Provincial Sanitary and Epidemiological Station in Olsztyn
Epidemiological and Clinical Research Laboratory
10-561 Olsztyn, ul. Żołnierska 16
contact telephone number: (0-89) 524 83 42
e-mail: m.stempniewska@sanepid.olsztyn.pl

Contract number 51442/2020/113/DG of 14-08-2020
Contract number 51443/2020/0130/ DMD of 14-08-2020
Contract number 51444/2020/114/DG of 14-08-2020
Contract number 51445/02020/0131/ DMD of 14-08-2020

SUBJECT: ASSESSMENT OF AIR AND SURFACE DISINFECTION BY MEANS OF OZONE TREATMENT

FACILITY: PROVINCIAL SANITARY AND EPIDEMIOLOGICAL STATION IN OLSZTYN, 10-561 OLSZTYN, UL. ŻOŁNIERSKA 16

	Name and surname	**Date**	**Signature**
Prepared by:	mgr Małgorzata Stempniewska	*28.08.2020*	***Stamp:*** ***[SENIOR ASSISTANT*** ***mgr Małgorzata Stempniewska]*** ***[illegible signature]***

Table of content

1. Introduction

Ozone is a naturally occurring tratomic oxygen molecule [trioxygen]. Similar to all other chemical substances, its presence can be beneficial or detrimental. Ozone is one of the most powerful oxidants, and as such its ability to eliminate bacteria, fungus, mould and their endospores quicker and using smaller dosages is more than ten thousand times that of conventionally used chlorine or fluorine compounds [1]. Ozone molecules are toxic to life forms despite being composed of oxygen atoms. As under normal conditions it is a gas, ozone also exhibits good penetration. On account of its reactivity it quickly interacts with molecules and organisms found in air and on surfaces. The extra oxygen atom attaches to a double bond in unsaturated organic compound molecules, causing a change to their chemical structure in order to eliminate odour. This means that ozone is also used for removing unpleasant smells (deodorisation process). Certainly, just as any other method, ozone treatment has its faults. As a highly corrosive compound, if used at too high a concentration and over prolonged periods it may lead to harmful reactions with some plastics and rubber elements (colour fading, matting, stiffening and accelerated wear and tear of elements). During the aforementioned chemical reactions and depending on the odorants present in the environment toxic chemical compounds can also arise (despite the fact that both the risk and volume of created toxins are lower than if other methods were applied).

All these factors mean that ozone treatment has to be performed by specialised contractors, able to determine the right concentration of ozone and treatment duration for the given circumstances [2]. Over the last century it has been used extensively within the scope of disinfection processes, pest control, deodorising treatments and also in agriculture, medicine and the industry [3,4]. The fact that ozone can be generated on-site is a significant advantage when it comes to using it for disinfection purposes. Ozone in a gas state is used for disinfecting residential and public use premises, archives and hospitals. It is effective within the scope of treating old, neglected flats or houses for fungus, where, poor ventilation and humidity have led to the growth of mould. Despite its unquestioned ability to deodorise, there is still a shortage of tests documenting its disinfectant properties.

An assessment of air and surface disinfection using ozone treatment has been carried out at the Epidemiological and Clinical Research Laboratory, a unit of the Provincial Sanitary and Epidemiological Station in Olsztyn. Bioindicators play an important role in a sanitary assessment of air and surfaces within enclosed premises. These include mould and yeast-like fungi (originating from the soil and organic substances – such as plant fragments or municipal waste) as well as bacteria naturally present on human skin and within the human respiratory system (Staphylococcus in particular).

2. Materials and methods

The tests were carried out at the Epidemiological and Clinical Research Laboratory, a unit of the Provincial Sanitary and Epidemiological Station in Olsztyn. Three representative premises of the tested building were selected for analysis: Growth medium lab – culture media dispenser, Cold store and laboratory room number 711.

2.1. Air testing methodology

Air samples were collected at three locations before disinfection 3 hours after completing the ozone treatment. Air samples were collected using the impact method by the Merc MAS-100 Microbial Air Monitoring System (calibration certificate number WO-01708493 of 20.11.2019). Five samples were collected at each sampling location in order to eliminate errors associated with the random character of microbial distribution in the air. The volume of aspirated air (100 litres) was configured for the expected microbial pollution of the tested environment. The air sampler collected the pre-set air volume into the air sampler head and onto contact plates with an agar medium:

1. Tryptone Soya Agar for the overall level of bacteria;
2. Sabouraud Dextrose Agar for the total number of mould and yeast-like fungi.

The samples were collected pursuant to Instruction I-O l/PO-03 "Collection, transport and storage of samples to be tested". All plates with growth media were subject to incubation at a temperature and for a time corresponding to the tested types of microbes in accordance with Test Procedure PB-OBP-019 "Collection, detection, identification and determination of the number of microbes in environmental samples". Once the colonies have been counted and the sample volume has been taken into account, the concentration of microbes in units making up colonies was determined per one cubic meter of air [CFU/m^3]. The final results constitute the probable total statistical number of units which make up the colonies, taking into account Feller's Statistical Correction Tables (pursuant to the guidelines set forth in the MAS microbiological air sampler user manual). Meteorological conditions were recorded at the time the samples were taken: temperature and relative air humidity.

2.2. Contact testing methodology

In order to determine the surface pollution levels, 5 sampling locations were established within the tested building. TSA LAB-AG AR + Tween + Letheen commercial contact plates were used to collect impressions from the selected surfaces. The samples were collected pursuant to Instruction I-01/PO-03 "Collection, transport and storage of samples to be tested". All plates with growth media were subject to incubation at a temperature and for a time corresponding to the tested types of microbes in accordance with Test Procedure PB-OBP-019 "Collection, detection, identification and determination of the number of microbes in environmental samples". Once grown colonies have been counted, the concentration of microbes per 25 cm^2 was determined.

3. Reviewing the results

Table 1 presents the micro-climate conditions on the test day. The obtained humidity level measurement results indicate moderate moisture content in ambient air. On the one hand this prevents the occurrence of bacterial and mould biodeterioration instances. On the other hand the effectiveness of ozone treatment increases together with relative air humidity.

Table 1. Micro-climate conditions at sampling sites before and after ozone treatment

Sample collection date	Sample collection site	Temperature (°C)	Humidity (%)
14.08.2020	Growth medium lab – Cold store	24.0	47.7
14.08.2020	Growth medium lab – Culture media dispenser	24.0	55.1
14.08.2020	Room No. 711	22.4	46.4
Sample collection date	**Sample collection site**	**Temperature (°C)**	**Humidity (%)**
14.08.2020	Growth medium lab – Cold store	23.3	54.0
14.08.2020	Growth medium lab – Culture media dispenser	22.9	56.8
14.08.2020	Room No. 711	18.6	64.6

3.1. Bacterial air contamination

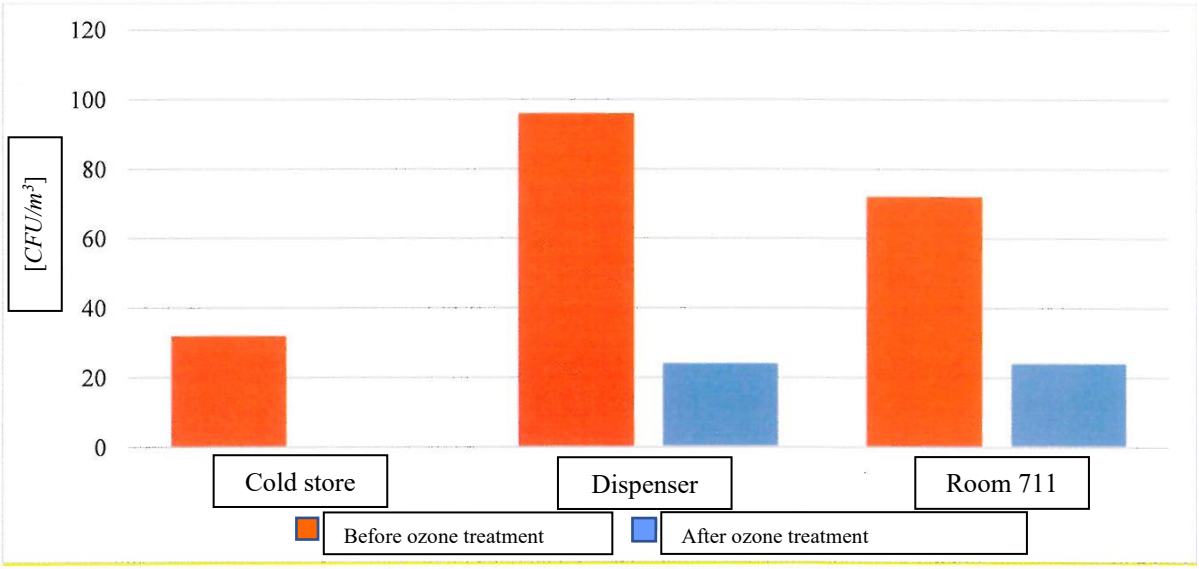
Atmospheric air comprises different types and species of microbes, including many bacteria (approximately 26%, out of which approximately 9% are *Staphylococcus* type bacteria). An increase in air temperature and no precipitation causes the number of microbes in the air to increase. Saprophytic microbes, resistant to humidity levels are primarily suspended in the atmosphere. However, pathogenic bacteria do also occur. The interior aerosol microflora is not qualitatively different from the external air microflora. Table 2 presents the results of qualitative and quantitative analysis of bacterial aerosol in the tested samples at sampling sites.

Table 2. Bacterial aerosol concentration and composition [CFU/m3] at sampling locations

Sample collection site	Type/species		Total number of bacteria [CFU/m3]		Reduction (%)
	Before ozone treatment	After ozone treatment	Before ozone treatment	After ozone treatment	
Growth medium lab – Cold store	*Acinetohacter johnsonii, Bacillus spp., Staphylococcus caprae*		32	<1	100
Growth medium lab – Culture media dispenser	*Acinetohacter ursingii, Bacillus spp., Micrococcus luteus, Staphylococcus epidermidis*	*Bacillus spp., Micrococcus luteus*	96	24	75
Room No. 711	*Bacillus spp., Micrococcus luteus, Staphylococcus epidermidis*	*Bacillus spp., Micrococcus luteus, Staphylococcus capitis*	72	24	67

Saprotrophic bacteria were grown in interior air of the tested premises. These included: *Bacillus spp., Micrococcus luteus, Staphylococcus epidermidis* as well as *Acinetohacter* type bacteria, which are commonly found within air in buildings. A human being Man is one of the sources that produce these microbes into the environment. Human pathogens (bacteria) according to the Resolution by the Minister of Health dated 22.04.2005 on the biological agents to health at work and the protection of health of workers exposed to these agents at work [*Dz. U. Journal of Laws* No. 81, item 716 as amended] were not found in internal air of the tested premises. The total number of bacteria in the tested samples before and after ozone treatment is shown in Figure 1.

Figure 1. Total number of bacteria [CFU/m3] at sampling locations



In both of the analysed cases, ozone treatment resulted in a decrease to the content of bacterial microbes in air by 100%, 75% and 66% respectively. It is significant that all the isolated types of bacteria were reduced by a similar degree. Under traditional disinfection methods, some microbes exhibit properties which increase their resistance to the disinfecting agent. For example, *Micrococcus* type bacteria have developed pigments which protect them against lethal ultraviolet radiation and *Bacillus* type microbes form endospores which ensure survival in unfavourable ambient conditions following chemical disinfection. Varied starting air pollution levels may explain the broad disinfection effectiveness results.

3.2. Bacterial surface contamination

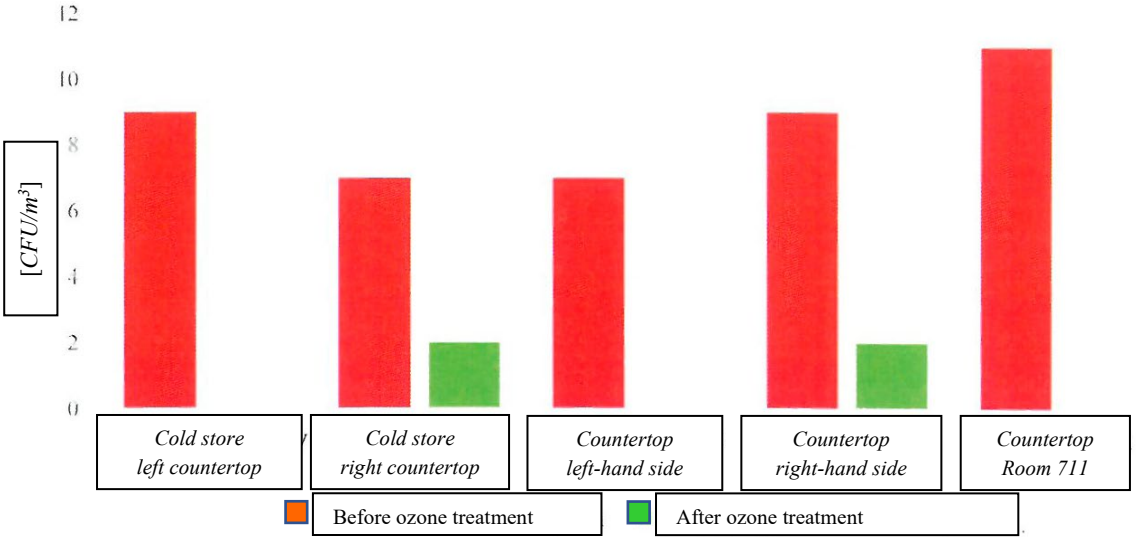
Ozone treatment is even more effective for contaminated surfaces. Out of the ten collected samples, the smallest recorded microbial reduction amounted to 71%. 100% disinfection result was achieved for three samples. An average ozone treatment resulted in an 89.8% reduction of microbes. Qualitative and quantitative analysis results are shown in Table 3.

Table 3. Qualitative and quantitative analysis of contact plates from the tested premises

Rooms where samples were collected	Sample collection site	Grown microbes		Total number of bacteria (CFU/25 cm^2)		Reduction (%)
		Before ozone treatment	After ozone treatment	Before ozone treatment	After ozone treatment	
Growth medium lab – Cold store	Middle shelf left side – countertop	*Bacillus species, Micrococcus luteus, Rhizobium radiobacter, Staphylococcus cohnii ssp. urealyticus*		9	0	100
Growth medium lab – Cold store	Middle shelf right side – countertop	*Bacillus species, Micrococcus luteus, Rhizobium radiobacter, Staphylococcus haemolyticus*	*Bacillus species, Micrococcus luteus*	7	2	71
Growth medium lab – culture media dispenser	Culture media dispenser table left hand side	*Bacillus species, Corynebacterium* group bacteria, Micrococcus luteus, *Staphylococcus haemolyticus*		7	0	100
Growth medium lab – culture media dispenser	Culture media dispenser table right hand side	*Bacillus species, Corynebacterium* group bacteria, *Micrococcus luteus*	*Bacillus species, Micrococcus luteus*	9	2	78
Room No. 711	Long laboratory countertop	*Aerococcus viridans, Bacillus spp., Micrococcus luteus*		11	0	100

The total number of bacteria in the tested samples before and after ozone treatment are shown in Figure 2.

Figure 2. Total number of bacteria [CFU/25 cm^2] at sampling locations



3.3. Mould contamination in the air

Microfungi are fungi whose morphology is determined using a microscope. They include mould and yeast-like fungi. Filamentous fungi are fungi whose thalli are primarily made out of hyphae. Fungal spores or fragments of hyphae are most common in atmospheric air (these make up approximately 70% of the composition of ambient air). Mould is common in the natural environment. Soil and dust are one of its permanent reservoirs. During growth its mould generates vast quantities of dry spores with diameters of a few m micrometres, perfectly adapted to disperse over long distances together with air movements. This means that many mould species settle on the surfaces of various materials. The ability of mould to grow and propagate is determined by three primary factors: humidity of the environment, temperature and the availability of nutrients. Due to the low requirements and a highly developed enzymatic apparatus facilitating the decomposition and use of various chemical compounds, humidity is the only parameter which strongly limits the growth of mould and yeast-like fungi. *Deuteromycota, Ascomycota* and *Zygomycota* class moulds are the most numerous in the microflora of air. They require small amounts of organic nutritional substances to grow and as such may develop on wood, construction materials, latex and rubber and also on fabrics at places with higher humidity. Pollution in the form of organic dust can be used by the fungi as a growth medium. Bacteria often occur together with fungi at places with high humidity. These are detrimental to the aesthetics of the infected materials, they destroy stored products and have an adverse impact on human health and well-being. Table 4 presents the results of qualitative and quantitative analysis of fungal bioaerosol.

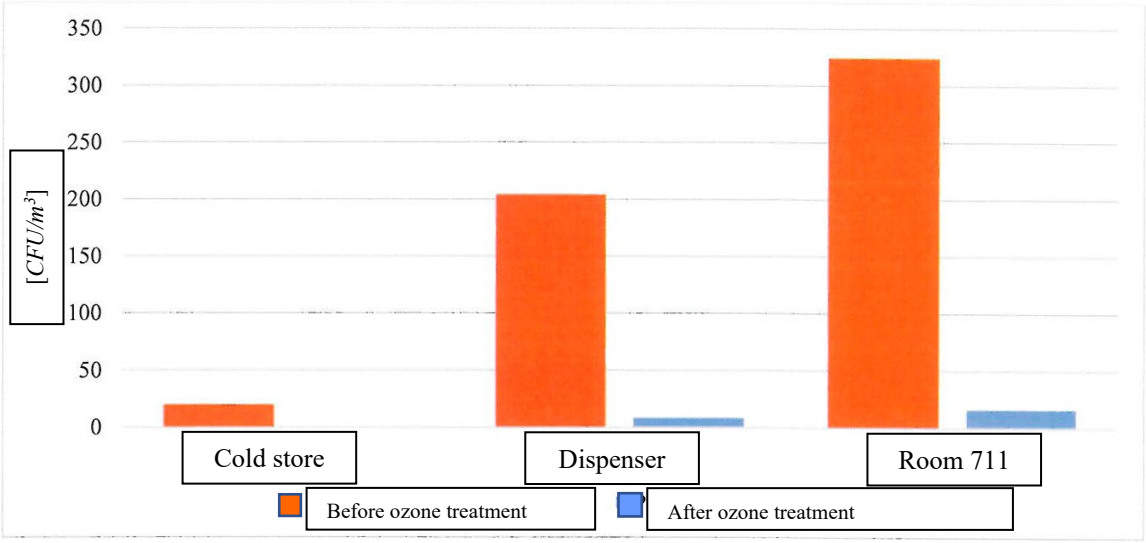
Table 4 Fungal aerosol concentration and composition [CFU/m^3] at sampling locations

Sample collection site	Type/species		Total number of fungi [CFU/m3]		Reduction (%)
	Before ozone treatment	After ozone treatment	Before ozone treatment	After ozone treatment	
Growth medium lab – Cold store	*Alternaria tenuissima, Cladosporium cladosporioides*	*Cladosporium cladosporioides*	20	4	80
Growth medium lab – Culture media dispenser	*Alternaria tenuissima, Cladosporium cladosporio ides, Talaromyces macrosporus*	*Alternaria tenuissima, Cladosporium cladosporioides*	204	8	96
Room 711	*Alternaria tenuissima, Cladosporium cladosporioides, Epicoccum nigrum, Pénicillium chrysogenum*	*Alternaria tenuissima, Cladosporium cladosporioides*	324	16	95

Ozone treatment returned positive results for mould and yeast-like fungi. The smallest recorded reduction was 80%, while 96% was the highest. On average the value was 90.3%. Human pathogens (fungus) according to the Resolution by the Minister of Health dated 22.04.2005 on the biological agents to health at work and the protection of the health of workers exposed to these agents at work [*Dz. U. Journal of Laws* No. 81, item 716 as amended] were not found in internal air of the tested premises.

The total number of fungi in the tested samples before and after ozone treatment are shown in Figure 3.

Figure 3. Total number of fungi [CFU/m^3] at sampling locations



Similar to bacteria, the reduction of mould and yeast-like fungi is uniform regardless of the ability to generate pigments (dematiaceous moulds) and resting spores in the form of chlamydospores (such as the *Fusańum* and *Paecilomyces* types).

4. Conclusions

The performed tests have demonstrated that ozone treatment may be a perfect complement to traditional disinfection methods. The gas ability to propagate freely, a properly performed treatment makes it possible to eliminate biological contamination from places which are hard to get to and which may be easily overlooked. The disinfection performance measured after ozone treatment demonstrated a high reduction to microbiological contaminants, with the average reduction rate of 86%. It also transpired that ozone treatment is effective when it comes to microbes more resistant to other methods, such as pigment producing organisms, those resistant to drying out or those which generate endospores. The treatment has significantly improved the microbiological quality of air and surfaces. The obtained results were convergent with those obtained whilst assessing the feasibility of ozone treatment for removal of fungus which grew during a flooding [5]. Deodorising properties are an added value, including decay odours.

5. Bibliography

[1] STRYCZEWSKA H.D., *Technologie plazmowe w energetyce i inżynierii środowiska.* Wydawnictwo Politechniki Lubelskiej. Lublin 2009

[2] FRĄCKOWIAK W., SŁAWIK K., SŁAWIK P., *Czy ozonowanie jest dobrą metodą w likwidacji korozji biologicznej?* Ochrona budynków przed wilgocią i korozją biologiczną. Polskie Stowarzyszenie Mykologów Budownictwa. Wrocław 2013, 12, pp. 95-99

[3] JURGOWIAK M., *Ozon w medycynie - tak czy nie?* TPS, 2003, 3, pp. 16-20

[4] LONCARB., *Ozone application in dentistry.* Archives of Medical Research, 2009, 40, pp. 136-137

[5] NOWAKOWICZ-DĘBEK B., KRUKOWSKI H., WLAZŁO Ł., BOJARCZYK M., *Ocena mikologiczna powietrza w domach zalanych w czasie powodzi na przykładzie gminy Wilków.* Mikologia Lekarska 2011, 18, No. 2, pp. 87-89