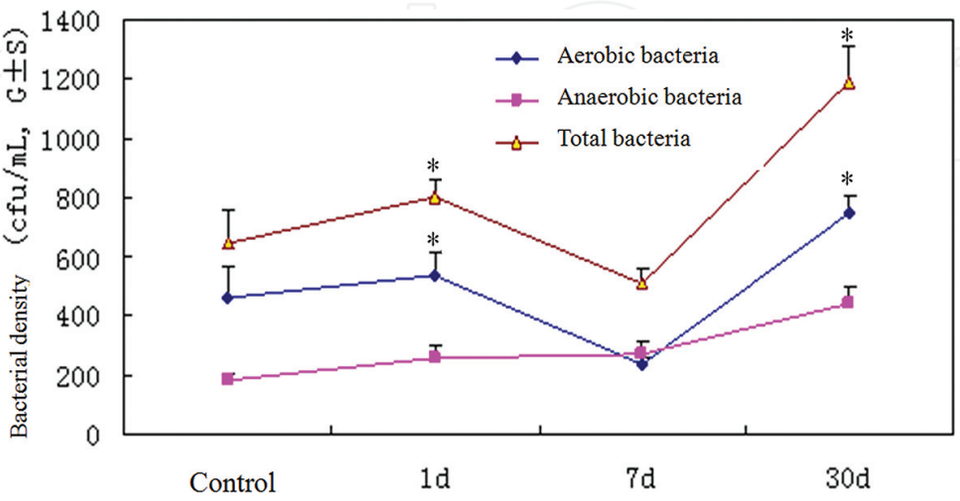

Figure 9. The changes of bacterial density in different time after $PM_{2.5}$ exposure(* P <0.05).

Bacterial species	No PM2.5 exposure		PM2.5 exposure		
	Number of rats	Detection rate	Number of rats	Detection rate	*p*
Escherichia coli	3	3.9	20	26.0	0.000$^{\Delta}$
Cellulomonas/Microbacterium	5	6.5	14	18.2	0.027*
Staphylococcus sciuri	2	2.6	17	1.3	0.000$^{\Delta}$
Klebsiella pneumoniae subsp pneumoniae	1	1.3	4	5.2	0.363
Streptococcus pneumoniae	0	0	1	1.3	0.156
Streptococcus anginosus	0	0	6	7.8	0.066
Staphylococcus aureus	0	0	1	1.3	0.156
Actinomyces naeslundii	2	2.6	10	12.9	0.016*
Bacteroides ureolyticus	5	6.5	17	22.1	0.027*
Gemella morbillorum	1	1.3	8	10.4	0.039*
Mannheimia haemolytica (Algae Pasteur bacteria)	0	0	3	3.9	0.061
Plesimonas shigelloides	0	0	1	1.3	0.156
Pasteurella pneumotropica	0	0	1	1.3	0.156
Staphylococcus gallinarum	3	3.9	9	11.68	0.071
Serratia marcescens	6	7.8	8	10.4	0.575
Enterobacter gergoviae	5	6.5	7	9.1	0.548
Staphylococcus lentus	5	6.5	11	14.3	0.113
Aerococcus viridans	3	3.9	6	7.8	0.303

*Compared with no $PM_{2.5}$ exposure, $P < 0.05$.
$^{\Delta}$Compared with no $PM_{2.5}$ exposure, $P < 0.01$.

Table 16. The comparison of bacterial detection rates before or after $PM_{2.5}$ exposure (%).

Group	Aerobic bacteria	Anaerobic bacteria	Total bacteria
No PM2.5 exposure	107.13 ± 27.69	122.54 ± 15.40	229.67 ± 430.83
PM2.5 exposure	512.71 ± 78.18$^{\Delta}$	330.95 ± 35.64$^{\Delta}$	843.65 ± 120.61$^{\Delta}$
P	0.000	0.008	0.000

$^{\Delta}$Compared with no PM2.5 exposure, $P < 0.01$.

Table 17. The comparison of bacterial density before or after $PM_{2.5}$ exposure (CFU/ml, G ± S).

detected only after $PM_{2.5}$ exposure also increased. In the anaerobic bacteria, the density of some bacteria such as *Clostridium tertium* and *G. morbillorum* which were detected only after $PM_{2.5}$ exposure increased too.

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6.3.3. The changes of flora density on the oropharynx of rats at different time after $PM_{2.5}$ exposure

Compared with the results before $PM_{2.5}$ exposure, the density of aerobic bacteria and the total population of bacteria increased significantly on 1st day ($P < 0.05$) but reduced significantly on 7th day while it increased obviously on 30th day ($P < 0.05$), however, the density of anaerobic bacteria had no significant difference in different time after $PM_{2.5}$ exposure (**Table 18**).

Group	*n*	Aerobic bacteria	Anaerobic bacteria	Total bacteria
Control	13	461.65 ± 101.23	182.58 ± 23.59	644.23 ± 116.37
1st day	12	539.25 ± 72.91*	260.08 ± 36.54	799.33 ± 63.54*
7th day	13	235.92 ± 24.91	274.81 ± 35.95	510.73 ± 44.15
30th day	14	746.96 ± 64.85*	443.82 ± 49.69	1190.79 ± 123.00*
P		0.038	0.145	0.029

*Compared with no $PM_{2.5}$ exposure, $P < 0.05$.

Table 18. The comparison of bacterial density in different time after $PM_{2.5}$ exposure (CFU/ml, G ± S).

On the basis of TSP and PM_{10}, the impact of $PM_{2.5}$ on respiratory tract micro ecology of rats was mainly focused on. In the research, the density of bacteria and the change of bacterial population were analyzed and the results showed that the detection rate of bacteria and bacterial amount especially aerobic bacteria increased significantly after $PM_{2.5}$ exposure. The density of aerobic bacteria on the oropharynx of rats fluctuated over time on compensated and decompensated states, which increased significantly on 1st day but reduced significantly on 7th day while it increased obviously on 30th day after $PM_{2.5}$ exposure. The total bacterial amount also showed the same trend, the density of most aerobic and anaerobic bacteria was higher than that before $PM_{2.5}$ exposure and some genera were detected after $PM_{2.5}$ exposure. All the results showed that $PM_{2.5}$ could change the microecology on the oropharynx of rats to microecological imbalance. Compared to previous experimental results of PM_{10}, the pathogenic bacteria of group B *Streptococcus* and *Staphylococcus intermedius* were not detected, only 1 case of *S. aureus* was detected after $PM_{2.5}$ exposure. The density of bacteria was higher than no $PM_{2.5}$ exposure, but the bacterial species were less than the previous PM_{10} experimental results, maybe this phenomenon was caused by the less sample size. In addition, some scholars thought that fine particulate matters were easy to deposit in the region of the bronchi and alveoli and possibly entered into blood circulation to damage the microecology of respiratory tract. Present studies have shown that the atmospheric pollutant particles with diameter less than PM_{10} can enter into the respiratory tract directly and keep in the deep lungs which are not easy to be discharged out of the body due to their small diameter without nasal block. $PM_{2.5}$ is also known as inhaled lung particulate matter and it has important effect on atmospheric visibility and air quality, which has attracted worldwide attention. Compared with the coarse atmospheric particulates, the particle size of $PM_{2.5}$ is small and there are a lot of poisonous and harmful substances in rich, moreover, $PM_{2.5}$ can stay for a long time in the

atmosphere and has a long conveying distance, so that it has more influence on the quality of atmospheric environment and human health.

7. Conclusions

Air pollution caused destruction of microecological balance in upper respiratory tract. The amounts of oropharyngeal aerobic and anaerobic bacteria were higher in children from heavily polluted area than those lightly polluted area. The detection rate of conditional pathogenic bacteria in children from heavily polluted area is higher than that in the control area. After 2 years of dynamic analysis, the detection rate of alpha streptococcus in children from heavily polluted area was lower than that in the control area and the difference was significant. Reduction of alpha streptococcus is bound to decrease the protective effect of the pharynx barrier, but increase susceptibility to infection.

Animal model of atmospheric pollutants was used to analyze the flora on the oropharynx of Wistar rats. The results showed that *K. pneumoniae* and *S. aureus* were not detected before air pollutant exposure, however, they were detected after air pollutant exposure and the detection rate of *K. pneumoniae* increased significantly. Thus Streptococcus pneumoniae, *S. aureus* and *K. pneumoniae* were thought as the common pathogens of bacterial pneumonia. The microecological environment on the oropharynx of rats changed after air pollutants exposure, it was easy for pathogens to colonize and cause respiratory infections.

After air pollutants exposure, the density of aerobic bacteria on the oropharynx of rats showed no significant change, however, the quantity of anaerobic bacteria decreased significantly and had significant decrease in trend over time. The average density of bacteria reduced with significant difference over time. The density of bacteroides and eubacterium of anaerobic bacteria were significantly reduced on the oropharynx of rats after air pollutants exposure compared with the results before air pollutants exposure. These results showed that air pollutants led to a decrease in respiratory total biomass and anaerobic bacteria and therefore declined the resistance of anaerobic bacteria, causing dysbacteriosis and colonization of pathogenic bacteria, which strongly proved that the anaerobic bacteria in normal flora played an important role in terms of colonization resistance.

The quantity of aerobic and anaerobic bacteria on the oropharynx of rats had no significant change with air pollutants but fluctuated with the concentration of air pollutants. The quantity of anaerobic bacteria on the oropharynx of rats had a downward trend in a high dose group (**Figure 8**). The density of Neisseria in aerobic bacteria increased significantly in high concentration group, however, the density of Veillonella in anaerobic bacteria reduced significantly. These results showed that the pathogenic and potential pathogenic bacteria were easier to colonize with the increasing concentration of air pollutants, but the quantity of total bacteria had no obvious change, which confirmed that the bacteria on the oropharynx of rats fluctuated within a certain range under certain conditions.

Air pollution could cause ecological imbalance and damage to the micro communities on the respiratory tract which led to the content of normal flora especially anaerobic bacteria decrease; therefore, the pathogenic bacteria colonized easily and caused respiratory diseases.

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