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ARTICLE

Influence of the 60 Hz Magnetic Field on the Airborne Microbial Distribution of Indoor Environments

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ABSTRACT

The aim of this work was to analyze the effect of the magnetic field generated by the household appliances on the airborne microbial surrounding these equipment located on indoor environments with particular interest in the environmental fungi. A simultaneous environmental study was carried out in locals of three different geographical places of Havana, Cuba, which have televisions, computers and an electric generator. The air samples were made by a sedimentation method using Malt Extract Agar. The concentration of total aerobic mesophilic as well as fungi and yeasts were determined in rainy and little rainy seasons by applying as factors: exposure time of dishes (5 to 60 min) and distance to the wall (0 and 1 m) at a height of 1 m above the floor. The predominant fungal genera were *Cladosporium, Penicillium and Aspergillus*. In the dishes that were placed at 0 and 0.5 m from the emitting sources were observed that some bacteria colonies formed inhibition halos, a great diversity of filamentous fungi and an increase in the mycelium pigmentation as well as the pigments excretion. In the rainy season, the highest amounts of fungi were obtained in all samples. In the little rain season the count of the Gram-negative bacilli increased three times the Gram-positive cocci.

1. Introduction

It is well-known that fungi and their propagules (spores and cellular fragments) are an important source of allergens. Its distribution is ubiquitous, universal and they behaving as allergens which contribute to triggering numerous diseases [1]. When the symptoms are described for the indoor occupants, caused by microorganisms and other contaminants, are referred to Sick Building Syndrome (SBS) [2]. Therefore, microbiological contamination of indoor air is of great importance because many people perform up to 90% of their activities in these spaces. The microbial contamination of the air of these places, mainly by fungi (aeromycobiota), is considered one of the greatest threats to health, since it can be ten times greater than the outdoor [3], although another author states that it is from six to seven times smaller [4].

The impact of the electric generators (EG) on the

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environment at different stages of their life cycle [5] and its location requires study which takes into account risks to the health of the population and the workers, in both phases construction and operation [6]. In this sense, most cases of EG studies are limited to the generation of electric and magnetic fields [7,8], noise emissions and physical-chemical air [9,10] but the microbiological quality of air is not studied and it is very important for human activity [11-15].

It is known that different physical factors influence in the dynamics of the behavior of biological aerosols [4]. It is known that different physical factors influence in the dynamics of the behavior of biological aerosols. Its characterization depends on the method of sampling and the analysis used but currently a standard protocol to evaluate the contamination and their effect in indoor environments is not available. Therefore, it is difficult to compare the results of a study with other one carried in the same country or in other geographical areas [3]. According to Gent (2003) [16] count can be classified into four levels: no detectable (0 CFU/m^3), low (1 to 499 CFU/m^3), means (500 to 999 CFU/m^3) and high (1000 or more CFU/m^3). In response to this classification, the private homes are considered with a mean value of contamination (600 - 800 CFU/m^3) whilst the dressing rooms have a high contamination (800 - 1500 CFU/m^3). In general, at indoors environments are acceptable between 100 to 500 CFU/m^3 but only it is accepted 50 CFU/m^3 if are pathogenic fungi such as *Aspergillus* spp.

Moreover recent researches show that environmental fungi were stimulated by nuclear radiation that persist in the surrounding areas to the power plant Chernobyl [17-19] and the environmental study made in a local closed showed that a similar phenomenon occurred with magnetic field density (B) of 10 mT and 60 Hz for 220 V [20]. In this investigation the fungal propagules were attracted and deposited on the Petri plates located near the magnetic field generator coil in the same way that occurred with the bacteria under a 4 kV electric field [21]. There is a great diversity of B in the environment of different electrical equipment, even of similar characteristics, whose value does not depend on its size, complexity, power or the noise that can make each domestic appliance [22]. In addition, it should be highlight that the extremely low frequencies the electric field is obstructed by any obstacle while the magnetic field only decreases as it moves away from the source.

It is proposed that electromagnetic waves can influence the distribution of the aeromicobiota in indoor environments. The trajectory of the spores of the fungus *Drechsrela turcica* was studied and it was shown that they have electric charge since they were attracted to the electrode through which circulated alternating current of 115 V/50 Hz [23]. More recent studies apply this property of fungal spores but under the action of an electrostatic field between 0.5 and 5 kV [24-26]. According to Jamieson and Jamieson (2006) [21] the electrical and magnetic fields generated by the equipment can attract pathogenic bacteria inside the locals like offices and hospitals increasing the risk of contamination by this way. For this reason the aim of this work was to analyze the effect of the magnetic field generated by electrical household appliances on the aeromicobiota surrounding these equipment located in indoor locals.

2. Materials and Methods

The environmental studies of interiors were carried out in three geographical areas of Havana, Cuba (Table 1). The premises studied are located in there municipalities Lisa, Arroyo Naranjo and East of Havana (at West, South and East of city, respectively). Two different days corresponding to the rainy and little rainy seasons of the years 2017 to 2018 were simultaneously sampled (Table 2).

Table 1. Description of the equipment used in this research according to the geographic location of the premises in the city

Equipment	Brand/ Country	Dimentions of the locals (m)	Neighborhood/ Municipality	Geographic location of the premises in the city
Personal Computer (PC)	AOpen/ Taiwan	3 x 2 x 2.2	Guatao/Lisa (LI)	West
Electric Generator (EG)	Genesal/ Spain	6 x 4 x 3.0		
Television (TV)	Atec Panda/ Korea	7 x 3 x 2.5	Alamar/ East of Havana (EH)	East
	LG/Korea	3 x 3 x 2.5	Electric/ Arroyo Naranjo (AN)	South

Note: *these acronyms were used to identify the equipments and their locations.*

Table 2. Thermo hygrometric values of outdoor environment each day studied according to report of weather station located in Havana city

Seasons	Day of microbiological indoor air sampling	Outdoor Temperature (ºC)			Outdoor Relative Humidity (%)
		medium	max	min	
Rainy	14th May 2017	26.6	31.4	23.2	75
	1st June 2017	24.9	30.2	22.8	89
Little rainy	27th December 2017	23.4	30.8	19.9	82
	4th January 2018	24.7	29.0	20.8	84

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Note:

Report of Weather Station 783250 "White House" (latitude: 23.16; longitude: -82.35; altitude: 50) of the Cuban Institute of Meteorology (http://www.tutiempo.net/clima/CASA_BLANCA/783250.htm).

The EG (Genesal, Spain, Power 47/51 kVA) was selected because it is located 6 m from away to the PC-LI in Guatao. TVs and computers were selected because they are the household appliances most used in the home and people stay in front of them for several hours and with similar daily frequencies for both [22].

The variables temperature and relative humidity of the locals were taken with a digital thermo hygrometer (Hygro-thermometer DHT-1, China) which scales of temperature is from 5 to 50°C and 0.1°C with accuracy and relative humidity from 10 to 100% with accuracy 1%. The measurements of density of the magnetic field were taken with a gaussmeter (Enertech, EmdexII, USA) of scale 1 to 3000 and 0.1 mG of precision.

As in most homes there are TVs, with the results obtained in the sampling of the premises with this appliance, a D-optimal multifactorial design was applied by the statistical program Design Expert version 6.0.1.0 (STAT Ease, Minneapolis). The experimental factors studied were: the exposures time of the Petri dishes, the distance from the PCs to the wall as well as the geographical location of the room where the TVs are located (Table 3). A total of 17 experimental runs were obtained and total aerobic mesophilic (TAM), fungi and yeasts (FY) were determined.

Table 3. Levels of the factors studied in the experimental design

Level	Factors		
	Exposure time (min)	Distance of the TV (m)	Localization of the TVs
1	5 (low)	0 (low)	Arroyo Naranjo (TV-AN)
2	60 (high)	1 (high)	East of Havana (TV-EH)

When exposing of the Petri dishes with Plate Count Agar and Malt Extract Agar to determinate TAM and FY, respectively it was taken into account that the household appliances are usually placed 5 to 10 cm from the wall. This distance was assumed as 0 m and from this was added up to 1 m in correspondence with the experimental design (Table 3) and according to Pasquarella et al. (2000) [4]. In this way the same conditions in the three locations were guaranteed. For the sampling, a diagonal design with 3 or 5 points was followed (Figure 1).

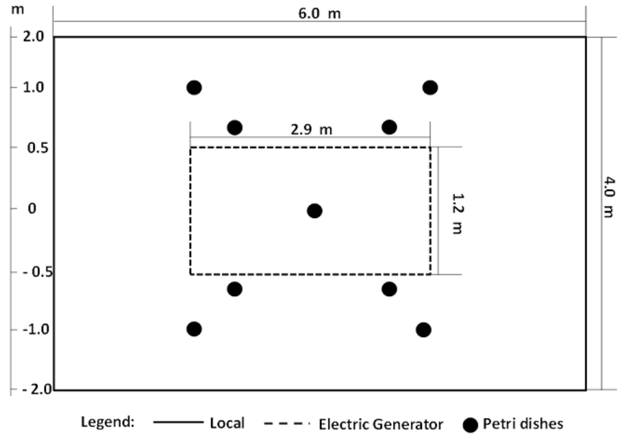

Figure 1. Representative scheme of the location of Petri dishes during the microbiological sampling of the air in a local (6 x 4 x 3 m) with an electric generator (EG) (2.9 x 1.2 x 1.3 m). The center of the generator was taken as distance 0 m, the Petri dishes were placed at five diagonal points and the microbiological environmental index was applied (1 m from the walls: 1 m from the floor: 1 h of opened Petri dishes)

After the incubation of Petri dishes at 30ºC for 5 days, the count was done to determine the microbial concentration expressed in Colony Forming Units per m^3 of air (CFU/m^3) according to the equation described by Omeliansky [27-29]:

$N = 5\ a.10^4\ (bt)^{-1}$

Where N: microbial concentration (CFU/m^3), a: number of colonies per Petri dish, b: dish surface (cm^2), exposure time (min).

With the values obtained the distribution a map of microbiological contamination in said premises using the Surfer v.8 software was built, according to a similar study [12].

Cultural and morphological characteristics of fungal colonies as well as conidiophores and conidia fungal structures were observed (stereomicroscope at 14X and trinocular Olympus microscope brand Samsung with digital camera coupled SCC-101 AP, 40X) and the identification was performed according to different manuals [30-33]. Bacteria were typified according to the morphology and Gram stain.

Analysis of relative frequency (RF) of occurrence of the detected fungal genera in the premises was expressed in percentage, using the following equation:

RF = Times a genus is detected/Total number of sampling realized x 100

At each point the sampling were performed in triplicate and the appliances turned off were took as control samplings. The data was analyzed with the program

Statgraphics Centurion XV.

3. Results and Discussion

In the colony count a concentration between 38 and 1060 CFU/m^3 of air was obtained for temperature and relative humidity average values of 27 ± 2°C and 82 ± 5%, respectively. Therefore, microbiological contamination of the premises can be classified with low to medium with counts of filamentous fungi (into the FY group) similar to the result informed for environmental indoors in Havana previously [15]. There were statistically significant differences ($p \leq 0.05$) between concentrations of TAM and FY but also between different levels of the factors studied.

For 5 minutes of exposure of the Petri dishes less variability of the colonies was obtained with respect to 1 h, which shows that the sensitivity of the technique can be increased by increasing the time of exposure, like was demonstrated in other similar research previously [20]. This is important for allergists, since a great deal of information can be obtained about prevailing fungi that may even contaminate food [34,35].

Figure 2 shows the average values of the microbial concentration in the Petri dishes exposed for 1 h at the three locations.

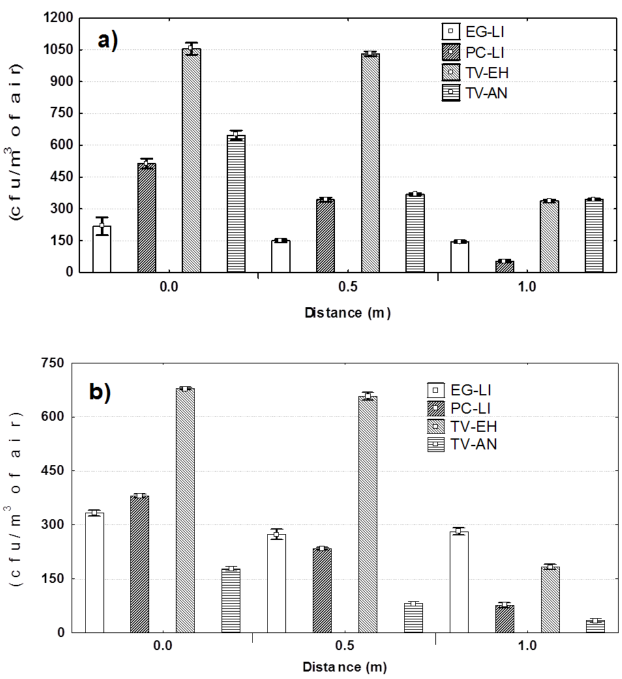

Figure 2. Microbial concentration (CFU/m^3 air) in three locals where the Petri dishes were exposed for 1 hour, 1 m above ground level and 1 m separated from walls in the rainy season of 2012. a) total aerobic mesophilic, b) fungi and yeasts

According to various studies, fungi count can vary within a large range depending on the methodology used and other determinants as the geographic area where the study was focused [1]. Since in all research the same methodology was used, statistically significant difference found in microbial concentrations obtained around appliances can be given by their geographic location. In that sense, the concentrations obtained at the same premises located in Lisa showed significant difference between the equipment groups (EG and PC), whilst there was significant difference between these premises and the others located in EH and AN consistent with those reported in an environmental study previously conducted in different locations in Havana [3].

At the distance of 0 m, all the studied premises had high pollutions, being the highest microbial concentration for TV-EH, followed in descending order: TV-AN, PC-LI and EG-LI. It should be noted that these values could be influenced by the sedimented dust on the equipment, since it has been suggested that microbial air pollution influences the microbial contamination of surfaces [13]. The particular behavior of the fungal concentration in front of TVs and EG-LI was studied in detail later.

By analyzing of the distance to the appliances (longer distance, lower density of OMF), similarity between the values to 0 and 0.5 m was observed, which they were higher than 1 m (Figure 2). This behavior was similar to those observed in other studies which showed that the bacteria were attracted up to the Petri dishes placed at 1 m distance to electric field of 4 kV [21] and the fungi to the Petri dishes located in front of the electric transformers which were emitting 0.4 to 1.2 mT [36]. Although in this investigation the observed behavior was similar for all appliances it is evident that the low values of OMF generated by these equipment cause the same effect and can be related to the increase of the electrostatic charge that concentrate the positive ions [37].

The settled dust is an important factor to be taken into account because this can have many species of fungi and yeasts xerophilic that commonly not float in the air [3]. In this sense, it is known that the genus *Cladosporium* is found in both indoor and outdoor environments and its presence in the dust could be due to fragments of spores that sediment [38] and they are present together with spores of the genus *Cephalosporium* and *Penicillium* even after cleaning with a vacuum cleaner [39]. However, considering the possible influence of the sedimented dust on the environmental microbial concentration, a contradiction is evident with the values obtained close to the EG-LI since a low microbial concentration was detected that contrasts with the large size and surface of this equipment (where a large amount of sedimented powder should be

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accumulated) with respect to the remaining equipments as well as the possible value of OMF it generates (0.1 and 0.6 μT for PCs, 0.8 and 23.5 μT for TVs and 0, 01 and 0.5 mT, for EG). This result can be explained taking into account the dispersion of the dust particles generated by the combustion gases during the EG operation, which makes it possible to suppose that these gases contributed to the decrease of the aeromicrobiota of the place where the EG is located.

Figures 3 and 4 present examples of growth in Petri dishes exposed for 1 h in different positions at the three locations. At a shorter distance the obtained bacterial colonies formed inhibition halos (Figures 3 c, 4 j, 4 o and 4 s) and a greater variety of filamentous fungi were observed whose colonies showed more pigmentation (Figures 4 c, f, g, h, m, and p). This variation in the colonies pigmentation was more significant for those isolated from the surroundings of the televisions and personal computers.

However, at 0 m distance the increase in the mycelial growth size and the pigmentation of the colonies could be influenced by the effect of the magnetic field, since in all the dishes close to the equipment the colonies pigmentation was observed: (orange color in Figures 4 b, c, h, and m) and coloring of the culture medium (fuchsia color in Figures 4 c, f, g, h, p, and q). In this regard, with 25 mT obtained from 50 Hz, stimulating effects were found in several fungi strains when were exposed for 4 h to 10 days [40,41]. It is suggested that this phenomenon of increased pigmentation occurs in fungi and other microorganisms to protect against the lethal effect of electromagnetic radiation [42]. This behavior is significant since it is an indicator of cellular metabolism (excretion of toxins and other metabolites that are allergenic and toxic), which shows that these fungal genera are present in similar environments including the environments where the electrical appliances studied are located.

Because pigmentation in fungal colonies is an adaptive response to the effect of such radiation [42] these strains of fungal species could be used in laboratory studies to evaluate the effects of higher values of B at 60 Hz frequency. Consistent with the above, to these distances from the EG-LI different fungal species was observed which excreted pigments and stained the culture medium. It should highlight that the dematiaceous and hyaline fungi that excrete pigments into the culture medium were detected more frequently at the 0 and 0.5 m distances of the EG-LI whilst at a distance of 1 m (lower magnetic field density or B) only were detected the hyaline fungi that do not excrete pigments (Figures 4 p and 4 q). Both the mycelium pigmentation and the pigments excretion to outside and therefore the staining of the culture medium are indicative of the fungal metabolic activity.

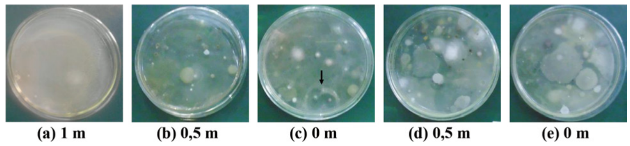

Figure 3. Colonies of total aerobic mesophilic (TAM) at different distances from a personal computers located in Guatao, Lisa (a, b and c) and a television located in Alamar, East of Havana (d and e) in the rainy season. The arrow indicates the formation of inhibition halos

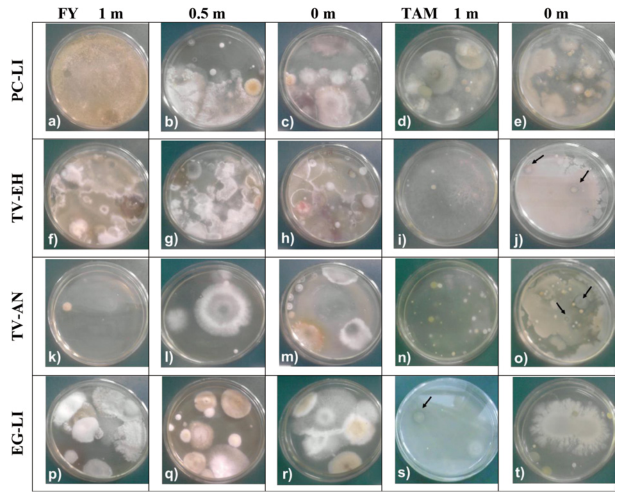

Figure 4. Microbial colonies obtained at different distances from personal computers and an electric generator located in a local of Guatao, Lisa (PC-LI and EG-LI), televisions located in homes of Alamar, East of Havana (TV-EH) and the neighborhood "Electric", Arroyo Naranjo (TV-AN) during the rainy season. FY: Fungi and Yeasts; TAM: Total Aerobic Mesophilic. The arrow indicates the formation of halo of inhibition

As in both studies this phenomenon occurred with a value of B in the order of the microtesla (μT) it is inferred that the value of B at 60 Hz must be in the order of militesla (mT) [20] to observe an effect of attraction or reorientation of the aeromicrobiota of indoor environments towards the sources of emission of the magnetic field. That is, for an electric current of 60 Hz the very low values of B are sufficient to affect the fungal metabolism in general while an increase of B can favor the formation of localized microenvironments and once they are colonized by fungi said magnetic field could also stimulate the fungal metabolism depending of the exposure time.

As for fungi, it was possible to observe similarity in their diversity and the predominant genera in the three geographic locations (RF of occurrence) were *Cladosporium* Link (100%), *Penicillium* Link (75%) and *Aspergillus*

Link (75%).

These genera coincide with those reported previously in environmental studies in Havana [15, 43-45], also they prevailed in the supermarket Chong Chom in Thailand [46], in the room of the Terracotta Warriors in Mausoleum of the First Emperor Qin in China [47], in other archives, libraries and museums [48,49] as well as in the halls of Yale University (New Haven, Connecticut) where RF of occurrence of the genera *Cladosporium, Penicillium* and *Aspergillus* were 62, 40 and 26%, respectively [16]. These are considered normal contaminants of indoor environments and potentially allergenic, consistent with that reported previously [16].

According to these results it is suggested that it is common to find these fungal genera in indoor environments [36,44,49] and the predominance of one or the other depends on the climatic region and the specific sampling conditions [3]. In that sense, there was no significant difference ($p \leq 0.05$) for the counts of these genera among the three locations coinciding with that observed in houses located in the Havana center and other areas of Cuba [2,15,50-52].

When comparing the effect of the OMF on the microbial concentrations in the locals with the EG (EG-LI) and the TVs it was possible to obtain significant differences in presence of the TVs ($p \leq 0.05$). Table 4 shows the average of these values for the different study conditions.

A linear models were obtained for both cases that explains more than 85 and 89% ($R^2 = 0.85$ and 0.89) of the behavior of concentration filamentous fungi and TAM, respectively. The probability that the linear models do not satisfactorily explain the behavior of the response variables was very low, which is considered adequate. The following equation is obtained:

$$RM = X_0 + X_1 \cdot A + X_2 \cdot B + X_3 \cdot C$$

Where RM: microorganisms concentration in CFU/m^3 air; X_0: constant model; X_1, X_2 and X_3: regression coefficients; A: exposure time; B: distance to the TV; C: TV localization.

Table 4. Results obtained for fungi and yeast (FY) and total aerobic mesophilic (TAM) concentrations in each experimental condition according to the matrix experimental of design

No.	A: time of exposition (min)	B: distance to the TV (m)	C: Localization of TV	FY (CFU/m3)	TAM (CFU/m3)
1	60	1	AN	39	308
2	5	1	AN	79	111
3	60	0	EH	675	1062
4	15	0.5	AN	72	229
5	5	1	EH	39	144
6	30	0.5	EH	505	754
7	60	0	AN	177	668
8	60	0.5	AN	85	393
9	60	1	EH	197	301
10	60	1	EH	197	304
11	5	0.5	EH	662	1029
12	5	0	AN	111	498
13	5	0	AN	113	495
14	60	0	EH	675	1060
15	5	0	EH	491	832
16	60	0	AN	177	668
17	30	1	AN	38	216

Table 5 shows that fungal concentration was significant ($p \leq 0.05$) in relation to the factors distance to the appliances (B) and location (C), and it was non-significant ($p \geq 0.05$) for the exposure time (A). This result shows that the exposure time does not affect the final count value (positive sign of the coefficients). That is, only a larger number of colonies will be obtained by exposing the dishes for 10 or 30 min without affecting the profile of the response surface graphs obtained which coincide with those obtained in a previous similar study [20]. This shows that the location factor (C) only influences the variety of genera and species of microorganisms, not in the quantity [3, 20]. In this case was obtained a higher count of TAM (47.29 CFU/m^3) than FY (15.55 CFU/m^3) as these also can grow in the first group and can increase total count, as explained above. Such behavior is beneficial as it will contribute to a more representative air sampling. Negative signs of the coefficient B indicate that lower values of CFU/m^3 are achieved while the Petri dishes are located away from the appliances (Figure 1).

Table 5. Results of experiment design obtained from the averages actual count

Factor	Estimated Coefficients (CFU/m3)		p	
	FY	TAM	FY	TAM
Intercept	255.67	522.90	-	-
A (time of exposition)	15.55	47.29	0.6680	0.2971
B (distance to the TV)	-134.23	-272.26	0.0031	0.0001
C (localization of the TV)	-172.42	-156.78	0.0001	0.0017

Note:

FY: fungi and yeasts; TAM: total aerobic mesophilic.

At the EG-LI site, a statistically significant difference ($p \leq 0.05$) was observed for the FY concentration between the two periods of the year and the concentration was higher in the rainy season due to the increase in the relative humidity of the air, a factor that is very important for the sedimentation of fungal propagules. Among both microorganisms groups (TAM and FY) also there were statistically significant difference and the count of FY was higher than TAM in all samples.

The Figure 5 shows the distribution of microbial air

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concentration at the EG-LI local and summarizes all information obtained during the study, and with it two factors were analyzed: operation of EG (turn on and turn off) and distance from the Petri dishes according theirs positions (0, 0.5 and 1 m). The formation of isolines (concentric rings) of more intense color centered surrounding the appliance is indicative of a higher microbial concentration in that area.

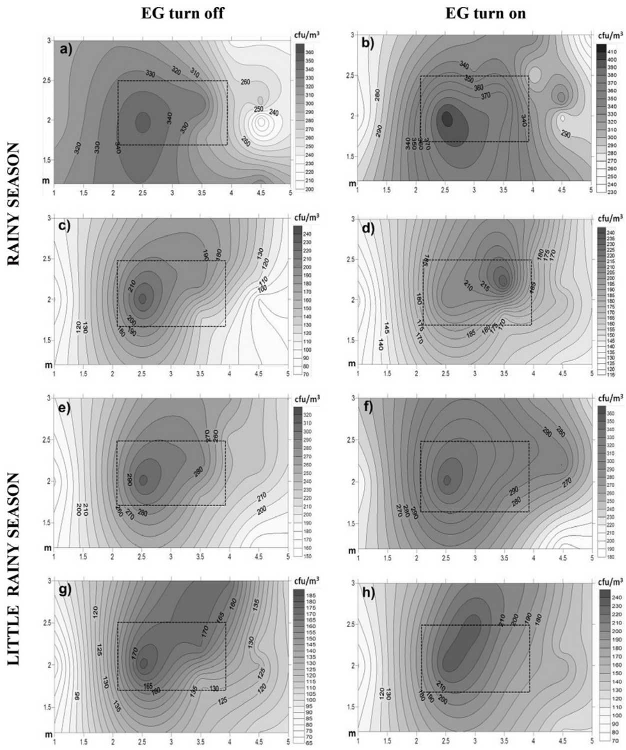

Figure 5. Distribution of microbiological concentration in the air (CFU /m3) on a local with the dimensions 6 x 4 x 3 m as well as an electrical generator (EG) of 47/51 kVA in different year seasons (rainy and little rainy seasons). Fungi - yeasts (a, b, e and f), total aerobic mesophilic (c, d, g and h). The rectangle of dotted line represents the area occupied by the EG in the room center

A similar behavior was observed regarding the increase of the air microbial concentration both in the experiments (EG turn on) and in the controls (EG turn off). This result evidences that there is influence of the equipment during its operation on the surrounding microbiota, probably due to the density of the magnetic field (B) existing in its vicinity or to the variation of aerodynamics that occurs (due to differences in temperature and relative humidity) when the EG is turn on. Although these variables were not controlled during the "in situ" experiment, their monitoring did not show statistically significant differences.

Figure 6 shows the relative density of each microbial group. Filamentous fungi outpaced the yeasts (Figure 6a) and among the bacteria, cocci predominated over the bacilli (Figure 6d). In the season of few rains the concentration of the filamentous fungi decreased but the yeasts increased (Figure 6c). It is important to highlight the low percentage detected of Gram negative bacteria (between 0 and 18%) as they are of great interest to public health because of its possible pathogenicity. In the rainy season (Figure 6e) the highest percentage of isolated bacteria were the Gram positive bacilli (59%) followed by the Gram positive cocci (29%). However, in the little rainy season no Gram negative cocci were found but the count of Gram negative bacteria was tripled (Figure 6f).

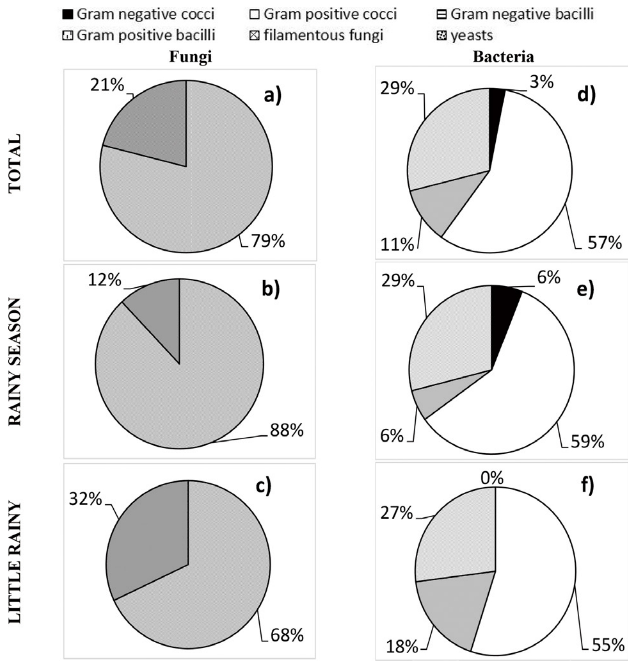

Figure 6. Relative density of the microbial concentration detected in the air of a local with dimensions 6 x 4 x 3 m as well as an electric generator of 47/51 kVA (turn on). Fungi and yeasts (a-c); bacteria (d-f) that are part of the total aerobic mesophilic group

A maximum colony count was obtained at the distance of 0 m and 1 h (60 min.) of exposure. As it is known within 3 cm of distance the televisions and computers generate a magnetic field of 50 and 30 μT, respectively [22]. With this result it is inferred that at densities less than 50 μT the microbial attraction towards the dishes is significantly lower than that observed in a similar environmental study with 10 mT [20]. In so far as Petri dishes are located away from the emitter center the incidence of the generated OMF density (B) by the household appliance decreases [22], so it is concluded that the value of B should be at least the double (100 μT), ie of the order of the militeslas (0.1 mT), to allow attraction of the existing aeromicrobiota in the indoor environment of any place.

4. Conclusions

The 60 Hz magnetic field generated by home appliances (0.01 to 50 μT) attracts many microbial colonies to the Petri dishes placed at 0.5 m of them, but these amounts are significantly lower than those attracted to 10 mT as observed in previous studies. Inhibition halos were formed by some bacterial colonies, a greater variety of filamentous fungi and a greater formation and excretion of pigments were observed in the Petri dishes that were located closer to the emitting source of OMF. A large variety of filamentous fungi was detected in the near of TVs and computers, which should be taken into account for the control of the microbiological quality of indoor air.

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