AIR QUALITY IN CELLARS: A CASE STUDY OF WINE CELLAR IN SĂLACEA, ROMANIA

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Received: November 29, 2019 | Revised: January 16, 2020 | Accepted: January 26, 2020
Paper No. 20-62/1-552

Abstract
The space, like cellars, where locals keep food products plays an important role. Cellars are ideal spaces because they provide balance and do not allow thermal shock. The purpose of a cellar is to extend the shelf life of the products, regardless of season and weather conditions. The cellar must meet certain conditions, such

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as constant temperature and humidity, lack of unpleasant odours, darkness etc. In this respect, this study has analysed air temperature, relative air humidity and fungi in a cellar located in the village of Sălacea, Bihor County, Romania. The results obtained show that the temperature inside the cellar during the monitored period falls within the optimal parameters for preservation of products and the constant presence of mould may develop pathologies in persons who spend more hours working in this environment. Bringing these parameters to normal values is an important task for cellar owners, whose solving is necessary for preserving products over a long period of time.

Key words
Cellar, authentic, fungi, temperature, humidity, heritage.

INTRODUCTION

Sălacea is the village of "the 1,000 cellars", dug directly into the bedrock. Microclimate monitoring and analysis of the airborne fungi in the underground cellars are extremely significant for human health (owners, visitors, etc.), as well as for the safety of the products that are kept inside these cellars. The approach is part of a vast plan of microclimate monitoring and fungi analysis inside wine cellars in order to support their preservation and their possible introduction into the national cultural heritage (Matlovicova et al. 2019).

Sălacea village, located in the northwest of Romania, Bihor county, is one of the most representative settlement, specific for the contact area between the Pannonian Plain (Ierului Valley subsidence plain) and the Western Hills (Sălacea Hills) (Figure 1). With an altitude ranging between 100 m in the north-western part and 160 m in south-east, the topography of Sălacea village has favoured building over 950 cellars, some of them dating back 200-300 years (Kéri and Kántor, 2009). These are evidence of the time when winegrowing represented a priority for the inhabitants of this area, the traces of such occupation being deeply dug into the ground in the form of cellars.

Together with an ethnography specialist and an architect, an authentic cellar model, made of wood, mud brick and reed, completely covered by earth, was identified and chosen for performing the monitoring between 02/04/2018 and 02/05/2018. The facade is entirely made of oak wood and it is not decorated (Figures 2 and 3). The cellar is dug at about 2-3 m deep. The facade focuses on the door which is made of oak wood and wrought iron (Figure 2).

The purpose of the study is to investigate the air quality in the identified cellar in order to observe the storage conditions of the products intended for consumption, but also the effects on the health of people who are working in such space for a longer period of time.

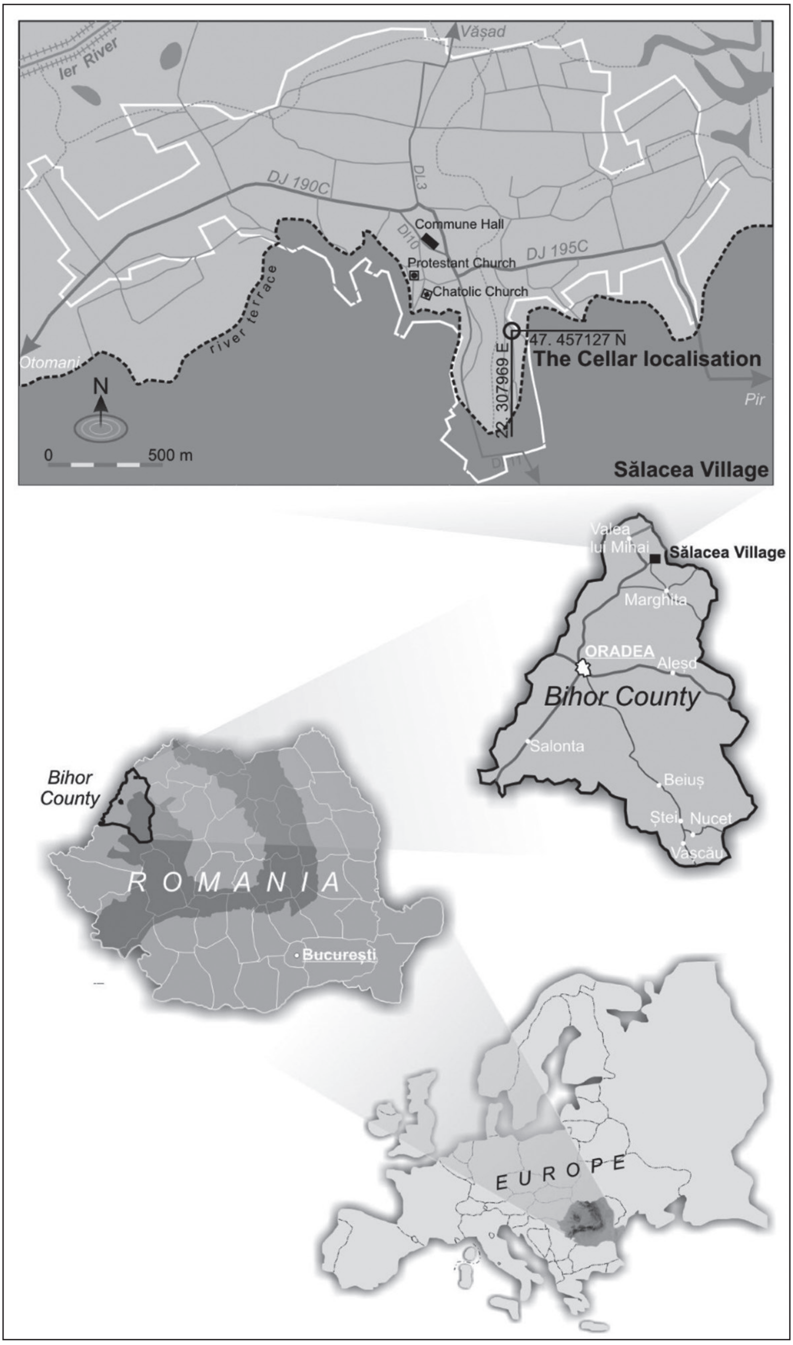

Figure 1
Geographical location of the monitored cellar

Figure 2
Facade of rebuilt traditional cellar, Sălacea

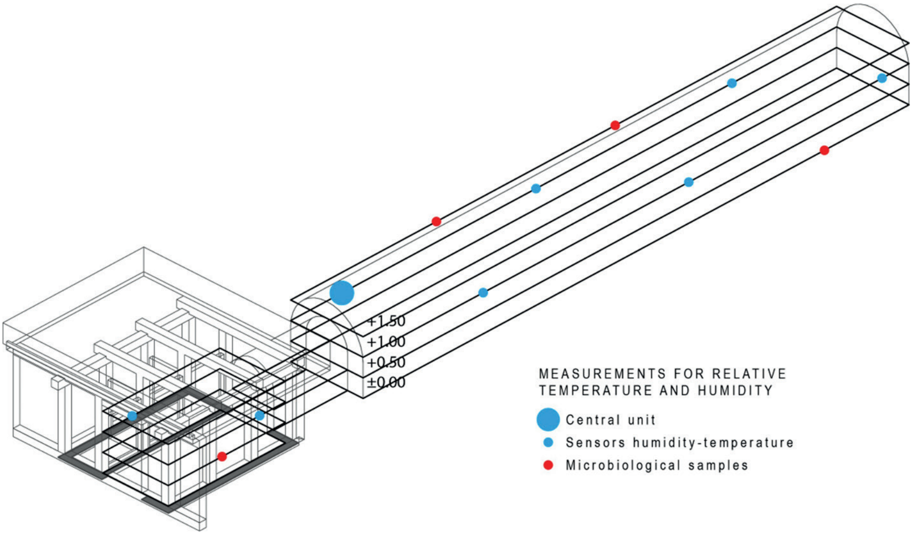

Figure 3
Cellar plan, location of the sensors used for temperature and humidity monitoring and microbiological samples (Author A. Lincu, 2019)

LITERATURE REVIEW

The international literature reveals several studies on the monitoring of indoor climate and bacterial and fungal aerosols in wine cellars. We mention in this sense, for cellars, the paper of Górny and Dutkiewicz (2002); Oneţ et al. (2018), Ilieş et al. (2018, 2019), Indrie et al. (2019) studied indoor air quality of museums, historic wooden churches and sport halls; concerning the airborne fungal in wine cellars, the studies of Haas et al. (2010) and Khan and Karuppayil (2012). Zavrl (2012) analyses indoor climate in the cellars from building heritage in Slovenia. Similar research has been done in other underground indoor environments such as caves, mines, etc. Concerning the caves, a series of studies were performed, of which we mention: Zhou et al. (2007) presenting the fungal world of cave ecosystems of China; Vaughan et al. (2011) regarding the caverns from Arizona. Other studies mentioned: Vanderwolf et al. (2013); Frączek and Kozdrój (2013); Frączek et al. (2013) studied airborne bacteria and fungi in subterranean and earth sanatoriums. Man et al. (2015) investigated fungi in caves located in central China. Other papers: Out et al. (2016) and more recently Rawat et al. (2017). Gębarowska et al. (2018) have analysed airborne bacteria and fungi in salt mines in Poland; Roohi et al. (2012) analysed bacteria from salt mines in Pakistan. Pusz et al. (2014) about airborne fungi in a copper mine and the study conducted by Rdzanek et al. (2015) in a coal mine.

Regarding fungi, yeasts and slime moulds in caves, papers were written by researchers such as: Shapiro and Pringle (2010) concerning anthropogenic influences on the diversity of fungi isolated in caves located in Kentucky and Tennessee. Other papers: Novakova (2009), Ogorek et al. (2013, 2014); Vanderwolf et al., (2013) etc. Docampo et al. (2011) wrote about fungal spore content of the atmosphere in the Cave of Nerja, Spain; Kuzmina et al. (2012) on microbiota of the Kinderlinskaya Cave (Russia) and Xianshu et al. (2008) about microorganisms deteriorating the earth heritage in Cambodia. Nieves-Rivera (2003) wrote on the mycological survey of a cave from Puerto Rico.

METHODS

The relative air temperature and humidity inside the cellar were measured using *Klimalogg Pro thermo-hygrometer* between April and May 2018, seven sensors and the central unit being installed. The sensors transmit the data from a maximum distance of 100 m with a frequency of 868 MHz. Also, the air temperature measurement range is -39.6°C to 59.9°C and air humidity ranges from 1% to 99% with 1% margin.

Inside the cellar, the sensors were located at a height of 1.5 m and the temperature measured by each sensor varied by 0.1°C.

The fungal contamination was determined using the conventional techniques of open plates called Koch sedimentation method (Cernei et al., 2013). The sedimentation method consisted in exposing the Petri plates with a diameter of 100 cm2 that contain Sabouraud agar medium with the addition of chloramphenicol (0.5 g/l) for the qualitative determination of fungi (Vanderwolf et al. 2013). The plates were exposed for 4 hours. A group of two plates were exposed in the middle of the storage cellar at the height of the table (60 cm from the floor), two plates in a corner, on the floor and two plates were exposed up, at the ceiling level. The exposure was carried out by opening the lids of Petri plates and their placement with opening part towards the ground, altogether with the culture medium plates. After exposure, the Petri plates were coated and transported to the laboratory where they were incubated in a thermostat for 10 days at 25oC in the dark to allow for the development of slow-growing colonies. For carrying out the plans, facades and volume measurements, the following software programs were used: Archicad (2018), Artlantis (2017), Adobe Illustrator CC (2017) and Adobe Photoshop CC (2015).

RESULTS AND DISCUSSION

Most of the cellars are made of burnt brick, but there are also cellars made of wood or mud brick and thatched roof. Their layout (Figure 3) includes: a hall where, if the surface allows, grapes are pressed and wine is made. Then, another room allows the passage to the cellar room, often named "the neck" by the locals, the storage place of various products (beverages, wine, juices, compotes, "pălincă" – traditional fruit spirit beverage) and different supplies (fruit and vegetables, pickles, etc.).

This study contains an analysis of the air temperature and humidity inside a cellar in Sălacea, Bihor county, between 02/04/2018 and 02/05/2018. Thus, the average air temperature inside the cellar was 10.2°C. The highest air temperature recorded inside was 15.1°C on 2nd May 2018 at 6:00 p.m., and the lowest air temperature was 3.9°C on 02/04/2018 and 03/04/2018 at 6:00 a.m. (Figure 4). It should be noted that the temperature pattern inside the cellar was determined by the outdoor temperature (Figures 4 and 5), the outdoor minimum and maximum air temperature values during the monitored period in Sălacea were between 4°C on 02/04/2018 and 03/04/2018 and 32°C on 02/05/2018. These values occurred against the background of a specific atmospheric circulation above Europe characterised on 03/04/2018 by high pressure in Romania, 1,015-1,020 hPa at the geopotential height of 500 hPa, meanwhile in the Western and Northern Europe two depresionary cores with centre pressure of just 885-900 hPa (map of 3rd March, with geopotential height of 500 hPa) were developing, situation similar with the one of 02/05/2018, when the maximum temperature for the reported period was recorded. At that time Romania was still covered by a high pressure field, 1,010-1,015 hPa in the troposphere of 500 hPa, and the Western and Northern Europe was under the influence of the depresionary baric condition with 985-990 hPa in the centre (map of 02.05.2018 at the level of 500 hPa). Therefore, we note that both thermal extremes of the reported period have occurred in the same type of atmospheric circulation, predominantly with the baric condition high in altitude, therefore they were not determined by a different atmospheric circulation but by the radiative warming of the crust which manifested stronger at the end of the analysed monitoring period, together with enhancement of the solar radiation and increase of the sunlight specific to spring months.

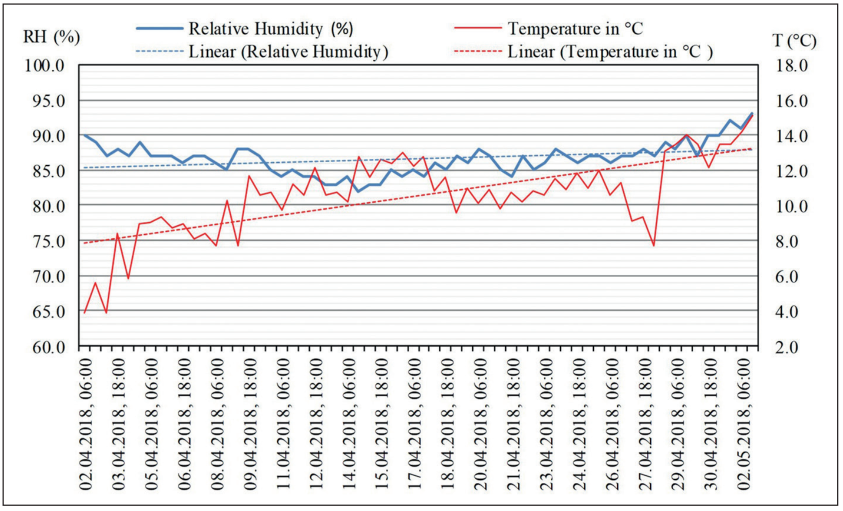

Figure 4
Variation of the air temperature and the concentration of relative humidity indoor of the cellar studied in Sălacea during the period 02/04/2018 – 02/05/2018.

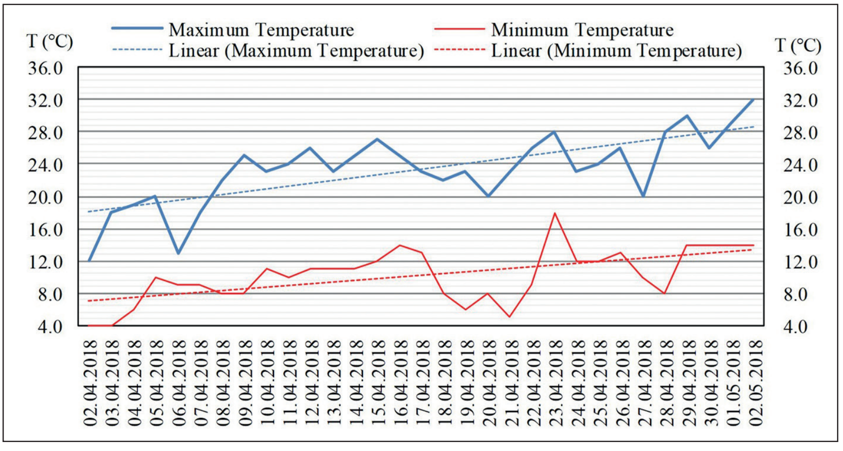

Figure 5
Variation of the outdoor air temperature in Sălacea during the period 02/04/2018 – 02/05/2018.

Moreover, the times of the day when the maximum and minimum temperatures occurred inside the cellar were determined by the moments they were produced outdoor, but they were delayed by 1-2 or even 3 hours due to the thermal inertia generated by the cellar; this inertia was caused by the topographical conditions where the cellar is located as well as the construction method and the materials used for building it, even if the cellar had the doors open for ventilation this time of the year.

The optimal storage temperature for fruit and vegetables ranges between +1 and +5°C, while for beverages and juices it ranges between 4-20°C and 7-12°C, for wine (8-10°C in the case of white wines and 10-12°C for red wine) (Budiș, 2004; Ieșeanu and Celac, 2007; Order no. 359/671/137 of 2002). A higher temperature speeds up the ripening of fruit and contributes to beverage spoilage.

Therefore, temperature inside the cellar during the monitored period ranges within the optimal parameters for the storage of wine, fruit and vegetable juice.

Humidity is another parameter that needs to be monitored. In rooms where fruits are stored, humidity must range between 85-90%, while for beverage storage (fruit and vegetable juice) this should be maximum 75% (Order no. 359/671/137 of 2002) and it may reach 80% for wine storage. A relative higher humidity allows mould to develop, thus contributing to the change of colour, taste and smell of the stored product.

The average value of air humidity inside the cellar was 86.9%, the biggest value of air humidity was 93% on 02/05/2018 at 6:00 p.m., and the minimum value was 82% on 14/04/2018 at 6:00 p.m. It should be noted that the maximum humidity value was recorded on the same date as the maximum temperature (02/05/2018) due to an advection of warm and humid air, as opposed to the minimum humidity value which occurred on a warm and dry day, as a result of a tropical dry-air mass originating above the Arabian Peninsula (map of 14th April at the geopotential of 850 hPa).

Humidity inside the cellar during the monitoring period is not covered by the optimal storage parameters for fruit and vegetable juices; however, it meets the parameters for storage of alcoholic beverages (spirits and wine) as well as for fruits, the recorded values are acceptable taking into consideration that the doors of the cellar were open for ventilation purposes, thus being created the conditions for a higher humidity than the one achieved in a closed space.

Fungi are well known components of the bioaerosols in wine production and storage cellars. They are usually found on the surfaces of the walls and wine bottles and may be released into the air during work operations and air movements in cellars (Piecková, 2016; Mandl et al., 2010).

Investigations on the diversity of fungi were made in the air inside the storage cellars from Sălacea village. A quantitative interpretation of the results describing the air quality inside the storage cellar is very difficult. After the morphological comparisons of the fungi on Sabouraud agar (phenotypic characteristics, such as colour, shape, size, colony surface texture, hyphal pigmentation) (Kirk et al., 2011), samples of fungal colonies were aseptically sampled for staining with lactophenol cotton blue and characterising spore and hyphae. Based on the microscopy analysis of fungi isolated colonies, the conidiophores and conidia fungal structures were examined with the Optical Microscope (Optic 40X). After this procedure members of the genus were microscopically identified: *Cladosporium*, *Penicillium*, *Aspergillus*, *Trichoderma*, *Ulocladium*, *Geotrichum*, *Fusarium* and *Alternaria* (Table 1).

Table 1 Cultural and morphological characteristics of the airborne fungi identified in the wine cellars in Sălacea

IDENTIFIED FUNGI	CHARACTERISTICS
Cladosporium sp.	Are the most frequently isolated airborne fungi, some species are pathogenic to humans; the colonies are olivaceous-black, suede-like to floccose; at microscope the conidiophores are straight and it can be observed the presence of shield-shaped conidia with a distinct hilum.
Penicillium sp.	Is a saprophytic fungus with green colonies, dense brush-like spore-bearing structures, simple conidiophores terminated by clusters of flask-shaped phialides.
Aspergillus sp.	Some species are saprophytes and other can be pathogenic to human beings; this fungus develops white colonies, brown to black with shades of green, distinctive conidial heads with flask-shaped phialides arranged in whorls on a vesicle.
Trichoderma sp.	Very common fungi, an opportunistic pathogen to humans, the colonies are white-yellow, conidiophores are highly branched and terminate in one or a few phialides, conidia are ellipsoidal.
Ulocladium sp.	Plant pathogens and food spoilage agents can cause serious infection in immune-suppressed individuals, the colonies are brown-black, woolly to cottony; present septate brown hyphae, brown conidiophores and brown conidia with round to oval shape.
Geotrichum sp.	Is a yeast that may cause opportunistic infections usually acquired via ingestion or inhalation; develop white, dry, powdery colonies, arthroconidia are unicellular, in chains, hyaline. The blastoconidia, conidiophores and pseudohyphae are absent.
Fusarium sp.	Opportunistic pathogens in humans, may cause storage rot, colonies are pale or bright-coloured with a cottony aerial mycelium, macroconidial are hyaline, fusiform, microconidia are one or two-celled, fusiform to ovoid, smaller than macroconidia.
Alternaria sp.	Plant pathogens, some species are causative agents of mycotic keratitis and onychomycosis, the colonies are olivaceous-black, conidia are oblavate, darkly pigmented, with short conical beaks.

The walls of the storage cellar were covered on a large surface by colonies of *Cladosporium* (*Zasmidium*) *cellare*, often referred to as a noble mould (Figure 6). This is a greenish-black sponge-like mould. According to the former practitioners, this mould is intended to provide optimal conditions for wine storage in cellars; in particular, it regulates air humidity and purifies it (Clemenz et al. 2008). *Cladosporium* is a potential allergenic mould.

Figure 6
Cladosporium cellare on the wall of the storage cellar in Sălacea

Fungi may develop in a wide variety of habitats. Different species of fungi require different conditions for optimal growth. Microbial metabolism is significantly influenced by the physical and chemical environment. Storage fungi, with lower requirements of humidity, are mainly the genus *Aspergillus* and *Penicillium*. Temperature is an important environmental factor affecting growth and mycotoxin production by moulds. Fungi are capable of surviving under the full range of temperatures normally experienced in environments in which they live. The temperature range usually reported for fungal growth is broad (10-35°C), with a few species capable of growth below or above this range. In general, the optimal temperature for mycotoxin production is below the optimal one for growth. The production of different mycotoxins by the same species is also related to the temperature level. For the food storage and wine production the temperature is the crucial variable. The rate of chemical reactions increases with temperature. The other impact of temperatures is variation, either on short term (e.g. diurnal variation) or long term (e.g. seasonal). Temperature variation is likely to be more critical. Moisture requirements of foodborne moulds are relatively low. Moisture control is the best and most economical means to control the environment in order to prevent mould growth and mycotoxin production. The simultaneous presence of different microorganisms, such as bacteria or other fungi, could disturb fungal growth and the production of mycotoxins. Therefore, several microorganisms have been reported as biological pest control agents (Centeno and Calvo, 2002). Diversity of fungi in the storage cellar may be influenced by environmental conditions to a certain degree (Magyar et al., 2016; Haas et al., 2010).

The pathologies which can develop in people working many hours a day in the cellar are those which result from work accidents (Murphy, 2014). Therefore, among the most frequent causes of these accidents we mention: incorrect handling of objects; presence of obstacles, low light etc. Also, animals getting inside the cellars may lead to contagious diseases such as *hydatid disease*, *toxoplasmosis*, *leptospirosis*. Intoxication with carbon monoxide caused by the fermentation process (Sami, 2006) may lead to loss of consciousness, sleep apnoea and arrhythmic disorders etc., thus carbon monoxide represents an invisible danger being a colourless and odourless gas which gets inside the body through the airway. Carrying out the professional activity in a high-humidity environment with a relative low temperature favours the development of rheumatic pathologies and it exacerbates the symptoms of already existing rheumatic suffering (Wilder et al. 2003; Zeng et al. 2017; Dahlberg and Grubb, 2008).

The role of indoor fungi in the onset and development of non-infectious diseases, such as allergy and asthma, is known for a long time (Khan and Karuppayil, 2012). Generally speaking, fungi can cause lung disease in two ways: either as airborne allergens or as infection which causes a pathogen. Some types of fungi can affect lungs in both ways, often simultaneously. The most frequent fungus which causes lung infections is *Aspergillus fumigatus*. The allergic fungal agents that can cause rhinitis and asthma but they can also determine infections include the spores belonging to *Cladosporium spp*. and *Alternaria spp*. The volatile organic compounds and microtoxins released by moulds, such as *Stachybotrys spp*. (Denning et al., 2014) represent a third potential cause of fungal disease.

Systematic microclimate monitoring and fungal analysis inside wine cellars may support their preservation, but also ensure optimal conditions for the safety of products which are stored indoor and for the health of the locals and their visitors. The research could be extended to a larger number of cellars and the indoor microclimate and fungi monitoring could become permanent. All these could contribute to the wider action of registering the cellars on the national cultural heritage list (in case it is resumed because it had failed a long time ago; Matlovicova et al. 2014, 2016, 2017). Taking into consideration the structural features and the conditions inside the cellar, there can be risks for the health of people (visitors, locals, workers etc.). Pathologies which can be developed in people who work several hours a day in the cellar represent the cause of work accidents; development or worsening of chronic pathologies. Humidity inside the cellar during the monitored period does not fit into the optimal parameters for fruit and vegetable storage. The control of humidity inside a cellar must not be neglected because humidity is one of the main factors which have an influence on the products stored. In this respect several measures may be taken in order to ensure an optimal humidity inside a cellar: cleaning the space of the cellar from the harvest that was not used the previous year; using furniture antiseptics; long-term storage of salt and lime; using professional dehumidifiers which can dry walls in depth. Moreover, the air quality inside the cellar and its constant freshening are significant for long-term storage of the fruits, vegetables, wines etc. under optimal conditions. Therefore, ventilation must be constantly provided so that the cellar is protected from strong air streams. It would be appropriately to have a ventilation system made of pipes: a pipe with the lower inlet should be installed near the floor in one corner of the cellar and another pipe with the lower inlet should be placed towards the ceiling of the cellar in the other corner so that the cold air gets in through the floor pipe and hot air gets out through the other pipe. This type of ventilation system may prevent the cellar from odours and becoming mouldy. The information obtained from qualitative analyses of fungi in the Sălacea storage cellar can be a useful tool for controlling indoor air quality. The constant presence of some moulds may be hazardous to human health and may potentially contaminate wines. Inside the Sălacea cellar the *Cladosporium cellare* is dominant genera among most of the common mycoflora in storage cellar, a potential allergenic mould.

CONCLUSIONS

One of the main contributions of the study is to provide the knowledge and data needed to support cellar owners in taking informed action to maintain an optimal microclimate in such a storage space.

The data obtained can be used by local authorities to support cellar owners in using the necessary technological devices to obtain the right product storage environment, thus contributing to the development of the local economy.

In the future it is intended to extend the research on a larger number of cellars.

Acknowledgement

The monitoring and sampling inside of the authentic cellar are not invasive. The research was possible by equal scientific involvement of all authors. The authors would like to thank to anonymous reviewers for their thoughtful suggestions and comments and to acknowledge the support of the grant PN-III-P1-1.2-PCCDI-2017-0686.

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