### ORIGINAL ARTICLE

# Passive air sampling: the use of the index of microbial air contamination

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Summary. Background: Bioaerosol plays an important role in human life with potentially infectious, allergic and toxic effects. Active and passive methods can be used to assess microbial air contamination, but so far there is not a unanimous consensus regarding the indications about methods to be used and how to interpret the results. The passive method has been standardized by the Index of Microbial Air contamination (IMA). Classes of contamination and maximum acceptable levels of IMA have been proposed, related to different infection or contamination risks. The aim of this study was to provide information about the use of the passive sampling method, with reference to the IMA standard. Methods: We searched PubMed and Scopus for articles published until January 2020 reporting the citation of the article by Pasquarella et al. "The index of microbial air contamination. J Hosp Infect 2000". Only studies in English language where the IMA standard was applied were considered. Studies regarding healthcare settings were excluded. Results: 27 studies were analyzed; 12 were performed in Europe, 8 in Asia, 5 in Africa, 2 in America. Cultural heritage sites, educational buildings and food industries were the most common indoor monitored environments; in 8 studies outdoor air was monitored. Conclusions: This review has provided a picture of the application of standard IMA in different geographic areas and different environments at risk of airborne infection/contamination. The analysis of the results obtained, together with a wider collection of data, will provide a useful contribution towards the definition of reference limits for the various types of environments to implement targeted preventive measures.

**Key words:** air sampling, bioaerosol, IMA, indoor, outdoor, passive method.

#### Introduction

Bioaerosol plays an important role in human life with potentially infectious, allergic and toxic effects (1-5). Measuring microbial air quality is a fundamental step for risk management (6-8): it allows to confirm the presence of biological agents, identify critical situations and validate the preventive measures adopted; air sampling is also a useful tool for scientific research, quality assurance and educational purposes. So far,

there is not a unanimous consensus regarding the indications for air sampling, what method should be used, and how to interpret the results in order to implement targeted preventive and control measures. Methods used for microbial air sampling can be classified in two categories: passive and active (6, 9). The active method allows the measurement of the concentration of culturable microorganisms in the air and is based on the use of some devices which collect a known volume of air, blown on to a nutrient media; the results are ex-

pressed as colony forming unit per cubic metre (CFU/ m³). Several types of devices are available, such as air impactors, impingers, centrifugal machines or filtration systems, which differ for biological and physical efficiency therefore providing different results, difficult to compare. The passive method measures the rate at which microorganisms settle on surfaces; it is based on sedimentation and relies on the use of settle plates being exposed to air for a defined period of time; results are expressed as CFU/plate/time. The passive method has been standardized by the Index of Microbial Air contamination (IMA) which corresponds to the number of CFU counted on a Petri dish (9 cm in diameter) left open to the air according to the 1/1/1 scheme (for 1 hour, 1 meter above the floor and about 1 meter away from walls and major obstacles) (10). The IMA can be expressed also as CFU/m² or dm² or cm²/time. Five classes of IMA have been defined, representing a different increasing level of contamination: 0-5 very good; 6-25 good; 26-50 fair; 51-75 poor; ≥76 very poor. Maximum acceptable values of IMA have been proposed, related to different infection or contamination risks; these are 5, 25 and 50, in places at very high, high and medium risk, respectively (10). It is up to whoever is in charge to state the level of infection risk and adopt the corresponding maximum acceptable IMA level.

The aim of this study was to provide information about the use and diffusion of the passive sampling method for assessing the microbial air quality, with reference to the IMA standard (10). This paper deals with the results regarding non-healthcare settings.

#### Methods

We searched PubMed and Scopus for articles published until January 2020 reporting the citation of the article by Pasquarella et al. "The index of microbial air contamination". J Hosp Infect 2000. Only studies in English language where the IMA standard was applied were considered. Studies performed in healthcare settings were excluded and will be object of a specific paper. Only studies using nutrient media for total bacteria and/or fungi count were included. When the exposure of settle plates was longer or shorter than one

hour, values measured in the sampling time considered were proportioned to one hour. The studies were analysed with reference to the Countries, settings, monitored environments and results obtained.

#### Results

Figure 1 shows the flow diagram of the review process. The reference "The index of microbial air contamination" was reported in n. 187 articles, 151 from Scopus and 36 from PubMed. After the screening by title, 29 duplicates were identified and removed. After the exclusion of the reviews (n. 29) and the studies performed in healthcare settings (n. 66), n. 63 articles studies performed in non-healthcare setting were considered for the review. Articles in which the citation of "The Index of microbial air contamination" was not referred to the air sampling method used, articles written in other than English language (11-16) and

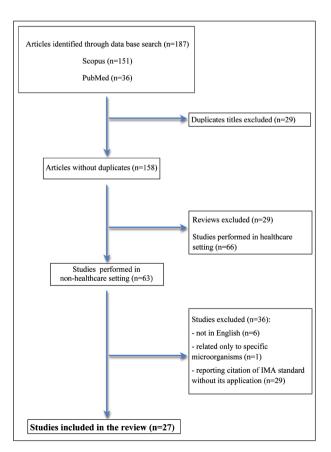


Figure 1. Flow diagram of study selection for review.

articles dealing with studies where specific microorganisms were searched (17), were excluded. A total of 27 studies were included in the review; in 25 studies quantitative or quantitative and qualitative air microbial contamination was evaluated (18-42); in 2 studies qualitative contamination only was evaluated (43,44).

Table 1 and Table 2 list the 25 studies yielding quantitative data, with reference, in particular, to the study setting, sampling period, sampling time and environments monitored, reporting the IMA values obtained for bacteria, fungi or total count. Ten studies were performed in Europe, including eight in Italy (18-22,24,26,31), one in Romania (39) and one in Norway (34); eight studies were performed in Asia, two in Malaysia (26,35) and one for each of the following countries: Iran (32), Israel (36), Japan (30), Thailand (33), Turkey (28), Vietnam (37); five studies were conducted in Africa, two in Ethiopia (38,41) and one for each of the following countries, Egypt (25), Nigeria (23), South Africa (29); two studies were conducted in America, one in the USA (40) and one in Cuba (42). Twenty-one studies (18-33, 38-42) evaluated indoor air contamination, mainly in cultural heritage sites, educational buildings and food processing plants; five of these studies assessed also the outdoor microbial contamination (25,31-34); three studies evaluated only outdoor air quality (35-37). In five studies (38-42), listed in Table 2, the air was sampled using the IMA standard scheme, but the results obtained (CFU/ plate/time) were transformed in CFU/m³ by using the Omelyansky's formula:  $N = 5a \times 10^4 \text{ (bt)}^{-1} \text{ where } N =$ CFU/m³, a = number of colonies per Petri dish, b = dish square centimeter, t = exposure time (min.) (45), based on the estimation that on the area of 100 cm<sup>2</sup>, in 5 minutes are deposited as many microbes as there are in 10 m³ of air. Table 2 reports both the original values in CFU/m³ and the IMA values obtained after the conversion based on the Omelyansky's formula.

As for cultural heritage sites, six studies were performed in Italy (18-22,24) and two in Africa (Nigeria and Egypt) (23,25). Considering the Italian studies, bacterial air contamination values ranged from 0 to 35 IMA without visitors (21); fungal contamination increased during opening time up to 48 IMA (20). Higher values were found in a museum library in Nigeria, where the heaviest microbial contamination means

both for fungi (73 IMA) and bacteria (30 IMA) were found during the rainy season compared with the dry season (23). Fungal contamination values found in an Egyptian museum, where six rooms were monitored, ranged from 1 to 256 IMA, with median values from 8 to 30 IMA (25); in this study also outdoor environment was monitored, and indoor /outdoor ratio confirmed that outdoor environment was the main source of indoor fungal pollution. Microbial air contamination in educational buildings was evaluated in 6 studies. Di Giulio et al. (26) performed a study in 14 University research laboratories located in three different buildings over a period of six months, in the morning and in the afternoon; the IMA values showed a seasonal fluctuation of total microbial contamination, which were always within the threshold values of 50 and 25 IMA defined respectively for the common laboratory rooms and for the bacteriology laboratory with a controlled microbial charge. An IMA value below 25 was also found in the University Tissue culture Laboratory in Malaysia by Chong et al (27); in this study the lowest bacterial mean contamination values were found in the Top Management Office, from 5.72 IMA in the morning to 3.81 IMA in the afternoon, while the highest mean IMA value (27.98) was found in the library. Other four studies, carried out in educational buildings, 2 in Ethiopia (38,41), 1 in Romania (39) and 1 in the USA (40), converted the IMA value to CFU/m<sup>3</sup> by using the Omelyansky's formula. Going back to CFU/ plate/time values (IMA) we found in a University microbiology laboratory in Romania (39) a mean charge converted value of 13.38; about classrooms, mean fungal contamination values ranged from 11.42 to 63.25. In a University dormitory in Ethiopia (38) very high values up to 760 IMA for bacteria and 501 IMA for fungi were reached. In Cuba, Anaya et al. (42) monitored the fungal contamination for a period of nine months in two food production plants, one for artisanal chocolate and one for products for special regime plant; IMA values, calculated from the CFU/m³ obtained by Omelyansky's formula, ranged from 0 to 125 IMA. In the study by Scholtz (29) fungal contamination was assessed along the pear export chain from South Africa to the UK over a three year period, obtaining a median range from 52 to 1725 IMA, with a median IMA value of 201. The assessment of indoor airborne fungal

on Study setting         Sampling period environments period         monitored environments environments         time/ an analy mean range/ median         Mean (SD)/ median           heritage         Museum         January         7 rooms         before opening time         2.86* 17         17           Library         April         8 rooms         during opening time         11.51* 3*         0*           Library         May         3 rooms         during opening time         8         3           Library         Spring         1 room         8         3           Library         Spring         1 room         5 (3) - 22 (4)           February         September         1 room         3 3.33           Library         September         1 room         3 3.33           April - June         14 rooms         3 3.33           April - June         Ab. B.C         in the morning (m)           University         Assearch         in the afternoon (a)	Country	÷	Jo.N.	Sampling	Bacteria (IMA)	IMA)	Fungi (IMA)	MA)	Microbial Total Count (IMA)
Ref   Fried   Fried   Fried   Reprintments   Ref   Fried   Reprintments   Ref   Fried   Research     18	ū	Sampling neriod	monitored	time/	Mean (SD)/		Mean (SD)/		
Indoor environments   Indoor environments	Ref)	portod	environments	condition	mean range/ <i>median</i>	Range	mean range/ <i>median</i>	Range	Mean/mean range/ <i>median</i>
tral heritage         before opening time         2.86*         1'           (19)         Library         April         8 rooms         during opening time         11.51*         3'           (20)         Museum         May         3 rooms         during opening time         11.51*         3'           (21)         Library         May         1 room         8         3         3         3           (22)         Library         Spring         1 room         7 (2) - 30 (3)         8         3         3           (23)         Library         Rainy season         1 room         5 (3) - 22 (4)         8         4           (24)         Library         May         1 room         3.33         0           Andal         Abril - June         14 rooms         1 rooms         3.33         0           Abril - June         Abril - Ju				Indoor envi	ronments				
(18)         Museum         January         7 rooms         before opening time         2.86*         1           (19)         Library         April         8 rooms         during opening time         11.51*         3°           (20)         Museum         May         3 rooms         during opening time         11.51*         3°           (21)         Library         July         1 room         8         3         3           (22)         Library         Spring         1 room         8         4         4           (23)         Library         Rainy season         7 (2) - 30 (3)         3.33         6           (24)         Library         Reprember         1 room         3.33         6           April - June         April - June         14 rooms         3.33         6           Abril - June           Abril - June         Research         in the morning (m.)         1.33         9           Abril - June         Research         in the afternoon (a.)         1.33         1.33	ral heritage								
(18)         Printed many Library         April April         8 rooms         during opening time         11.51*         3*           (20)         Museum         May         3 rooms         during opening time         0°           (21)         Library         July         1 room         8         3           (22)         Library         Spring         1 room         8         4           (23)         Library         Rainy season         7 (2) - 30 (3)         3.33         0           (23)         Library         Rebruary         3.33         0         3.33         0           (24)         Library         September         1 room         3.33         0         3.33         0           Abril - June         A, B, C         A, B, C         A, B, C         1.33         0           Abril - June         A, B, C           Abril - June         Research         In the afternoon (a)         A, B, C	M		7	before opening time	$2.86^{*}$	1* - 7*	0.45*	0* - 2*	
(20)         Library         April         8 rooms         during opening time         0°           (20)         Museum October October         July July December         1 room         8         3           (21)         Library Library Cason         Spring Dry season         1 room         7 (2) - 30 (3)           (23)         Library Cason         Rainy season         7 (2) - 30 (3)         3.33         6           (24)         Library December         May December         1 room         3.33         6           April - June April - June December         April - June Buildings April - June April - June April - June Buildings April - June April		January	/ rooms	during opening time	$11.51^{*}$	3* - 21*	*68.0	0* - 3*	
(20)         Museum         May Doctober         3 rooms         during opening time         8         3           (21)         Library         December         1 room         8         3           (22)         Library         Rainy season         7 (2) - 30 (3)           (23)         Library         Rainy season         7 (2) - 30 (3)           (23)         Pebruary         5 (3) - 22 (4)           Resument         1 room         3.33         0           April - June         14 rooms         1.33         0           Abril - June         14 rooms         A. B. C.         in the morning (m.)           Research         1aboratories         in the affermoon (a.)         Research		April	8 rooms			0* - 31*		0* - 3*	
(20)         Mutuseum         October         3 rooms         during opening time           (21)         Library         July         1 room         8         3           (22)         Library         Spring         1 room         7 (2) - 30 (3)           (23)         Library         Rainy season         7 (2) - 30 (3)           (24)         February         5 (3) - 22 (4)           Catomary         1 room         3.33         0           April - June         14 rooms         3.33         0           ational Buildings         A, B, C         in the morning (m.)         A, B, C           Coctober - December         A, B, C         in the afternoon (a.)         Buildings		May	6					1 - 480	
(21)         Library         July         1 room         8         3           (22)         Library         Spring         1 room         7 (2) - 30 (3)           (23)         Library         Rainy season         7 (2) - 30 (3)           (23)         Library         Rebruary         5 (3) - 22 (4)           (24)         Library         May         1 room         3.33         0           April - June         14 rooms         A, B, C         In the morning (m.)         1.33         0           Ab         October - December         A, B, C         in the afternoon (a.)         A, B, C         In the afternoon (a.)		October	o rooms	auring opening time -				0 - 100	
(21)         Library         Spring         1 room         8         4           (22)         Library         Spring         1 room         7 (2) - 30 (3)           (23)         Library         Rainy season         7 (2) - 30 (3)           (23)         February         5 (3) - 22 (4)           Rebruary         May         1 room         3.33         0           April - June         14 rooms         3.33         0           ational Buildings         A, B, C         in the morning (m.)         A, B, C           A, B, C         in the afternoon (a.)         Research           October - December         Iaboratories         in the afternoon (a.)	; I	July	<del>,</del>		8	3 - 35	1	0 - 3	
(22)         Library         Rainy season         1 room         7 (2) - 30 (3)           (23)         Library         Rebruary         Anay         1 room         3.33           (24)         Library         September         1 room         3.33           ational Buildings         April - June         14 rooms         A, B, C         in the morning (m.)           Research         October - December         Iaboratories         In the afternoon (a.)		December	1 room		8	4 - 15	2	0 - 3	
(23)         Library         Rainy season         7 (2) - 30 (3)           (24)         Library         May         1 room         3.33           ational Buildings         April - June         14 rooms         1.33           Abril - June         A, B, C         in the morning (m.)           Buildings         A, B, C         in the afternoon (a.)           Coctober - Iaboratories         Iaboratories		Spring	1 room				0 - 28*		
(23)         Library         February         5 (3) - 22 (4)           (24)         Library         May         1 room         3.33           ational Buildings         April - June         14 rooms         A, B, C         in the morning (m.)           Research         October - Iaboratories         Iaboratories         In the afternoon (a.)         Essence (d.)		Rainy season			7 (2) - 30 (3)		22 (3) - 73 (4)		
Tebruary   Rebruary   April - June   April - June   Buildings   April - June   Buildings   A. B. C. Cotober - Joecember   A. B. C.		Dry season			5 (3) - 22 (4)		13 (2) - 27 (5)		
(24)         Library September September         1 room Accember         1 room September         3.33           ational Buildings         April - June Buildings         14 rooms Buildings in the morning (m.) Research Buildings and Buildings Buildings and Buildings Buildings and Buildings Buildings and B		February			3.33	- 1	0.5	0 - 2	
(24)         Library September         1 room December         3.33           ational Buildings         April - June Buildings         14 rooms Buildings         in the morning (m.) Research December           (26)         University October - Iaboratories         A, B, C in the afternoon (a.) Research Iaboratories         In the afternoon (a.) In	F	May	<del>.</del>		3.33	1 - 6	3.67	6 - 0	
ational Buildings         April - June         14 rooms         in the morning (m.)           (26)         University         A, B, C Research December         in the afternoon (a.)         A Boratories		September	1 room		3.33	0 - 5	4.33	1 - 9	
ational Buildings  April - June 14 rooms Buildings A, B, C Research October - laboratories December		December			1.33	- 1	0.67	0 - 2	
April - June 14 rooms Buildings  University — A, B, C Research October - laboratories December	ıtional Buildings								
April - June 14 rooms Buildings Cotober - A, B, C Research December laboratories				'					A m. 1.1 - 41.8 a. 0.1 - 6
University ————————————————————————————————————		April - June	14 rooms						B m. 0.5 - 3.8 a. 0.5 - 4.3
October - laboratories  December			Buildings	in the morning (m.)					C m. 1.1 - 20.3 a. 0.7- 29
		-	Research	in the afternoon (a.)					A m. 0 - 11 a. 0 - 45
		October - December	laboratories	'					B m. 2.1 - 5.7 a. 0.5 - 4.2
									C m. 1.3 - 17 a.0.5 - 10.3

Table 1. Ch	Table 1. Characteristics of studies using the Index	lies using the In	idex of Microbial A	of Microbial Air contamination (IMA) standard	VIA) standard				
Country		.1:	N. of	Sampling	Bacteria (IMA)	IMA)	Fungi (IMA)	MA)	Microbial Total Count (IMA)
Publication	n Study setting	Sampining	monitored	time/	Mean (SD)/		Mean (SD)/		
year (Ref)		perion	environments	condition	mean range/ median	Range	mean range/ <i>median</i>	Range	Mean/mean range/ <i>median</i>
			5 environments i	in the morning (m.) in the afternoon (a.)					
	ı		University		m. 12.08*				
			Service Center		a. 5.09*				
			Top Manage-		m. 5.72*				
Malaysia			ment Office		a. 3.81*				
2017 (27)	University -		Tissue Culture		m. 6.99*				
			Laboratory		a. $17.8^*$				
			Cafè		m. 22.25* a. 15.26*				
	1		Library		m. 27.98* a. 27.98*				
Other environments	ronments								
				1 - 6	Spring 9.1 (5.7)		Spring 2.7 (1.7)		
				perore autopsy	Summer 27 4 (22 1)		Summer 16.7 (26.3)		
			ļ		(1.77) 1.77		(6.05)		
Turkey	Morgue	Spring -	Touchting I	***************************************	Spring 51.1 (17.1)		Spring 117.8 (271.6)		
2011 (28)	Department	Summer	ı autopsy toom	uming autopsy	Summer 60.9 (65.7)		Summer 99.3 (175.6)		
			l		Spring		Spring		
				after autonsy	21.6 (49.3)		13 (28.4)		
				arter autopsy	Summer 19.7 (21.6)		Summer 9.2 (10.5)		
South Africa	Fruit handling environments	Over three years period	11						27 - 900• 105•
7011 (73)									
Japan 2019 (30)	Animal housing system	November - January	1		0.3•		9.3•		

Country		÷	Jo.N.	Sampling	Bacteria (IMA)	[MA]	Fungi (IMA)	MA)	Microbial Total Count (IMA)
Publication year (Ref)	Study setting	Sampling period	monitored environments	time/ condition	Mean (SD)/ mean range/ median	Range	Mean (SD)/ mean range/ median	Range	Mean/mean range/median
				Indoor and outd	Indoor and outdoor environments	ts			
Norway 2009 (34)	Dry-cured meat production facility	February, August, December	16 rooms indoor outdoor	operational			15		
Iran 2014 (32)	School dormitory and retirement home	One year period	1 1			indoor 10 - 112 outdoor 15 - 96		indoor 11 - 36 outdoor 8 - 40	
	Fitness centre A (indoor)				2.09 (1.50)		0.97 (1.69)		
Thailand 2016 (33)	Fitness centre B (indoor)		3		8.44 (5.74)		5.07 (2.34)		
	Fitness centre C (outdoor)				7.52 (3.73)		5.59 (3.57)		
				at rest			10 - 76		
Italy	םמ.1. ל		3 Feeding rooms (outdoor)	operational			39 - 76		
2017 (31)	Dunaio iarms			at rest			92 - 9		
			3 Milking rooms (indoor)	operational			12 - 24		
Egypt 2020 (25)	Museum	Two years period	6 Buildings	working hours				1 - 256**	
				Outdoore	Outdoor environments				
Malaysia	Residential areas: Case study: built on		C		• *8		36•		
2015 (35)	Control: at 20 km from case study		7		27•		36•		

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	able
	<b>Table 1.</b> Characteristics of studies using the Index

Country			N. of	Sampling	Bacteria (IMA)	MA)	Fungi (IMA)	MA)	Microbial Total Count (IMA)
Publication year (Ref)	Publication Study setting year (Ref)	Sampung period	monitored environments	time/ condition	Mean (SD)/ mean range/ Range	Range	Me	Range	Mean/mean range/ <i>median</i>
	Areas closeness			early in the morning	шевати		medudi		
Israel 2016 (36)	to domestic GW-treatment	to domestic GW-treatment June - February	8	0.3 m away from GW-t systems	0.22 - 616.7**				
	systems (RVFCWs)		I	1 m away from GW-t systems	0 - 15.2**				
			4						
j			road area		80.3**		4.37**		
Vietnam	Ho Chi Minh Cire	Three years	zoo area	four times a day	33.3**		52.48**		
(16) (107			residential area		42.26**		3.02**		
			rural area		99.27**		14.17**		

Legenda: \*calculated from CFU/dm²/h; \*\*calculated from CFU/m²/h; • IMA calculated for 1 h; only fungal count on Saburand Dextrose Agar medium was considered

Table 2. Characteristics of studies using the Index	of studies using t	the Index of M	Iicrobial Air con	tamination (	(IMA) standar	d with va	of Microbial Air contamination (IMA) standard with values expressed as CFU/m³ calculated by Omeliansky's formula	FU/m³ calculate	ed by Omeli	ansky's formula
Country	Studysetting	Sampling	N. of monitored	Sampling time/	Bacteria	ria		Fungi		Microbial Total Count
Publication Year (Ref.)	0	period	environments	condition	Range	e,	Mean	Range	şe	Mean
Educational Buildings										
					CFU/m³	IMA*	CFU/m³ IMA* CFU/m³ IMA*	CFU/m³	$IMA^*$	CFU/m³ IMA*
Fthionia				at 6 a.m.	at 6 a.m. 747 - 9960 57 - 760	27 - 760		531 - 6568	41 - 501	
2015 (38)	University	University April, May	30 dormitory rooms	at 7 p.m.	at 7 p.m. 511 - 4010 39 - 306	39 - 306		730 - 6403	56 - 489	
Romania 2016 (39)	University (U) High school (Hs) Primary school (Ps)	March, April, May	5 rooms	between 12 a.m. and 5 p.m.						
			U - A3				497.3 37.94			

Country	Study setting	Sampling	N. of monitored	Sampling time/	Bacteria	eria			Fungi		Microbial Total Cour	Microbial Total Count
Publication Year (Ket)	0	period	environments	condition	Range	ge	Mean	u	R	Range	Mean	an
Educational Buildings												
					CFU/m³	IMA*	CFU/m³ IMA*	[MA*	CFU/m³	IMA*	CFU/m	CFU/m³ IMA*
			U - A5				414 3	31.59				
			U - microbiol lab				175.3 1	13.38				
			Hs - classroom				829 (	63.25				
Romania			Ps - classroom				149.7	11.42				
2016 (39)									March 122 -862	March 9.31 - 65.67		
			All monitored						April 145 - 830	April 11 06 - 63 33		
									May 176 - 795	May 13.43 - 60.66*		
HSA	High school	October -	8 100008								135	10
2019 (40)	Primary school	February									293	22
Ethiopia 2019 (41)	<u></u>	March - Apri	March - April 51 classrooms	at 6:30 a.m. and at 5:00 p.m.			613.29	47 1	136.5 - 2164.5	10 - 164		
Food industry												
Cuba	2 Food production plants:	March - November		between 1:00 p.m. and 2:00 p.m.								
2019 (42)	Artisanal chocolate plant		5 rooms						0 - 1507	0 - 115		
	Product for special regime		2 rooms						39 - 1638	3 - 125		

Table 3. Fungi and bacteria isolated in the different environments

																										Fu	ngi						
Environments Country Publication year (Ref)	Acremonium spp.	Alternaria spp.	Ascocbyta spp.	Aspergillus spp.	Aureobasidium pullulans	Beauveria spp.	Bipolaris spp.	Botryotinia spp.	Botrytis cinerea	Candida spp.	Chaetomium spp.	Chrysonilia sitophila	Chrysosporium spp.	Cladosporium spp.	Cochliobolus sp.	Curvularia spp.	Eurotium spp.	Fusarium spp.	Geotrichum spp.	Maya benzeri mantar	Microsporum spp.	Monilia sitophila	Mucor spp.	Neurospora spp.	Paecilomyces spp.	Penicillium spp.	Phaeospheria spp.	Pithomyces spp.	Pseudopestalotiopsis spp.	Rhizopus spp.	Rhodotorula spp.	Scedosporium apiospermum	
Cultural heritage																																	
Italy 2015 (21)		<b>√</b>												√												√							
Italy 2015 (22)				√					√					√												√	√						
Nigeria 2018 (23)				√												√								√		√			<b>√</b>				
Educational building	s				•																												
Italy 2010 (26)*				√										<b>√</b>									√										
Poland 2013 (43)**		<b>√</b>		<b>√</b>	√				<b>√</b>	<b>√</b>	√		√	<b>√</b>					<b>√</b>		√		√		√	√				<b>√</b>	<b>√</b>		
Italy 2014 (20)*				√										<b>√</b>				√								<b>√</b>							
Romania 2016 (39)		<b>√</b>		√				√						√		√		√	√				√			<b>√</b>				√			
USA 2019 (40)		<b>√</b>	√	√											<b>√</b>											√		<b>√</b>			√		
Ethiopia 2019 (41)		<b>√</b>		√						√								√			√		√			<b>√</b>				√			
Food industry					•																												
Norway 2009 (34)	V													√			√	√							√	<b>√</b>							
South Africa 2017 (29)																										<b>√</b>							
Portugal 2017 (44)																																	
Cuba 2019 (42)				<b>√</b>										<b>√</b>				√					√	√		<b>√</b>							
Autopsy room																																	
Turkey 2011 (28)	<b>√</b>	<b>√</b>		√	√		<b>√</b>			<b>√</b>		√	√					√		√		√	√		√	√				<b>√</b>	<b>√</b>	<b>√</b>	
Outdoor																																	
Israel 2016 (36)																																	
Vietnam 2019 (37)				√		√					<b>√</b>					<b>√</b>		√					√		√	<b>√</b>				<b>√</b>			

\*Genera most frequently found; \*\*Other isolated microorganisms: Acanthurus blochii, Artrographis Kalrae, Arxula adeninivarans, Bipolaris spicifera, Bjerkandera adusta, Blastomyces dermatididis, Cladophiarophora boppi, Corynespora cassiicola, Cystfilobasidium informominiatum, Debaryomyces hansenii, Debaryomyces polimorphus, Debariomyces occidentalis, Debariomyces vanrijiae, Emericella quadrilineata, Emmonsia crescens, Epidermophyton floccosum, Gymnoascus dancaliensis, Hormographiella aspergillata, Hormographiella verticillata, Kluyveromyces lactis, Kluyveromyces marxianus, Kluyveromyces thermotolerans, Kluyveromyces varrowii, Kluyveromyces wickerhamii, Lipomyces starkeyi, Madurella grisea, Mrakia frigida, Nadsonnia commutata, Oosporidium margaritiferum, Phialophora bubakii, Phoma cruris-hominis, Pichia anomala, Pichia farinosa, Pichia membranifaciens, Rhizomucor pusillus, Rhodosporidium dacryoideum, Saccharomyces cerevisiae, Saccharomycopsis capsularis, Saccharomyces fructuum, Scytalidum lignicola, Yarrovia lipolytica

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Scopulariopsis spp.	Sporotrichum spp.	Stemphylium spp.	Syncephalastrum spp.	Thielaviopsis spp.	Trichoderma spp.	Trichophyton spp.	Trichotecium spp.	Ustilago spp.	Verticillium spp.	Wallemia spp.	Yeasts	Sterile mycelia	Acinetobacter spp.	Actynomyces spp.	Aerococcus spp.	Aeromonas spp.	Alcaligenes faecalis	Bacillus spp.	Corynebacterium spp.	Cochliobolus indoltheticum	Curtobacterium spp.	Escherichia coli	Enterobacteriaceae	Enterococcus spp.	Klebsiella spp.	Microbacterium spp.	Micrococcus spp.	Mycobacterium smegmatis	Myroides spp.	Neisseria meningitidis	Pseudomonas spp.	Rathayibacterium caricis	Staphylococcus spp.	Streptococcus spp.	Vagococcus spp.	Heterotrophic
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contamination was also performed in a dry-cured meat production facility, and outdoor in a study by Asefa et al. in Norway (34); overall, in the production rooms, the mean value of 15 IMA was observed with the heaviest contamination in the brining, smoking, and sorting processes rooms, showing the last one the highest IMA value (about 90 IMA, graphic data); the outdoor fungal contamination was about 25 IMA (graphic data). In Italy, Vella et al. (31) carried out a study in three buffalo farms, including indoor and outdoor air microbial evaluation, at rest and in operational conditions: mean IMA values for fungal contamination ranged from 6 to >76 IMA in indoor milking rooms and from 10 to >76 IMA in the outdoor areas (feeding rooms). In the study by Sonmez et al. the presence of bacteria and fungi was determined in an autopsy room, in summer and spring seasons, before, during and after autopsy. The microbial air contamination was significantly higher at the time of the autopsy than that found in pre and post- autopsy sessions, reaching the highest values of 117.8 IMA for fungi in spring and 60.9 IMA for bacteria in summer; maximum acceptable IMA values were considered 75 for bacteria and 19 for fungi. In Japan, Tasaki et al. (30) monitored, for a period of thirteen months, a cargo van rabbit housing system obtaining a mean IMA value of 0.30 and 9.30, for bacteria and fungi, respectively. Other two studies, one in Iran (32) and one in Thailand (33), monitored the indoor and outdoor microbial contamination. In the first one a school dormitory and a retirement home were monitored, and bacterial IMA values for the two environments ranged from 10 to 112, while fungal contamination from 11 to 36; outdoor bacterial and fungal IMA values ranged from 15 to 96 and from 8 to 40, respectively. The second one dealt with three fitness centers, two indoor and one outdoor, locating settle plates at 1.5 m from the floor considering this height representing the human breathing zone; indoor mean IMA values ranged from 2.09 to 8.44 for bacteria and from 0.97 to 5.07 for fungi, while in the outdoor center bacterial and fungal mean IMA values were 7.52 and 5.59 respectively. Studies dealing with only outdoor microbial air sampling were carried out in Malaysia (35) and in Israel (36), both regarding waste treatments areas, and in Vietnam (37) where air was sampled in Ho Chi Minh city. In the study by Ithnin et al. (35), air sampling was performed around a former area dumping site, the case location, and 20 kilometers away, the control location; the mean bacterial air contamination values were 48 and 27 IMA, respectively, while mean fungal contamination was the same at both sites (36 IMA). Benami et al. sampled bioaerosols emitted from domestic grey water (GW) treatment systems; low amount of bacteria, with mean values ranging from 0 to 15.2 IMA were found to aerosolized up to 1 m away from the GW treatment system, while at the 0.3 m distance the mean values reached value of 616.7 IMA. In Ho Chi Minh city, airborne bacteria and fungi in the atmosphere were assessed from 2014 to 2016, covering two wet and dry seasons, at four sites of the city (zoo, road, rural and urban areas). The highest bacterial contamination was found at rural area while the lowest at zoo (33.3 IMA), where the heaviest fungal contamination was found (52.48 IMA).

Table 3 shows bacteria and fungi isolated in the different monitored environments by using settle plates according to IMA standard; in two studies (43,44) only qualitative evaluation was performed. Studies in which both active and passive air sampling were performed, but microorganisms isolated were reported without distinguishing which method allowed their isolation were not considered (21,24,25,32). Among bacteria, *Bacillus* spp., *Staphylococcus* spp., *Micrococcus* spp., *Pseudomonas spp.* and *Enterococcus* spp., were the most frequently isolated genera, while *Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp. and *Fusarium* spp. were the predominant fungi.

#### **Conclusions**

This review has provided a picture of the application of IMA standard in different geographic areas and in different environments at risk of airborne infection/contamination. The use of settle plates, whose sampling efficiency is not influenced by engineering factors, standardized with the IMA, yields comparable results wherever and whenever they were obtained, providing the basis for the definition of threshold limits towards an effective risk prevention. In some studies (26,27,28,29,31,33), the IMA threshold values initially proposed for the different environments (10) were considered, and proved to be useful for the

interpretation of results. A wide range of microbial contamination has been observed, in the same settings of several studies; a larger collection of data, recording also variables which can affect the microbial air contamination, will provide a useful contribution towards the definition of limit values referred to specific environments. In particular, exposure times and incubation temperature for fungal search need to be defined, for a complete standardization of the air sampling.

A consideration should be made regarding the use of Omelyansky's formula which was applied in order to convert the CFU/plate values (IMA) in CFU/m³. Both active and passive sampling can be used for a general evaluation of microbial air quality, but they have specific aims: while active sampling measures the concentrations of microorganisms, passive sampling measures the fall-out of the biological particles, as a mirror of the airborne risk for critical surfaces (e.g. object, material, food). In any case, considering the relationship provided by the EC GGMP Guidelines to Good Manufacturing Practice (46), it can be observed that the CFU/m³ results obtained with the Omelyansky's formula are much higher, giving an overestimation of the risk. It could be suggested to keep the IMA value without converting in CFU/m³, and to use the EC GGMP active and passive methods relationship for a possible estimation of the CFU/m<sup>3</sup>. However, it is questionable to assume that a predefined correspondence between active and passive sampling exists, as some Authors do when using specific formulae to obtain the number of CFU/m³ from the number of CFU/settle plates.

In a context in which there are no generally accepted protocols for the evaluation of microbial contamination of air, the use of IMA standard, for the relevance of data providing the estimation of the airborne risk of contamination for critical surfaces and the cumulative measurements of microbial contamination, as well as for its characteristics of economy and simplicity of use, represents a valid tool in the identification of situations at risk and in the evaluation of effectiveness of prevention interventions.

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