Comparison of the degree of microbiological contamination of selected closed rooms using the method of fast detection with a multi-sensor matrix

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Abstract. The problem of microbiological contamination has been an important issue in the construction and environmental industry for many years. The concept of Sick Building Syndrome (SBS) means a combination of different health conditions, which can be eradicated by being in a specific interior. The basic prerequisite for an effective analysis of the issue is a detailed knowledge of its causes and consequences for people. In many cases, the reason for SBS is an increase in humidity of building partitions, but also excessive humidity in closed rooms, which becomes the cause of microbiological contamination of building objects. These factors create appropriate conditions for the development of microorganisms, adversely affecting the durability of the building, its technical condition, and above all the health of residents. This article presents a comparison of two rooms of technical nature, characterised by a different degree of microbiological load. For the analysis, a room with a high degree of microbiological contamination and a room in which high quality internal air quality is maintained were selected. The degree of a microbiological load will be determined on the basis of sensory measurements carried out with the use of microbiological, chemical, and rapid detection method using a multi-sensor matrix. The multi-sensor matrix forms the basis for electronic nose technology, which can also be used to assess internal air quality.

1. Introduction
The problems of internal air quality in recent years have become the subject of special interest, especially in the light of research documenting that man spends more than 80% of his time inside [1]. The increase of the society’s awareness of the dangers associated with inadequate internal air quality increasingly often forces actions aimed at improving it [2]. It should be emphasised that the internal air quality is affected not only by internal pollution but also from the external environment [3]. The air may contain physical, chemical, and biological pollution, but taking into account the pollution generated inside the buildings, it is worth noting that the air quality is also affected by construction materials, finishing materials, and furnishings and the pollution emitted by them [4].

Hazards resulting from the presence of microorganisms in the air are not only direct health hazards but also hazards in the industry (food, pharmaceuticals, cosmetics), agriculture (plant and crop production, animal husbandry), and even construction (ground infections, structural damage, sick building syndrome) [5]. The term Sick Building Syndrome refers to the syndrome of ailments related to...
the impact of harmful and health-improving factors on humans in internal spaces, such as chemical, dust, and microbiological contamination, noise, mechanical vibrations, electromagnetic fields, lighting, static electricity at improper air parameters (relative air temperature and humidity, air velocity, carbon dioxide concentration) and microclimate [6,7].

The composition of the internal air depends on external factors, i.e.: equipment, finishing materials used, amount of dust, quality of the ventilation and air conditioning system, and even on the man himself and his activities [6,8,9]. Fungal spores, allergens, bacterial spores present on surfaces or suspended in the atmospheric air, additionally emit chemical substances such as mycotoxins, endotoxins, enterotoxins. All these have proven toxic or carcinogenic effects. It is therefore important to maintain a low concentration of micro-organisms in every living, public, or working area so that users of the facility are not exposed to the deterioration in their health. Wherever there is potential exposure to microorganisms, it is advisable to perform appropriate analyses to help establish internal air quality and identify possible pathogenic foci [10-12].

This article presents a comparison of two rooms of technical nature, characterised by different degrees of microbiological load. For the analysis, a room with a high degree of microbiological burden and a room where high internal air quality is maintained were selected.

2. Materials and methods
This study is a part of a larger scope of research. This article attempts to assess the possibility of using a sensor matrix to assess indoor air quality mainly related to microbial infection on the basis of rooms with significant levels of biological contamination. Therefore, only one result is presented as exceeding the limit values.

2.1. Description of the tested rooms
The study was conducted in two technical rooms located in public buildings in Lublin. These buildings are located in the central part of the city. The selected rooms have limited access only for authorised persons. The measurements were taken in May, which could have caused high relative humidity, as the outside air already had a high moisture content, an average of 7.45, and a maximum of 11.23 g / kg of dry air.

Room 1. After preliminary visual inspection, room 1 was classified as microbiologically infested. The assessment was made on the basis of visible raids, discolourations, and changes in the structure of the building substance, as well as perceptible smell characteristics for damp rooms. Room 1 is located on the lowest storey of the building and its area is 12 m² and the cubic capacity is 36 m³. The floor is made of concrete flooring, lined with tiles. The technical room is illuminated with artificial light, no access to natural light. During the measurements, the internal air temperature and humidity were measured as follows: internal temperature: 22 °C; room humidity: 65%

Room 2. During the preliminary examination of the room, the overall condition was found to be satisfactory and it was considered to be microbiologically clean. In addition, the purpose of the room in which the experiment was carried out requires particular cleanliness of the internal air.

The technical room analysed is located on the 2nd floor of a four-storey building. The area of the room is 20 m² and the cubic capacity is 56 m³. The floor is made of carpet material, which enables washing and disinfection. The room has access to natural light through 2 windows with dimensions: width 2320 mm, height 1780 mm, located on one external wall. Air quality is ensured by a ventilation system combined with filters for incoming and outgoing air. During the measurements, the internal air temperature and humidity were measured as follows: internal temperature: 20 °C; room humidity: 60%

2.2. Test methods for rooms
The degree of the microbiological load was determined on the basis of sensory measurements carried out with the use of microbiological, chemical, and fast detection methods using a multi-sensor matrix. Research methods:
- microbiological method
The collision method consists in the fact that the air, flowing at high speed, which hits the surface of the nutrient medium, is forced to suddenly change the direction of movement and that, as a result, a microorganism falls out of the air current and settles on the surface of the medium. The Petri dishes with media are incubated, the number of colonies on each plate is counted, and the number of colonies in 1 m$^3$ of air is calculated afterwards [6].

The cascade impactor is a device for the determination of specific size distributions of any aerosol-forming dust. Employing properly profiled channels leading the aerosol, the dust of different diameters settles in different places [6]. The impactor that was used in the measurement - Stoplex MAS-6A 6 stage microbial air sampler, airflow during the measurement 28 L/min. The impactor was placed in the centre of the room 1.5 m from the floor. 6 Petri dishes with Sabouraud’s medium were inserted into the impactor and air was taken for 10 minutes with a pump. The plates were then placed in an incubator at 27°C. The incubation time of the samples was 2 weeks.

Measurements were made in various city districts, with different meteorological conditions. Microbiological tests have not been performed for the outside air, because such measurements would be difficult to compare.

- rapid detection method with a multi-sensor matrix

The e-nose device’s construction was based on 17 metal oxide semiconductors (MOS-type gas sensors) manufactured by TGS Figaro. The gas sensor array includes items described in table 1. Although the matrix contains sensors that are sensitive to different gases, the purpose of the e-nose is not to identify the individual components of the gas mixture. The aim is to determine the overall response profile of the sensors to different types of polluted air.

| Name       | Destination                                      | Range [ppm]          |
|------------|--------------------------------------------------|----------------------|
| TGS-2600-B00 | General air pollution sensor                     | 1÷30 (for H$_2$)     |
| TGS-2602-B00 | Toxic air pollutant sensor (NH$_3$, H$_2$S, C$_2$H$_5$OH, C$_6$H$_5$-CH$_3$) | 1÷30 (for ethyl alcohol) |
| TGS-2610-D00 | Propane and butane sensor with a carbon filter (C$_3$H$_8$, C$_4$H$_10$) | 500 ÷ 10000          |
| TGS-2611-E00 | Methane sensor with a carbon filter (CH$_4$)      | 500 ÷ 10000          |
| TGS-2620-C00 | Ethyl Alcohol Sensor                             | 50 ÷ 5000            |
| TGS-4161    | carbon monoxide                                  | 350 ÷ 10000          |
| TGS-2444    | ammonia                                          | 10 ÷ 100             |
| TGS-2442-B02 | Carbon monoxide sensor CO                        | 30 ÷ 1000            |
| TGS-800     | general air pollutants                           | 1 ÷ 1000 (for isobutane) |
| TGS-825-A00 | hydrogen sulfide                                 | 5 ÷ 100              |
| TGS-813-A00 | Combustible Gas Sensor                           | 500 ÷ 10000          |
| TGS-821     | Hydrogen                                         | 10 ÷ 5000            |
| TGS-823-A00 | solvent vapor                                     | 50 ÷ 5000            |
| TGS-812     | Explosive and toxic gas sensor (methane, isobutane, hydrogen, CO) | 100 ÷ 5000          |
| TGS-830     | chlorofluorocarbons                               | 100 ÷ 3000           |
| TGS-832-A00 | chlorofluorocarbons                               | 100 ÷ 3000           |
| TGS-2106    | sensor of exhaust gases, exhaust gases from diesel engines | 0.1 ÷ 10 (for NO$_2$) |

The performance of the sensors is based on changes in the electric conductivity of sensing elements due to surface chemical reactions between gas molecules and the semiconductor. The intensity of this reaction is proportional to the gas composition and concentration. Each sensor provides a different signal response according to its own sensitivity characteristics [13,14]. Each measurement lasted 15 minutes and included a 5 minutes phase of gas sensor purging with synthetic air and 10 minutes of measurement. The purge and sampling airflow was adjusted to 0.5 L/min. For further analysis, stabilised data from the last minute of measurement were used.
Due to the fact that the portable gas chromatograph tests did not bring the expected results, it was decided to apply the microextraction technique on SPME fibres, which were then tested by gas chromatography coupled with a mass spectrometer GC-MS TraceUltra – PolarisQ, Thermo. Fibers used for microextraction were placed in selected rooms to absorb fungal metabolites in the air. It was used Supelco PDMS fibers (phase thickness 100 µm). The pure fiber chromatogram is shown in the figure 1. During each measurement in a given room, one fiber was used. Absorption time – 60 minutes.

The parameters of the chromatograph were as follows:
- dispenser: Blue Sky Restek 1mm – temp: 250°C, in splitless mode 2 min.
- chromatographic column: Equity 5MS from Supelco 30m x 0.25mm ID x 0.25µm df with helium flow of 1.2ml/min with purity 99.999% from BIP
- Column temperature program: start 40°C - isotherm 2 min, 8°C/min to 150°C, 12st/min to 250°C - isotherm 5 min.
- transfer line (auxalilleries) – 250°C
- ion source temperature (filament) – 220°C
- detector – Full Scan 35-350 m/z
- comparison library – NIST 08 willey8
- data collection program Xcalibur 1.4

3. Results and discussion
As a result of microbiological tests, many fungi species were found. They are listed in table 2.

The fungi species were identified visually, after comparing them with the reference samples. As a result of the tests carried out with the cascade impactor, it was possible to determine the number of fungal colonies at particular levels of the impactor together with the average number of colonies in each of the tested rooms. These are summarised in table 3.

According to PN-EN 13098:2007, the permissible number of CFU in rooms should be less than 500 cfu/m³. There is no possibility to calculate the sum of fungal colonies in each room because in level 2 of the cascade impactor in room 1 the number of colonies is uncountable. What is more the average from each room is necessary to calculate CFU.
Table 2. Identification of fungi species.

| Identification of fungi species | Room 1                                      | Room 2                                      |
|---------------------------------|---------------------------------------------|---------------------------------------------|
| Penicillium chrysogenum         |                                             | Mucor racemosus                            |
| Mucor racemosus                 |                                             | Aspergillus fumigatus                       |
| Aspergillus fumigatus           |                                             | Aspergillus ochraceus                       |
| Aspergillus flavus              |                                             | Alternaria alternata                       |
| Aspergillus ochraceus           |                                             | Cladosporium sphaerospermum                |
| Aspergillus Niger               |                                             | Fusarium sporotrichioides                  |
| Aspergillus ustus               |                                             | Penicillium chrysogenum                    |
| Penicillium expansum            |                                             | Cladosporium cladosporioide                |
| Stachybotrys chartarum          |                                             |                                             |
| Alternaria alternata            |                                             |                                             |
| Cladosporium cladosporioide     |                                             |                                             |
| Cladosporium sphaerospermum     |                                             |                                             |
| Chaetomium globosum             |                                             |                                             |
| Fusarium sporotrichioides      |                                             |                                             |
| Cladosporium herbarum           |                                             |                                             |
| Alternaria alternata            |                                             |                                             |

Table 3. Number of fungal colonies at each level of the cascade impactor.

|                  | Room 1      | Room 2     |
|------------------|-------------|------------|
| Level 1          | 146         | 3          |
| Level 2          | 174         | 11         |
| Level 3          | uncountable | 10         |
| Level 4          | 53          | 45         |
| Level 5          | 196         | 19         |
| Level 6          | 23          | 16         |
| Average          | 118.4       | 17.3       |
| CFU              | 470         | 550        |

As a result of the matrix tests, a multidimensional signal was obtained from 17 sensors, the averages of which are shown in Table 4, in order to visualise the results, a PCA analysis was performed. The Statistica 13.1 Statsoft program was used for the statistical analysis of the e-nose measurement data. In a further step, the multivariate data were subjected to the main components of PCA analysis, based on the covariance matrix. The use of PCA is to:
- Searching for significant dependencies in the multidimensional data set, invisible due to information overload;
- Reduction of the variables number in order to enable the graphical visualisation of measurement data.

As a result of reducing the number of variables, completely new dimensions (principal components) are determined, showing the largest variance of the data set. Practically the first few principal components explain most of the total variance of the data set, so further analysis was limited to only two principal components, explaining 81.5% of the variance. The use of two variables enables convenient...
representation in a two-dimensional graph. Summary of eigenvalues and basic parameters of PCA analysis are presented in table 4.

**Table 4. Summary of eigenvalues and basic parameters of PCA analysis.**

| Variable | Eigenvalue | Variance [%] | Cumulated eigenvalue | Cumulated variance [%] |
|----------|------------|--------------|----------------------|------------------------|
| 1        | 10.55308   | 65.95678     | 10.55                | 65.95                  |
| 2        | 2.49704    | 15.60648     | 13.05                | 81.56                  |
| 3        | 1.74024    | 10.87652     | 14.79                | 92.43                  |
| 4        | 0.88757    | 5.54728      | 15.67                | 97.98                  |
| 5        | 0.17564    | 1.09772      | 15.85                | 99.08                  |
| 6        | 0.07744    | 0.48401      | 15.93                | 99.56                  |
| 7        | 0.02423    | 0.15144      | 15.95                | 99.72                  |
| 8        | 0.02005    | 0.12531      | 15.97                | 99.84                  |
| 9        | 0.00921    | 0.05755      | 15.98                | 99.90                  |
| 10       | 0.00543    | 0.03392      | 15.98                | 99.93                  |
| 11       | 0.00351    | 0.02194      | 15.99                | 99.95                  |
| 12       | 0.00279    | 0.01743      | 15.99                | 99.97                  |
| 13       | 0.00226    | 0.01411      | 15.99                | 99.99                  |
| 14       | 0.00107    | 0.00670      | 15.99                | 99.99                  |
| 15       | 0.00036    | 0.00224      | 15.99                | 99.99                  |
| 16       | 0.00006    | 0.00038      | 15.99                | 99.99                  |
| 17       | 0.00003    | 0.00019      | 16.00                | 100.00                 |

The results of the PCA analysis are presented in figure 2. The measurement data can be grouped into clusters representing the different categories of rooms. The degree of contamination correlates only with the first principal component of PC1, and the rooms affected by a mycological problem are located on the left side of the PC1 axis. However, there is no significant relationship between the degree of contamination and the second principal component. Classification of data in PC1/PC2 space was performed using k-means clustering. For PC1 between SS (sum of squares) variance is significantly larger than within SS variance (table 5). This outcome indicates the possibility of e-nose for evaluation of indoor air quality in buildings or SBS syndrome. But it should be noticed that the used sensors have low sensitivity compared to the level of contaminations typically occurs in rooms. In this experiment, a meter with a sensor array optimised for other purposes and applications was used, in which the pollutants were characterized by higher concentrations. Nevertheless, the used sensors are not highly selective only for target chemicals and also react to a wide spectrum of interfering compounds with different concentration levels. This research is a test, whether universal and therefore inexpensive sensors can be used to detect microbial contamination. However, the positive results of the research do not constitute an unequivocal answer to this question, because bad air quality could have been caused not only by microbial contamination but by accompanying sources emitting pollutants with higher concentrations. There is also an opposite possibility: poor conditions in the tested room additionally influenced the development of mould.

**Table 5. Basic summary of k-means clustering.**

| Variable | Between SS df | Within SS sf | F         | p         |
|----------|---------------|--------------|-----------|-----------|
| PC1      | 479.1416 1    | 3.999642 34  | 4073.068  | 0.000001  |
| PC2      | 3.1371 1      | 8.388315 34  | 12.715    | 0.001101  |
In the contaminated room 1, a large number of organic compounds was found. Among them are the compounds that may be associated with microbial contamination, such as hydrocarbons and aromatic hydrocarbons, alcohols, ketones, terpenes [15-16]. They either can indicate the influence of other sources, e.g. green areas or the use of household cleaning chemicals. The most common compounds found in room 1 were listed below (retention time in bracket). Compounds were recognized by TIC (total ionic current) as well as by characteristic ion detection.

- alcohols: 1-butoxy-2-propanol (8.02), dihydromyrcenol (10.71), 2,3-dimethyl-1-pentanol (11.4), 10-undecanol (13.29), p-menthan-1,2-diol (13.45), 2-phenoxyethanol (13.6)
- aromatic hydrocarbons: benzene (2.93), toluene (4.45), ethylbenzene (6.35), m-p-xylene (6.52), o-xylene (7.0), 2-octene (9.88), styrene (6.97), metoxymethyl-benzene (7.89)
- bicyclic monoterpenes: pinene (9.07), delta-carene (9.5), camphene (13.09), delta-3-carene (14.19)
- carboxylic acids: acetic acid (12.63)
- ketones: 2-methylcyclopentanone (7.19), p-menhant-3-one (13.45)
- esters: linalyl acetate (12.8), methyl-acetate (13.96)
- terpenes: limonene (9.9)
- cyclic ethers: cineol (9.93), eucalyptol (9.92)
- other organic compounds: P-cymene (9.79), 3-buthylo-2,5-dibuthylopyrazine (11.12), carvacrol (13.81), 1-3 butenyl-1,3-cyclopentadiene (10.03).

Room 2 is characterised by good internal air quality and there are no signs of microbial contamination. It does not mean, however, that there will be no chemical pollution in the room. It is a technical space where laboratory equipment is located, as well as various chemical compounds can be used. However, in comparison to room 1, there are significantly fewer compounds indicating the presence of fungi. The most common compounds found in room 2 were listed below:

- alcohols: phenoxyethanol (13.63), menthol (12.73), 1-undecyn-4-ol (13.29),
- aromatic hydrocarbons: 1,4-dichlorobenzene (9.54), 3-methyl-2-butyl-benzene (14.94)
- acyclic alkanes: docosane (16.6)
- alkyne hydrocarbons: 1-decyne (11.35)
- cyclic ether: cineol (9.92)
- terpenes: limonene, camphor (12.23)
- monoterpenes: menthone (13.14)
- aromatic hydrocarbon: naphthalene (12.99)
- other organic compounds: triacetin (15.79), dimethyloctadiene (14.87).

Example of chosen GC analysis from Room 1 (contaminated) and Room 2 (clean) is shown respectively on the upper and lower diagram of figure 3. This is a spectrum of total ion current (TIC) for m/z 35-350 IU on Trace GC Ultra gas chromatograph with PolarisQ mass spectrometer (ion trap) by Thermo for sample sorbed od fiber PDMS 100μm Supelco.

![GC Chromatograms](image)

**Figure 3.** Example of chromatograms from contaminated room 1 (upper diagram) and clean room 2 (lower diagram).

### 4. Summary

All methods used can distinguish between infected and non-infected rooms. In rooms that were sensorially considered to be uninfected, CFU was not exceeded. Chromatographic studies have allowed the detection of compounds that may be associated with microbial contamination. PCA analysis from measurements with the use of a sensor matrix allows to distinguish a room with and without biological contamination, and thus with a different state of internal air quality. The chromatographic analysis shows, however, that this may be an indirect relationship.
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