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RESEARCH ON THE MICROBIOLOGICAL QUALITY OF AIR IN NAVAL FACILITIES

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ABSTRACT

Harmful biological factors accumulated in the ambient atmosphere are a very important and increasingly recognised problem of both occupational medicine and public health. The quantitative and qualitative assessment of harmful biological agents in the working environment is a very important element of the exposure assessment and therefore of an assessment of workers' health risks. In 2018, a pilot study on the microbiological quality of air was carried out at two facilities of the Polish Navy.
Keywords: work environment, air contamination, risk assessment.

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INTRODUCTION

With the development of civilisation, the state of cleanliness of atmospheric air, as well as the air in enclosed spaces where people live, is constantly drastically deteriorating. The atmospheric air contains numerous components that create pollution. One of the elements having a significant impact on the air quality are biological pollutants, forming bioareosol, which includes: fungal spores, pollen, bacteria and viruses. The assessment of air pollution by harmful biological agents is an important problem for human health [1,2,3].

The European Community issued Directive 2000/54/EC, which deals with the protection of workers from risks related to exposure to biological agents at work. The Directive, which sets out the employer's obligations with regard to the protection of workers from exposure to biological agents, includes a classification of biological agents that pose a risk in the workplace and describes safety measures and places where exposure to biological agents is particularly dangerous. Due to the possibility of an occurrence of harmful biological agents in indoor air, a qualitative and quantitative control of the level of microbiological air pollution is necessary [2]. In 2018, we conducted a pilot study on the microbiological quality of air in two facilities of the Polish Navy.

HAZARDS ARISING FROM THE PRESENCE OF BIOLOGICAL POLLUTANTS IN THE AIR

Biological harmful factors accumulated in the atmosphere around us are a very important and an increasingly recognised problem of both occupational medicine and public health. The quantitative and qualitative assessment of harmful biological agents in the working environment is a very important element of the exposure assessment and therefore of workers' health risks. Exposure to biological agents in the occupational and non-occupational environment is common and often leads to a wide range of adverse health effects, from simple irritations and ailments to allergic reactions and the occurrence of infections, infectious diseases and toxic reactions. The most common hazard in the working environment is posed by biological harmful agents as components of bioaerosols, which are airborne or air-drop transmitted and penetrate the body through the respiratory system [4,5].

Most health problems related to air quality in the workplace are associated with exposure to mould, which accounts for about 70% of the total indoor air microflora. Moulds are a common cause of allergies in humans, leading to allergic rhinitis, conjunctivitis and asthma, as well as gastrointestinal infections. Many of the fungi we find in the air from the genera *Aspergillus*, *Penicilinum*, *Fusarium* or *Stachybotris* have toxigenic properties. There are many indications that mycotoxins and volatile metabolites of fungi may be the cause of the disease referred to as "chronic fatigue syndrome". [6,7,8] Bacteria generally make up 19-26% of the microflora in buildings. Most bacteria do not pose a health risk at low concentrations of these microorganisms in the air, but some have pathogenic, allergenic or toxic properties even in small quantities. Ventilation and air-conditioning systems are conducive to the growth of *Legionella* bacteria, which can cause legionellosis or Pontiac fever.

Biological particles suspended in the air may be a direct cause of allergies, asthma, and many other diseases [9]. Diseases, whose aetiological factors are transmitted through the air, include:

- viral: measles, chickenpox, influenza, mononucleosis, rubella, mumps (parotitis), herpes, meningitis,
- bacterial: bronchitis and pneumonia, rhinitis; lung tuberculosis, diphtheria, pertussis, scarlet fever, actinomycosis,
- fungal: aspergillosis of the lungs, lung mucorrhiza, lung cryptococcosis, bronchial mycosis, lung geotrichosis, fungal pneumonia, pleural mycosis and other [6,10].

Attention should be paid to the fact that it's not only the presence of pathogenic micro-organisms or toxins of microbiological origin in the air that are a threat, but also an excessive amount of saprophytic micro-organisms, especially if their composition is non-diverse and they are dominated by organisms of a single species [11,12,13].

SOURCES OF BIOAEROSOL

Microorganisms in the air most often occur in the form of bioareosols, i.e. suspended in the air on tiny particles of liquids, or dusts of plant, animal or mineral origin, which may contain spores and conidia of fungi, as well as bacteria and their spores [3]. Among harmful biological factors present in the air we can distinguish:

- Microorganisms causing infectious and invasive diseases - viruses, bacteria, fungi. Published results indicate the presence of fungi in air samples of the following genera: *Aspergillus*, *Penicillium*, *Cladosporium* and yeasts of the genus *Candida*. The bacterial microflora is poorer with prevalence of Gram-positive bacteria (*Micrococcus spp., Staphylococcus spp., Bacillus spp*.) [1,5,6].
- Biological allergens - microscopic plant and animal particles. Among the components of bioaerosol it is possible to distinguish pollen, plant remains, animal dandruff, particles originating from peeling of the epidermis in humans and animals.
- Biological toxins - the presence of certain species of bacteria and fungi is also associated with the presence in the air of immunotoxic substances produced by these microorganisms, which include endotoxins, mycotoxins, glucans, volatile organic compounds or peptidoglycan. Endotoxins and (1→3)-β-D-glucans are the products of cell wall degradation of Gram-negative bacteria and fungi respectively [1,6].
- Biological vectors, i.e. arthropods carrying diseases such as mosquitoes, ticks, flies.

Internal air pollution can derive from both external and internal sources. External sources of microbiological air pollution can be divided into natural (soil, water, decomposition of organic matter, plant phyllosphere) and anthropogenic (landfills, sewage treatment plants, composting plants, livestock farms, agricultural holdings, car traffic and derivatives) [3].

Internal pollution can be caused by people, dusts of organic origin, materials accumulated in buildings and

air penetrating through ventilation and air conditioning systems. One of the main sources of bioaerosols in rooms is people - sweat drops, saliva. Man is the main source of bacteria, because of the natural flora of human skin. The production of biological aerosol can occur by sneezing, coughing, as well as physical effort. Toilets are also a source of bioaerosols, owing to the high humidity, which contributes to the growth of mould fungi. Washbasins, showers and drains are a source of gram-negative bacilli.

Microbiological contamination may be present in construction and finishing materials. Fibrous materials, insulating materials, gypsum boards can be an important source of living airborne microorganisms that can accumulate on filters and filtering equipment. As long as there is sufficient moisture, fungi can grow in almost all materials. The high cellulose content in some materials (e.g. ceiling panels) makes them an ideal environment for their growth. An important factor influencing indoor air quality is heating, ventilation and air conditioning, which is mainly provided in newly built buildings [4,5,9].

LEGAL REGULATIONS

The control of microbiological purity of air in Polish and world legislation is insufficiently regulated at present. Unlike most chemical and physical agents, there are no commonly accepted criteria for assessing exposure to biological agents, as well as generally accepted normative values and methodological recommendations. This is primarily due to the fact that:

- still no satisfactory epidemiological data are available to establish the relationship between exposure to the agent and the health effect of the agent,
- the susceptibility of each organism exposed to a given biological harmful agent is an individual characteristic of that organism, which makes it difficult to clearly determine the effects of such an effect,
- source (measurement) data on the most common bioaerosols in the environment are still insufficient,
- there is a lack of standardisation of measuring methods (e.g. lack of standard samplers) and experimental methods [2].

At present, there are no legal acts in force in Poland which define the permissible content of microorganisms in the air, both atmospheric and indoor. The previously binding standards [PN-89/Z-04008/01; PN-89/Z-04008/08; PN-89/Z-04111/02; PN-89/Z-04111/03] concerning atmospheric air pollution were repealed in 2015, but have not been replaced by new ones; therefore, in many studies on the assessment of microbiological purity of the air, the information on limit values of microbiological contamination contained therein is still used to interpret the obtained results.

The proposals of the team of experts on biological agents of the interministerial committee on Threshold Limit Values (TLV) and Permissible Exposure Limits (PEL) also deserve special attention. They present proposals of acceptable concentration values for groups of microorganisms commonly occurring in the air of working, residential and public spaces, developed on the basis of the results of environmental measurements, taking into account the potential harmfulness of particular biological factors. These proposals may form the basis for the development of generally accepted standards for harmful biological agents in indoor air and, in the meantime, may be considered as optional standards or reference values for the interpretation of microbiological air test results [2,3,5].

Provisions contained in Directive 2000/54/EC of the European Parliament and of the Council of the European Union "on the protection of workers from risks related to exposure to biological agents at work" was implemented in Polish law by a relevant provision in the Labour Code. This resulted in the adoption by the Polish Committee for Standardisation of European acts PN-EN 13098 "Air at workplaces. Guidelines for measurement of microorganisms and endotoxins suspended in the air". (2002) and EN 14031 "Air at workstations. Monitoring of airborne endotoxins" (2004). A shortcoming of both legal acts and Directive 2000/54/EC is the absence of limit values for concentrations of micro-organisms and endotoxins in the air, which calls into question their effectiveness in protecting workers from risks related to exposure to biological agents at work. The implementation of quantitative standards based on an epidemiologically and experimentally proven relationship between the concentration of a given biological agent and the resulting health effects would make it possible to take precautionary measures and, in certain situations, preventive action [2].

PILOT STUDIES ON MICROBIOLOGICAL AIR QUALITY

Pilot studies were conducted in two Naval facilities: the Diver Training Centre and the vessel ORP "Kościuszko". The aim of the research was to test the developed method and preliminary estimation of the level and type of microbiological contamination of air and surrounding areas in selected places in these two facilities.

Air samples were taken using the impact method, which involves sucking an air stream onto a solid substrate intended for the isolation of the microorganisms to be determined. Tests were carried out using the Microflow α90 Microbiological Air Sampler. The apparatus automatically drew 100 dm3 of air within 1 minute. Before sampling, the inside of the aeroscope was disinfected with sterile gauze soaked in 70% ethyl alcohol.

After suction, the air was directed through narrow holes to the head with a petri dish containing a suitable microbiological medium. Solid media suitable for isolated groups of microorganisms were used in the study. The total number of microorganisms was determined on a TSA medium, incubation was performed at 37°C for 24-48 hours. In order to isolate staphylococci from the air, an MSA medium was used, which together with the air sample was incubated at 37°C for 24 hours. Moulds and yeasts were isolated on an SDA medium and then incubated at 28°C for 72-96 hours. The number of colonies of the microorganisms to be determined was converted into the total number of colony-forming units (cfu) in 1 m^3 air using the formula:

$$L = (Pr \cdot 1000) / V$$

where:
L – total number of microbial colony-forming units (cfu) in 1 m^3 air,
Pr – umber of colonies grown on the substrate used, with statistical correction according to the aeroscope manufacturer's table,
V – volume of air drawn (dm^3),
1000 – converted to 1 m^3 air.

Surface samples were taken using 25 cm^2 imprint plates with a TSA (for total microbial count), Sabouraud Dextrose Agar (for mould and yeast counting) and Baird Parker Egg Yolk Tellurite (for Staplylococcus). Three to five samples were taken from the vicinity of the air sampling point to confirm the presence of the microbial types detected during the air test. Surface samples were incubated at 35°C for 24-48 hours - bacteria and at 28°C for 72-96 hours - fungi.

The results obtained were calculated as cfu/m^3 and cfu/100 cm^2, respectively, the arithmetic mean of all the repetitions performed at a given location was taken as the final result.

RESEARCH RESULTS

The microbiological contamination varied, the number of microorganisms did not exceed the quantitative thresholds contained in PN-89/Z-04111/02 and PN-89/Z-04111/03 standards and the developed proposal of the Team of Experts on Biological Agents of the Interministerial Commission on TLV and PEL. The dominant microorganisms in the air were filamentous fungi, which often constituted more than 60% - 70% of the isolated organisms. The imprint samples taken from surfaces adjacent to the air sampling point also showed large amounts of mould fungi. In addition, the presence of *Staphylococcus* bacteria was detected in almost all the samples taken from the surfaces, and more than 50% contained *S. aureus*.

Tab. 1

Air test results - samples taken at the Diver Training Centre on 16.10.2018.

Place of sampling	Total number of micro-organisms TSA substrate	Total quantity of yeasts and moulds SDA substrate	Notes
Main hall - point no. 1 at the suits dryer	260 cfu/m^3	680 cfu/m^3	presence of microorganisms of such genera as: *Aspergillus, Penicilinum, Cladosporium, Rhizopus, Micorococcus*
Main hall - point no. 2 at the suits dryer	150 cfu/m^3	400 cfu/m^3	presence of microorganisms of such genera as: *Aspergillus, Penicilinum, Cladosporium, Micorococcus*
Main hall - the edge of the pool	140 cfu/m^3	450 cfu/m^3	presence of microorganisms of such genera as: *Aspergillus, Mucor, Penicilinum, Cladosporium*
Main hall - running tracks	640 cfu/m^3	540 cfu/m^3	presence of microorganisms of such genera as: *Aspergillus, Penicilinum, Cladosporium,*
Shower room 1	100 cfu/m^3	330 cfu/m^3	presence of microorganisms of such genera as: *Aspergillus, Mucor, Penicilinum, Cladosporium*
Shower room 2	230 cfu/m^3	440 cfu/m^3	presence of microorganisms of such genera as: *Staphylococcus, Aspergillus, Mucor, Penicilinum, Cladosporium*
Toilet	100 cfu/m^3	190 cfu/m^3	presence of microorganisms of such genera as: *Mucor, Penicilinum, Cladosporium*
Office space at the Main hall	230 cfu/m^3	330 cfu/m^3	presence of microorganisms of such genera as: *Staphylococcus, Aspergillus, Rhizopus, Penicilinum, Cladosporium*

Tab. 2

Air test results - samples taken on the ORP Kościuszko on 19.10.2018.

Place of sampling	Total number of micro-organisms TSA substrate	Total quantity of yeasts and moulds SDA substrate	Notes
Engine room with engine running	140 cfu/m^3	210 cfu/m^3	presence of microorganisms of such genera as: *Aspergillus, Penicilinum, Cladosporium*
Mess - at lunch	445 cfu/m^3	600 cfu/m^3	presence of microorganisms of such genera as: *Aspergillus, Mucor, Penicilinum, Cladosporium*

Tab. 3

Results of surface cleanliness tests - samples taken at OSN on 16.10.2018.

Place of sampling	Total number of micro-organisms TSA substrate cfu/100 cm²	Total quantity of yeasts and moulds SDA substrate cfu/100 cm²	Notes	
Main hall - areas near the diving suit warehouse	80 cfu	240 cfu	presence of microorganisms	Staphylococcus
Main hall - the interior of the "chopper"	120 cfu	280 cfu	presence of microorganisms	Staphylococcus
Main hall - areas at the edge of the pool	96 cfu	300 cfu	presence of microorganisms	Staphylococcus
Main hall - areas around the running tracks	160 cfu	320 cfu	presence of microorganisms	Staphylococcus
Main hall - treadmill control panel	72 cfu	256 cfu	presence of microorganisms	Staphylococcus
Shower room no. 1	100 cfu	60 cfu		
Shower room no. 2	280 cfu	240 cfu	presence of microorganisms	Staphylococcus
Toilet flat surfaces	360 cfu	92 cfu	presence of microorganisms	Staphylococcus
Office space at the Main Hall	504 cfu	320 cfu	presence of microorganisms	Staphylococcus

Tab. 4

Results of surface cleanliness tests - samples taken on the ORP Kościuszko on 19.10.2018.

Place of sampling	Total number of micro-organisms TSA substrate cfu/100 cm²	Total quantity of yeasts and moulds SDA substrate cfu/100 cm²	Notes	
Mess – table surfaces	352 cfu	384 cfu	presence of microorganisms	Staphylococcus
Mess – seat-back	> 1200 cfu	88 cfu	presence of microorganisms	Staphylococcus
Mess – surface by coffee thermos	> 1200 cfu	68 cfu	presence of microorganisms	Staphylococcus
Mess – refrigerator door surface	200 cfu	16 cfu		

Fig. 1 Diver Training Centre – air sampling point – Main Hall, edge of the pool.

Fig. 2 Diver Training Centre – air sampling point – toilets.

Fig. 3 ORP "Kościuszko" – air sampling point – Mess.

CONCLUSIONS

The data presented are preliminary studies. It is advisable to further monitor the degree of air pollution in these and other Naval facilities in order to assess the impact of microbial concentrations in the air on the health of soldiers and civilian workers. It was found in the presented studies that the average total number of microorganisms in the air did not exceed the values presented in the standards no longer in force or the limit values presented in the proposal of the Team of Experts. It was shown that in both facilities the concentration of moulds and yeasts in $1m^3$ of air is higher than the concentration of bacterial bioareosol, which can be explained by increased humidity, especially in the Diver Training Centre.

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