



Airborne microorganisms in a subterranean archaeological area of the basilica of San Lorenzo in Lucina (Rome)

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Abstract

The basilica of San Lorenzo in Lucina in Rome was built in the fifth century on the foundations of a Roman insula used for dwellings and commercial activities, where the first Christian communities had held their meetings. This archaeological area is still rather isolated since it can only be visited by groups for one/two hours once a month. Such a situation is ideal for studying both the quantity of microorganisms in the air under undisturbed conditions and/or conveyed by visitors. The biodeterioration of subterranean remains is determined by the development of bacteria, cyanobacteria, actinomycetes, algae and fungi. On the walls of this underground archaeological area the main form of alteration is the efflorescence.

In order to compare the stone and airborne microflora, the microorganisms living on stone and the airborne ones have been detected. Air samples were taken both during the visits and under non-turbulent conditions utilizing three sampling methods: gravitational, intake by multi-stage impact and intake by single-stage impact.

The results have made it possible to determine the microbial content of the air, as well as the sampling schedule required for rarely visited underground environments. Furthermore, a comparison of the three methods used showed that the adoption of one or the other sampling method gave different complementary information on airborne microflora.

Introduction

Downtown Rome rises on the archaeological remains of a subterranean city, from which only a few areas have been excavated and made accesible to visitors.

Such areas have high relative humidity during both summer and winter, with a more or less constant temperature, and clearly show signs of biodegradation caused by the development of microflora suited to a subterranean environment (Agarossi et al., 1985; Agarossi et al., 1994; Albertano and Grilli Caiola, 1989).

As early as 1974, Lefèvre called attention to the risk of the degradation of subterranean environments as a result of excessive public access. On the walls of the Lascaux cave, covered with prehistoric drawings and paintings, a patina of biological origin had

formed after a period during which 500–600 people had visited the caves every day. The visitors were so many that the temperature and CO₂ of air increased so that algae growth was favorable. A systematic study is therefore necessary to discover the effects produced by the presence of visitors, with the aim of reaching a regulated visitor flow so that no negative effects can take place (Monte et al., 1997).

The present study gives data for the microbial load both in the air and on the walls of the archaeological area below the Basilica of *San Lorenzo in Lucina* in Rome. This area has been open to the public for about ten years, having been excavated between 1982 and 1987 (Bertoldi, 1994). It consists of a Roman insula, where the first Christian communities used to meet, and urban buildings of the II and V centuries. The upper part includes two small slits which open

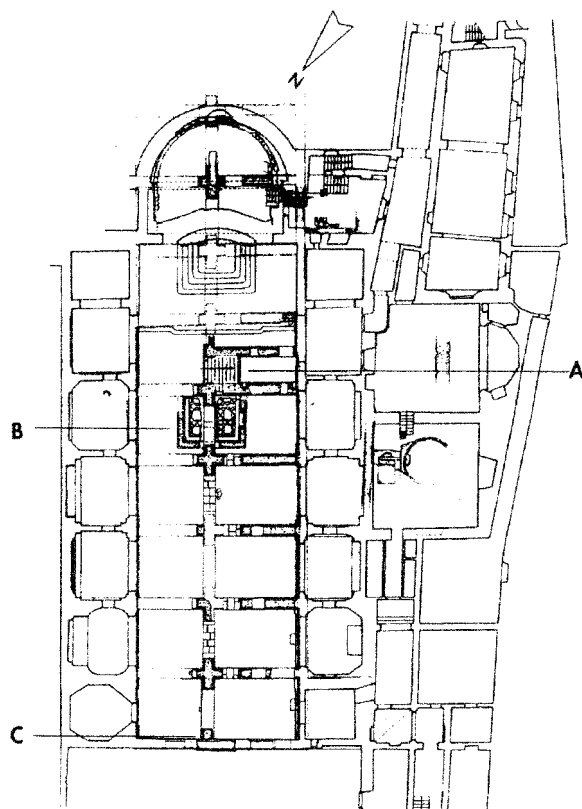


Figure 1. Map of archaeological subterranean area of *San Lorenzo in Lucina*: – area for sampling 1–2 (A); – area for sampling 3–4 (B); – area for sampling 5–6 (C).

on the square, acting as air intakes and ensuring a slow change of air (Figure 1). The area is reasonably isolated, since it can only be visited by groups once a month, which makes it ideal for ascertaining the quantity of microorganisms in the air under undisturbed conditions and/or produced by the presence of visitors. In order to compare microbial concentration in the air and on the stone surface, air samples, both prior to and during visits, and stone samples were analysed. Indeed, besides estimating of the microbial air-load of a rather isolated environment, the aim of the work was also to verify the most suitable sampling methods for biological air research for the purpose of studying the colonization of underground structures by microorganisms.

Materials and methods

Cultural analysis were performed with the aim of discovering:



Figure 2. Type of deterioration: whitish efflorescence.

- the degree of biodeterioration of the stone structures
- airborne microflora under normal conditions and after the presence of groups of visitors

Biodeterioration of the stone substratum

Cultural and microscopic tests were performed on samples taken from the deteriorated archaeological remains of the underground environment. The type of deterioration found was a widespread whitish efflorescence (Figure 2, 3).

The microscopic and culture techniques are as indicated by Normal 9/88 and Normal 19/85. The following culture media were used:

- mycological agar (Difco) for developing fungi
- nutrient agar (Oxoid) for developing heterotrophic bacteria
- Kuster & Williams medium for developing actinomycetes (Normal 9/88).

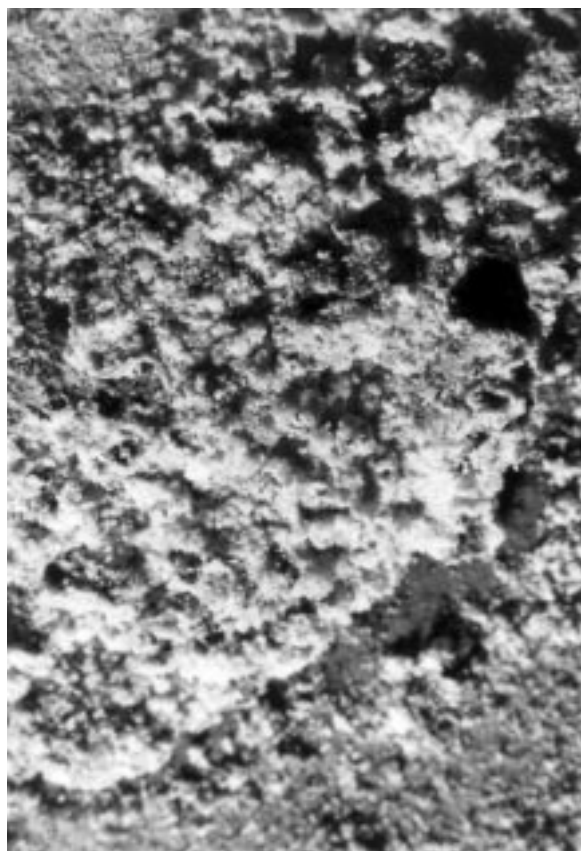


Figure 3. Whitish efflorescence: detail.

Analysis of microbial airload

The analysis of airborne microorganisms was performed by means of air intake over culture media and by gravitational settling of the spores on 10 cm diameter Petri dishes left open for one hour (Normal 39/93). For air intake, the following sampling systems were used:

- SAS (surface air system), in which the air is drawn from 6 cm diameter Petri dishes containing culture media for developing micro-organisms. Air intake speed was 180 liters/minute.
- Andersen, with the air intake speed of 30 liters/minute on 10 cm diameter Petri dishes placed at six different stages. The air flow allows the particles to settle progressively according to spore size.

Table 1. Microbial load of the stone structures of the archaeological area in San Lorenzo in Lucina.

Sample	Bacteria	Actinomycetes	Fungi
1.	12×10^3	12×10^5	4.6×10^3
2.	53×10^3	49×10^5	37×10^2
3.	77×10^4	39×10^5	25×10^3
4.	32×10^4	69×10^5	51×10^3
5.	71×10^2	115×10^5	43×10^3
6.	25×10^2	67×10^5	49×10^3

the number of microorganisms refers to 1 gram of sample. Triple tests were performed.

Table 2. Number of CFU settling by gravity in 1 hour on the culture medium.

Sample	Bacteria	Actinomycetes	Fungi
1.	7	50	27
2.	6	48	31
3.	5	67	33
4.	8	71	35
5.	3	81	21
6.	5	79	25

Results

The stone structures belonging to the archaeological site show an advanced degree of colonization by microorganisms causing biodeterioration (Table 1). The actinomycetes in particular have found an ideal habitat for their development: a high degree of relative humidity and relatively stagnant air.

The number of CFU deposited gravitationally in one hour on sedimentation dishes could be compared to the proportion found on the stone structures: a higher number of actinomycetes and, progressively, a lower number of fungi and bacteria (Table 2).

The number of CFU/m³ of air, using the SAS sampling method as compared to the value found using the Andersen equipment, is considerably lower for actinomycetes and fungi. Proportions are similar only for bacteria, most probably due to their limited number. The smaller diameter of dishes used for air intake SAS clearly limits CFU development (Table 3).

The presence of visitors gives rise to a change in airborne microflora amount. An analysis performed using the multi-stage Andersen sampling method shows double the number of fungi and actinomycetes, whereas the number of bacteria remains almost constant. Evidently, rather than introducing other micro-

Table 3. Number of CFU/m³ of air according to the SAS and Andersen sampling methods.

Sample	Air-samplers	Bacteria	Actinomycetes	Fungi
1.	SAS	233	588	825
	Andersen	570	2.070	1.035
2.	SAS	270	650	769
	Andersen	625	1.990	1.325
3.	SAS	350	527	990
	Andersen	390	1.890	1.375
4.	SAS	370	621	879
	Andersen	310	1.975	1.281
5.	SAS	366	483	925
	Andersen	420	1.290	1.380
6.	SAS	372	575	890
	Andersen	380	1.340	1.268

Table 4. Number of CFU/m³ of air under normal conditions and after the passage of visitors.

Sample	Sampling conditions*	Bacteria	Actinomycetes	Fungi
1.	A	540	2.070	900
	B	480	4.110	2.250
2.	A	311	2.250	780
	B	403	3.450	1.571
3.	A	557	1.997	990
	B	438	2.371	2.340
4.	A	627	2.121	1.217
	B	650	3.458	2.371
5.	A	320	2.290	1.380
	B	340	2.960	2.160
6.	A	271	2.525	1.978
	B	250	2.851	2.986

Legenda

A: samples taken without visitors.

B: samples taken after the passage of a group of Visitors.

flora from outside, the presence of visitors stirs the air inside as well as floor dust, facilitating the dispersion of fungi and actinomycetes conidia (Table 4).

The ratio of settled microflora to the airload shows greater difficulty in gravity sedimentation for bacteria than for fungi and actinomycetes, of which one settles for every 20-40 CFU in the airload. The ratio of substratum microflora to the airload shows that fungi have greater diffusion capacity, whereas the number of actinomycetes on the substratum are about 2000 times higher than airload (Table 5).

Table 5. Numerical ratio of microorganisms: CFU in 1m³ of air to settling microbes in 1 hour (A) CFU in stone structures to airborne microbes (B).

Sample	Bacteria		Actinomycetes		Fungi	
	A	B	A	B	A	B
1.	81	21	41	579	38	4.4
2.	104	84	41	2462	42	2.7
3.	78	95	28	2063	42	18
4.	39	99	28	3493	37	39
5.	60	98	16	4914	65	31
6.	76	14	17	5000	51	39

Conclusions

The above data show an advanced state of biodegradation in the stone structures of this particular underground environment. Its microbial ecosystem shows a prevalence of actinomycetes. This indicates a strong specialization by the actinomycetes, with a consequently high degree of substratum colonization. A comparison of the number of CFU settling by gravity in one hour with the airload and with the ones colonizing the stone substratum gives a different ratio according to the microflora analyzed. Bacteria, which are rather numerous in the substratum, are not widely diffused in the air and have difficulty in settling. Fungi show greater interchangeability between air and substratum, as well as settling more easily. The behaviour of actinomycetes is halfway between fungi and bacteria. These data represent an initial attempt to establish a numerical proportion among both airborne and settling biodeteriorating microflora. This was possible because the environment, which is rather isolated, does not undergo major fluctuations due to external disturbances. A comparison of the two air intake sampling methods demonstrates that the SAS method is not very suitable for research on total microflora, since plate diameter is too small for colony development. It can be used in environments where airborne microflora is rather rare, or else to highlight particular groups of microbes by means of highly selective culture media. The Andersen method was found more suitable for general research on airborne microflora.

The presence of visitors certainly increases the microbic airload in particular where fungi and actinomycetes are concerned. The analysis have showed that the shape and colour of microbial colonies present

in the air were similar to those isolated from stone substrate. This can mean that, rather than an introduction of microorganisms from outside, the colonies are being more widely dispersed in the air due to the movement of visitors.

Indeed, such an increase only involves those microorganisms which produce vegetative spores, such as fungi and actinomycetes. To what extent this can accelerate the deterioration phenomena can be seen over a period of time which is long enough to highlight significant changes in microflora from both a quantitative and qualitative point of view, a task that will be dealt with by future research.

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