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Original article

## IMPACT OF TECHNOGENIC AIR POLLUTION FACTORS ON THE IMMUNE SYSTEM IN RESPIRATORY DISEASES

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**Background.** Suspended solid particles (SSPs) of atmospheric air contribute to the development and progression of respiratory diseases. One of the adaptive mechanisms, allowing for adjustment under the impact of pathogenic factors of the environment is the immune system.

**Purpose.** The objective of the present research is to reveal triggering micro-toxicants of the surface layer of the atmospheric air in Vladivostok city on the immune system of population with bronchopulmonary pathology.

**Materials and methods.** Overall 360 patients (105 healthy humans, 130 patients with bronchial asthma (BA), and 125 patients with chronic obstructive pulmonary disease (COPD) were included in the study. Percentage content of SSP fractions and absorbed heavy metals (Pb, Cr, Mn, Fe, Co, Ni, Cu, Zn) were assessed in the surface layer of atmospheric air. Integral immune system response indices (Dp%, Din%,  $\sum Np$ ) were determined using the Multiple Correlation module.

**Results.** The maximum integral response (Din%=1.74) was observed in the group of patients with COPD at minimal total immune responses ( $\sum Np=28$ ), in the group with AD, Din% was 1.54 ( $\sum Np=31$ ). The lowest immune response was revealed in healthy individuals (Din%=1.22) at the maximum total activity of immune responses ( $\sum Np=32$ ).

**Conclusion.** The microtoxicants of the surface layer of the atmospheric air in Vladivostok city have a negative effect on the immune system of all the studied population cohorts. A more pronounced immune response was observed in individuals with bronchopulmonary pathology. The analysis highlighted the trigger factors (fractions of 0-1 and 50-100 microns, as well as presence of Fe, Mn, Cr, Cu, Co) of the atmospheric air of Vladivostok city, as well as the ranges of their impacts on the immune system of healthy population and individuals with bronchopulmonary pathology, which allows predicting the severity of environmental bronchopulmonary pathology and initiate preventive measures.

**Keywords:** *suspended solid particles; heavy metals of atmospheric air; bronchopulmonary pathology; immune system*

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Научная статья

## ВОЗДЕЙСТВИЕ ФАКТОРОВ ТЕХНОГЕННОГО ЗАГРЯЗНЕНИЯ ВОЗДУШНОЙ СРЕДЫ НА ИММУННУЮ СИСТЕМУ ПРИ ЗАБОЛЕВАНИЯХ ОРГАНОВ ДЫХАНИЯ

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**Обоснование.** *Твердые взвешенные частицы (ТВЧ) атмосферного воздуха способствуют развитию и прогрессированию заболеваний органов дыхания. Одним из приспособительных механизмов, осуществляющих адаптацию при воздействии патогенных факторов окружающей среды, является иммунная система.*

**Цель.** *Выявление триггерных микротоксикантов приземного слоя атмосферного воздуха г. Владивостока на иммунную систему населения при бронхолегочной патологии.*

**Материалы и методы.** *В исследование включено 360 человек (105 практически здоровые, 130 с бронхиальной астмой (БА), 125 с хронической обструктивной болезнью легких (ХОБЛ)). В приземном слое атмосферного воздуха оценивали процентное содержание фракций ТВЧ и содержание абсорбированных тяжелых металлов (Pb, Cr, Mn, Fe, Co, Ni, Cu, Zn). Интегральные показатели отклика иммунной системы ( $Dn\%$ ,  $Din\%$ ,  $\sum Nr$ ) определяли с использованием модуля «Множественная корреляция».*

**Результаты.** *Максимальный интегральный отклик ( $Din\%=1,74$ ) отмечается в группе лиц с ХОБЛ при минимальной суммарной активности иммунных реакций ( $\sum Nr=28$ ), в группе с БА  $Din\%$  составляет 1,54 ( $\sum Nr=31$ ). Наименьший иммунный ответ выявлен у здоровых лиц ( $Din\%=1,22$ ) при максимальной суммарной активности иммунных реакций ( $\sum Nr=32$ ).*

**Заключение.** *Микротоксиканты приземного слоя атмосферного воздуха г. Владивостока негативно влияют на состояние иммунной системы всех*

*исследованных когорт населения. У лиц с бронхолегочной патологией наблюдается более выраженный иммунный ответ. В результате анализа выделены триггерные факторы (фракции 0-1 мкм, 50-100 мкм, Fe, Mn, Cr, Cu, Co) атмосферного воздуха г. Владивостока и диапазоны их воздействия на иммунную систему здорового населения и лиц с бронхолегочной патологией, что позволяет прогнозировать утяжеление экологозависимой бронхолегочной патологии и задействовать профилактические мероприятия.*

**Ключевые слова:** твердые взвешенные частицы; тяжёлые металлы атмосферного воздуха; бронхолегочная патология; иммунная система

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## Introduction

According to the World Health Organization (WHO), 99% of the world's population breathes air that exceeds the air pollution limits, recommended by WHO [23]. Air pollution is an important cause of non-communicable diseases worldwide, with suspended solid particles (SSPs) being one of the main air pollutants. SSPs represent a complex mixture of organic and inorganic components [6, 9, 12, 16]. The effect of SSPs depends on their nature, chemical composition, as well as particle size and physical characteristics [7]. Technogenic contamination of the air environment with SSP fractions and heavy metals, absorbed on them, poses a significant health risk [16, 19]. It is known that SSPs contribute to the development and progression of cardiovascular, central nervous, and other human systems diseases; however, airborne microtoxics have the greatest pathogenic effect in respiratory diseases [5, 7, 13, 16]. Contact with the surface layer of atmospheric air is the most important in respiratory diseases; however, studies in this segment are extremely limited.

Impact of SSPs of atmospheric air activates the formation of reactive oxygen species (ROS), which leads to the development of oxidative stress and, subsequently, inflammation [7, 13]. Intensification of inflammatory and oxidative processes also underlies chronic bronchopulmonary diseases. Inflammation further enhances the production of ROS, causing changes in transcriptional factors that mediate the pathways of cellular response to stress [7, 25]. The immune system is one of the body's adaptive mechanisms provides its adjustment to the impact of pathogenic factors, both external and internal. However, its sensitivity to

anthropogenic air pollution components is different [3, 8]. Therefore, isolating trigger factors and determining exposure ranges of airborne technogenic pollutants on immune system will allow providing timely therapeutic and preventive measures to reduce the risk of bronchopulmonary diseases exacerbations.

### **Purpose**

The objective of the present research is to identify trigger microtoxics of the surface layer of atmospheric air in Vladivostok city and their impact on the immune system of the patients with bronchopulmonary pathology.

### **Materials and methods**

The study objects were the fractional composition of suspended solid particles and heavy metals, contained in the surface layer of Vladivostok urban air, as well as the immune system of healthy urban residents and individuals with bronchopulmonary pathology (bronchial asthma, chronic obstructive pulmonary disease, COPD).

Atmospheric pollution was assessed by aerosol suspensions of solid particles, collected in the surface layer of atmospheric air, using PU-4E electric aspirator (CJSC KHIMKO, Russia) into a liquid absorbent medium using a high-speed Richter's absorber. The study was conducted in residential areas of the city, where the polluting objects were located (high-traffic freeways, energy and industrial facilities located within 200-800 m by the prevailing wind rose).

Suspended solids were assessed by the percentage of fractions (within the ranges of 0-1, 1-10, 10-50, 50-100, 100-400, 400-700, and >700  $\mu\text{m}$ ) relative to the total weight of suspended solids in the sample. The content of absorbed heavy metals in air (Pb, Cr, Mn, Fe, Co, Ni, Cu, Zn) was measured in  $\mu\text{g}/\text{l}$ .

The study included 360 people living in the areas, where air samples were taken, including 105 practically healthy individuals, 130 people with controlled and partially controlled bronchial asthma (BA) of mild severity, 125 people with chronic obstructive pulmonary disease (COPD) of a stable course of mild severity. The mean age of the subjects was  $48.5 \pm 4.9$  years. BA and COPD were diagnosed according to the Global Strategy for Asthma Management and Prevention, Global Strategy for Prevention, Diagnosis and Management of COPD, and the International Classification of Diseases (the 10<sup>th</sup> revision). The study was conducted taking into account the requirements of the World Medical Association Declaration of Helsinki (2013) and approved by the local ethical committee. Voluntary informed consent was obtained from each patient for being

involved in the study. Individuals, residing for at least 5 years in the study area, within a radius of 1 km from the air sampling point, were included in the study. Exclusion criteria for subjects from the study were the presence of acute infectious diseases, chronic diseases of internal organs in the acute phase, chronic heart failure in decompensation, and contact with harmful and hazardous occupational factors.

Venous blood was used as biological material. Cellular immunity parameters ( $CD3^+$ ,  $CD4^+$ ,  $CD8^+$ ,  $CD4^+/CD8^+$ ,  $CD19^+$ ,  $CD16^+56^+$ ) were estimated by flow cytometry using BD FACS Canto II cytometer (USA). Serum cytokine levels (tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interferon  $\gamma$  (IFN- $\gamma$ ), and interleukin 4 (IL-4), IL-6, IL-10, IL-17A) were assessed by Cytometric Bead Array test system (BD, USA). The level of total IgE in blood serum was assessed by enzyme immunoassay using Chema Medica kit. Phagocytic activity of neutrophils was determined using Phagotest kit (BD, USA). Oxygen-dependent bactericidal mechanisms of peripheral blood neutrophils were assessed using Burstest (Phagoburst) reagents (BD, USA).

The effect of suspended solid particle fractions on the immune system of the subjects was determined using the Multiple Correlation module of the STATISTICA 10 software. Inter-system matrices (environmental factors – immune status indicators) were formed, to which statistically significant ( $p < 0.05$ ) correlation relations ( $r$ ), characterizing the most significant impact factors on the immune system, were included. The immune response activity index ( $N_p$ ), represented by the number of statistically significant  $r$  ( $p < 0.05$ ) in the formed matrix was determined.

To determine the ranges of air pollution indicators ( $M \pm m$ ), the table of results of the Paired and Partial Correlations module was used.

To separate air pollution trigger fractions, a component-wise response index  $D_p\%$  ( $D_p\% = \sum \text{of absolute values of } r / \sum R \times 100$ ) was determined, which characterized the ratio of the sum of statistically significant connections to the total sum of hypothetically possible values in the rectangular connection matrix (at  $R=1.0$ ). The integral index  $D_m\%$  was calculated similarly to  $D_p\%$ , which allowed identifying the immune response to the combined impact of air pollution fractions in different groups of testees (BA, COPD, and control group).

## Results

The calculations, carried out in the patient groups with bronchial asthma, chronic obstructive pulmonary disease, and in the control group are presented in the Table below.

Table.

**Factors of technogenic impact of airborne microscale pollutants  
in Vladivostok city on the immune system of healthy individuals and patients  
with bronchopulmonary pathology**

BA			COPD			Control		
Ranges of air pollution indicators M±m (%)	N <sub>p</sub>	D <sub>n</sub> %	Ranges of air pollution indicators M±m (%)	N <sub>p</sub>	D <sub>n</sub> %	Ranges of air pollution indicators M±m (%)	N <sub>p</sub>	D <sub>n</sub> %
<b>0-1</b> 1.20-2.19	4	<b>0.17</b>	<b>0-1</b> 1.24-6.24	3	<b>0.17</b>			
<b>1-10</b> 12.25-16.95	1	0.05	<b>1-10</b> 4.33-20.98	2	0.14	<b>1-10</b> 7.97-21.13	2	0.11
<b>10-50</b> 36.20-40.32	2	0.1	<b>10-50</b> 32.63-67.16	2	0.13	<b>10-50</b> 15.8-72.47	2	0.07
<b>50-100</b> 1.58-4.02	5	<b>0.25</b>	<b>50-100</b> 3.02-4.67	3	<b>0.20</b>	<b>50-100</b> 1.79-5.12	3	<b>0.14</b>
			<b>100-400</b> 0.44-0.58	1	0.05	<b>100-400</b> 4.96-14.02	2	0.09
			<b>400-700</b> 0.43-0.64	1	0.06	<b>400-700</b> 0.48-26.22	5	0.10
<b>&gt;700</b> 0.45-0.52	1	0.04	<b>&gt;700</b> 7.79-8.96	1	0.07	<b>&gt;700</b> 0.56-9.66	2	0.08
<b>Fe</b> 16.95-26.47	3	<b>0.2</b>	<b>Fe</b> 11.98-13.53	1	0.06	<b>Fe</b> 12.71-33.58	2	0.09
<b>Ni</b> 0.3-0.45	3	0.16	<b>Ni</b> 0.26-0.52	1	0.05			
<b>Mn</b> 4.66-6.31	5	<b>0.24</b>	<b>Mn</b> 5.14-6.22	1	0.06	<b>Mn</b> 4.77-9.05	2	0.09
			<b>Cu</b> 0.51-3.13	4	<b>0.25</b>	<b>Cu</b> 1.09-4.01	4	0.16
<b>Cr</b> 0.42-0.51	5	<b>0.23</b>	<b>Cr</b> 0.45-1.03	2	0.12	<b>Cr</b> 0.62-0.74	1	0.04
			<b>Co</b> 0.02-0.1	3	<b>0.2</b>	<b>Co</b> 0.05-0.09	2	0.09
<b>Pb</b> 1.0-1.3	2	0.1	<b>Pb</b> 0.97-1.56	2	0.12	<b>Pb</b> 0.83-1.11	1	0.04
			<b>Zn</b> 1.3-14.64	1	0.06	<b>Zn</b> 23.84-45-85	4	0.12
Number of affecting factors 10	∑N <sub>p</sub> 31	D <sub>n</sub> % <b>1.54</b>	Number of affecting factors 15	∑N <sub>p</sub> 28	D <sub>n</sub> % <b>1.74</b>	Number of affecting factors 13	∑N <sub>p</sub> 32	D <sub>n</sub> % <b>1.22</b>

The conducted analysis allowed revealing the peculiarities of immune response in the studied groups. The maximum number of responses to the impact of various external factors was noted in the group of COPD patients (15 factors), while the minimum response was noted in individuals with BA (10 factors).

The activity of the immune response also differed between the study groups. Individuals with BA have shown the greatest response to nanofractions of 0-1  $\mu\text{m}$  (4 responses), 50-100  $\mu\text{m}$  (5 responses), as well as exposure to Mn (5 responses) and Cr (5 responses). In individuals with COPD, maximum immune activity was also noted for the fraction of 0-100  $\mu\text{m}$  (10 responses), as well as exposure to Cu (4 responses) and Co (3 responses). In addition, responses to large fractions >100  $\mu\text{m}$  and exposure to Zn were detected. In the control group, an increased response activity to larger fractions (400-700  $\mu\text{m}$ ), as well as exposure to Cu (4 responses) and Zn (4 responses) was revealed.

The component-wise indicator  $D_p\%$  was used to identify trigger factors. In a group of BA patients, the maximum  $D_p\%$  was observed when exposed to fractions of 50-100  $\mu\text{m}$  ( $D_p\%=0.25$ ), as well as to Fe ( $D_p\%=0.2$ ), Mn ( $D_p\%=0.24$ ) and Cr ( $D_p\%=0.23$ ). In a group of COPD patients, the highest  $D_p\%$  was revealed for 0-1 and 50-100  $\mu\text{m}$  fractions ( $D_p\%=0.17$  and  $D_p\%=0.20$ , respectively), as well as for Cu ( $D_p\%=0.25$ ) and Co ( $D_p\%=0.2$ ). In healthy individuals, the maximum component-wise indicator  $D_p\%=0.21$  was detected when exposed to fractions of 400-700  $\mu\text{m}$ .

Analysis of the integral indicator  $D_{in}\%$  allowed identifying contingent, the most vulnerable to the impacts of external factors. The maximum  $D_{in}\%$  was noted in the group of patients with COPD ( $D_{in}\%=1.74$ ) at the minimum total activity of immune responses ( $\sum N_p=28$ ). In the group of patients with BA,  $D_{in}\%$  was 1.54 ( $\sum N_p=31$ ). The lowest immune response was detected in healthy individuals ( $D_{in}\%=1.22$ ) at the maximum total activity of immune responses ( $\sum N_p=32$ ).

## Discussion

Determining the nature, intensity, and factors of the impact of technogenic air pollutants on the immune system was carried out based on the assessment of the combined response of the parameters to a component-wise effect of microtoxics of the surface layer of atmospheric air.

The conducted analysis has revealed that microtoxics of the surface layer of atmospheric air exert the greatest integral pathogenic impact on the individuals with COPD and AD, while the least one, on the individuals of the control group. The obtained data indicate that air pollution with SSPs is unfavorable for all studied population cohorts, including healthy population. Exposure to SSPs activates various cellular mechanisms, and pro-inflammatory intracellular signaling cascades [13]. Individuals with bronchopulmonary pathology are most susceptible to SSP exposure [15, 18]. Exposure to microtoxics can contribute to the development and progression of respiratory diseases [20, 21].

The analysis of the impact of microtoxics of the surface layer of atmospheric air on the immune system has shown their maximum pathogenic effect in the group of individuals with COPD ( $n=15$ ), which indicates the greatest vulnerability of the immune system at chronic obstructive pulmonary disease. This is due to the fact that a chronic systemic inflammatory response is formed in COPD [2]. Atmospheric air pollution plays a major role in the pathogenesis of both COPD and BA. Despite the fact that patients with BA actively respond to cumulative anthropogenic exposure ( $D_{in}\%=1.54$ ), the number of trigger factors is reduced ( $n=10$ ). In BA, stimulation and formation of the atopic component occurs, and the immune response has a selective nature to individual damaging environmental agents. Increased content of metals, associated with microsize fractions, capable of penetrating more deeply into the respiratory tract, creates prerequisites for forming metal allergies [11, 17]. The most adequate response of the immune system to the impact of external factors was noted in the control group, which indicates an increased adaptive capacity of a healthy body [13]. However, the results indicate that the technogenic pollution of Vladivostok city has a pathogenic effect on the healthy population of the city as well.

The activity of the immune response and the component-wise indicator of the response differ in the studied groups depending on the ranges of microtoxicant fractions. The fractions of 50-100  $\mu\text{m}$  have the greatest pathogenic impact on all groups. The fractions in the 0-1  $\mu\text{m}$  range also have an active impact on the immune system of patients with BA and COPD. However, according to the integral immune response level ( $D_{in}\%=1.22$ ), healthy individuals much easier tolerate exposure to atmospheric air microtoxics, indicating a higher level of compensatory-adaptation mechanisms. Literature analysis indicates a multi-directional mechanism of impact of microtoxics on the immune system for the studied nosological entities, which may be due to a different type of formed systemic inflammatory reaction. Exposure of SSPs to the patients with BA promotes the formation of IL-1, IL-8 and maturation of B-lymphocytes, as well as the production of atopic inflammatory mediators (IgE) [14]. Proinflammatory mediators, such as IL-6, IL-1 $\beta$ , TNF- $\alpha$  and IL-8 increase in patients with stable COPD. Impact of SSPs activates NF- $\kappa\text{B}$ , which regulates TNF- $\alpha$  and IL-1 $\beta$  transcription and, consequently, increases the inflammatory processes [22].

The response of immune system to heavy metals varies significantly: maximum activity in the group of patients with BA was observed when exposed to Fe ( $N_p=3$ ,  $D_p\%=0.2$ ), Mn ( $N_p=5$ ,  $D_p\%=0.24$ ), Cr ( $N_p=5$ ,  $D_p\%=0.23$ ); in patients with COPD, to Cu ( $N_p=4$ ,  $D_p\%=0.25$ ), Co ( $N_p=3$ ,  $D_p\%=0.2$ ); while component-wise response rate in healthy individuals did not exceed  $D_p\%=0.16$ . The interaction of metallic



elements with functional groups of macromolecules is an important mechanism of their toxicity. The result of exposure to heavy metals depends not only on the nature of the causative agent, physicochemical form, dose, exposure conditions, but also on the individual factors of the body [10]. Heavy metals can have a specific impact on immunity. It has been shown that exposure to some heavy metals leads to an increase in TNF- $\alpha$ , IL-6, IL-8, activates NF- $\kappa$ B, disrupts immune homeostasis and activates inflammation processes [10, 24]. In individuals with chronic respiratory pathology, air pollution by heavy metals cause a pronounced response, characterized by the intensification of inflammatory processes [1, 4].

### **Conclusions**

In the course of the conducted study, correlation relationships have been revealed, which allowed establishing the intensity of the response of the immune system to the impacts of different SSP fractions and absorbed heavy metals. It was revealed that microtoxics have a negative impact on all the studied population cohorts. Individuals with bronchopulmonary pathology have shown a more pronounced immune response. The analysis allowed identifying the trigger factors of the surface layer of atmospheric air in the city of Vladivostok (fractions of 0-1 and 50-100 microns, as well as occurrence of Fe, Mn, Cr, Cu, Co) and the ranges of their impacts on the immune system of healthy population and individuals with bronchopulmonary pathology, which allows predicting the formation and exacerbation of environmental-dependent bronchopulmonary pathology, and initiating a set of preventive measures.

**Conclusion of the Ethics Committee.** The study was conducted in accordance with the principles of the Declaration of Helsinki, and approved by the Institutional Review Board and approved by the Ethics Committee of the Vladivostok branch of the Far Eastern Scientific Center of Physiology and Pathology of Respiration – Research Institute of Medical Climatology and Restorative Treatment (protocol no. 9, 24.11.2021).

**Informed Consent.** Informed consent was obtained from all subjects who participated in the study. Written informed consent was obtained from patients for the publication of this article.

**Conflict of interest information.** The authors declare that there is no conflict of interest.

**Sponsorship information.** The study had no sponsorship.

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