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Microbiological Analysis, Cytotoxic and Genotoxic Effects from Material Captured in PM2.5 and PM10 Filters Used in the Aburrá Valley Air Quality Monitoring Network (Colombia)

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***Abstract*—**This study aims to evaluate the diversity of microorganisms in filters PM2.5 and PM10; and determine the genotoxic and cytotoxic activity of the complex mixture present in PM2.5 filters used in the Aburrá Valley Air Quality Monitoring Network (Colombia). The research results indicate that particulate matter PM2.5 of different monitoring stations are bacteria; however, this study of detection of bacteria and their phylogenetic relationship is not complete evidence to connect the microorganisms with pathogenic or degrading activities of compounds present in the air. Additionally, it was demonstrated the damage induced by the particulate material in the cell membrane, lysosomal and endosomal membrane and in the mitochondrial metabolism; this damage was independent of the PM2.5 concentrations in almost all the cases.

***Keywords*—**Cytotoxic, genotoxic, microbiological analysis, PM10, PM2.5.

I. Introduction

THE atmospheric pollution, for both urban and rural environments, has increased in the last years, due to anthropogenic activities like the combustion of fossil fuels, sewage treatment plants, solid waste management facilities, the agricultural practices and the compost manipulation, among others. The human exposure to this pollution has been related with increase of the morbidity and mortality rates [1]. Among the critical pollutants, we can find the particulate material PM10 and PM2.5. This is because they can be easily inhaled according to their size, generating adverse effects to the human health [4]. In the case of PM10 material, these mainly affect the superior respiratory track causing allergic symptoms, nasal congestion, sinusitis, cough, fever, eye irritation; in contrast, the materials caught by the PM2.5 attack the inferior respiratory track causing bronchitis, asthma and emphysema [1]. For these materials, besides their size, their chemical composition is one of the factors that determine the risk for the health. This is because many cases are related with toxic substance sprays or aerosols. They can be the reason of a series of pathologies, due to their capacity to induce oxidative stress in the cells throughout the formation of reactive oxygen species and to interact with cell macromolecules, as the nucleic acids and proteins, generating tumorigenic, carcinogenic and mutagenic effects [2], [3].

The bioaeresols are microscopic particles suspended in the air, and in many cases they are found attached to the particulate material [10]. For the ones, that imply risks to the health, we can find, pollen grains, microbial spores, small size insects (for example mites), viruses, fungi, bacteria and the products of their metabolisms (for example microtoxins). In this way, the particulate material is a complex and heterogeneous mixture of chemical and biological elements, which can be attached to a carbon nucleus forming a condensate [4], [5]; and due to low research about the bioaerosol presence in the Aburrá Valley and the effect of the chemical substances associated to the particulate material, this research pretends to assess the diversity of microorganisms in the PM2.5 filters (bacteria) and PM10 (fungi); and determine the genotoxic and cytotoxicity of the complex mixture present in the PM2.5 filters used in the Aburrá Valley Air quality monitoring network (Colombia).

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II. Methodology

A. Sampling

For the microbiological bacteria analysis, semiautomatic BGI PQ200 air sampling equipment was used, Partisol 2000 and Partisol Plus 2025. The sampler absorbs the environmental air by means of a bomb with a constant volumetric flow rate, through a special entrance and an inertial size particle separator (impactator), where the suspended particulate material is separated in the range of PM2.5 to be collected in a 47mm diameter Teflon filter, with a pore size of 0.2 µm (micrometers). The filter exposure time in the sampling equipment was 24 hours [6].

The sampling for the fungi microbiological analysis was performed by means of semiautomatic Hi-Vol (high volume) sampling equipment that classifies the particles according to the desired size, and provides a measurement for the mass concentration of particulate material with an aerodynamic diameter equal or less to 10 µm in the air, during a 24 hour period. The air is dragged to the equipment with a constant flow speed; the particles with diameter equal or less to 10 µm continue the air flow current moving towards the equipment´s

injector tubes and they are deposited in a quartz filter. The bigger particles get out from the current line, they impact against a dish and become suspended. The filter remains in a horizontal position so that the air sample is dragged in a descendent way through the filter.

Three sampling points of the air quality monitoring network were used as:

- MED-UNFM: National University of Colombia- Mining Faculty, this station was considered as a strategic point for the air quality measurements due to its proximity to the routes with the highest vehicle flow in the city.
- MED-PJIC: Jaime Isaza Cadavid Polytechnical Institution is a traffic station that has the purpose to do the tracking in direct influence areas of the vehicle traffic.
- BAR-PDLA: For comparison effects (qualitative), the reference station located in "Parque de las Aguas" (a thematic water park) was targeted, in the municipality of Barbosa (Antioquia), which is intended to deliver information about the pollutants that enter the Aburrá Valley

B. Microbiological Analysis for Bacteria

Four different growing methods were used, with the objective of achieving the isolation of a great diversity of bacteria: chocolate agar (Merck) and blood agar (Merck) for opportunist pathogen microorganisms. EMB agar (Merck) enterobacteria; and nutritive agar (Merck) for the bacteria isolation with low demanding nutritional requirements. These growing media were used with the purpose of finding microorganisms that can be pathogenic and their presence can generate health risks [7]. The isolates were characterized by conventional microbiology (macroscopic and microscopic morphology and Gram staining) and molecular characterization (Ribosomal RISA Intergenic spacer region analysis between the DNAr 16S-23S region, using the first primers G1 (50-GAAGTCGTAACAAGG-30) and L1 (50-CAAGGCATCCACCGT-30)). The ITS product amplification patterns were solved by polyacrylamide gel electrophoresis - PAGE using the GelCompar II software (Applied Maths Biosystems, Belgium) and the grouping analysis by the DICE correlation method, with the likeness coefficient UPGMA.

The isolates obtained from each ITS group were identified by partial sequencing of the gen 16S rDNA, the amplification products of the 16S rDNA amplification were sent to sequence in both directions (with the separators 27F and 1492R) to Macrogen Incs. The identification of sequenced products, were edited with the ChromasPro 1.7.7 (Technelysium) software and contrasted with the BLAST server from the "National Center for Biotechnology Information" (NCBI) and the rRNA Database Project (RDP II) [8] [9]. After this, the phylogenetic trees were built with the Mega 4.0 software [10].

C. Fungi Microbiological Analysis

To achieve the isolation, the Saboraud and YGC growing methods were used. Each fungi culture was incubated at 25 ºC for 7 days. After this, a countdown and macroscopic descriptions were done of the grown cultures and later they were inoculated in PDA agar, keeping the same incubation conditions. Each isolate was characterized by morphological and macroscopic (diameter, color and appearance) and microscopic (lactophenol blue staining and observation of the reproductive structures at 10X and 40X) methods; this allowed the identification of compatibles genres. Then, a molecular identification was made with the objective of confirming the genres or species that were identified from the macro and microscopic description.

The fungi DNA extraction was performed with pure cultures that were incubated in a liquid media Sabourod during 8 days (120 rpm, 28 °C), by liquid nitrogen. The DNA amplification was performed with the polymerase chain reaction (PCR), using universal molecular tracker primers of the ribosomal DNA region ITS1 (5′ TCCGTAGGTGAACCTTGCGG 3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) according to the description in [11]. The sample visualization of the ribosomal RNA ITS regions was done by 1% agar gel electrophoresis.

The amplified product sequencing of the ribosomal gen of each of the fungi was done by the company MacroGen Inc (Korea). The results were compared with supplied information by the BLAST database from the National Center of Biotechnological information (NCBI) server. The phylogenetic analysis used by the Neighbor-Joining method with 1000 repetitions, was performed with the Mega 6.1 software [9], it allows to compare found sequences with the stored ones, also it allows to know the microorganisms' genre and even in some cases the species [8].

D. Genotoxic and Cytotoxic Analysis

For the genotoxic and cytotoxic analysis, an extraction with the Soxhlet equipment was done [11], in order to perform the extraction by ultrasound [12]. For the genotoxicity and cytotoxicity tests, the cell line CHO–K1 (ATCC CCL- 61) was used, this is a subclone of the parental CHO line derived from a Chinese hamster ovary, that grows attached to the substrate [13] and the cell line Jurkat (ATCC TIB-152) form leukemoid origin, established from peripheric blood cells of a 14 year old child with severe leukemia. These cells grow in suspension, adopt linfoblast morphology and form clusters [14].

The cytotoxic activity of the chemical component of the PM2.5 filters was assessed by means of the following attempts: MTT test [15], Trypan blue test, neutral red catchment test [16]. The genotoxic activity was performed by means of the following: T lymphocyte chromosomal aberration analysis of peripheral blood, sister chromatid exchange (SCE) and alkaline gel electrophoresis of individual cells (Cometa test) [17].

III. Results

A. Microbiological Analysis of Bacteria

Based on the Gram staining, it was observed that the Gram-positive bacteria predominated over the Gram-negative. However, for the BAR-PDLA station, the Gram-negative

bacterial growth was not observed (Fig. 1). These results match the ones obtained by [18], in the city of Mumbai, in which 82% of the culturable bacteria in their sampling were Gram-positive. According to the bacteria morphology the bacillus predominated over coccobacilli and cocco bacteria, in the three sampling points (Fig. 2). Regarding the presence of hemolysin, a predominance of the beta type hemolysis was observed in the three sampling points, besides a small percentage that did not manifest growth in blood agar for the station MED-UNFM (Fig. 3)

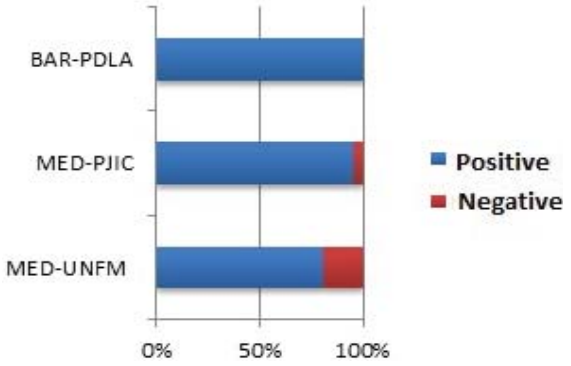

Fig. 1 Gram-positive and Gram-negative bacteria for each monitoring station

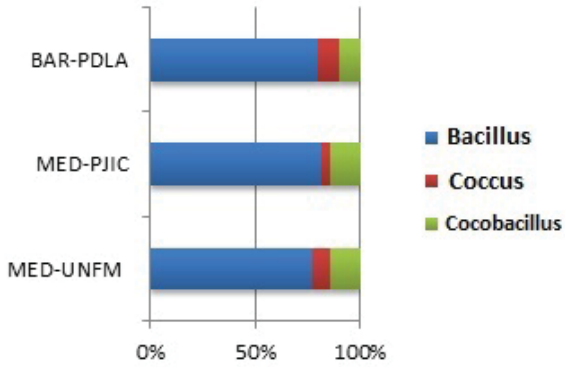

Fig. 2 Bacterial cell morphology

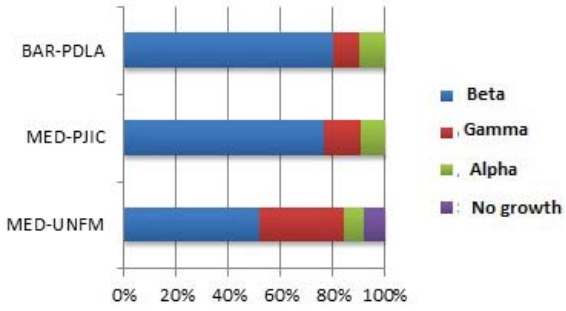

Fig. 3 Isolated group hemolysis in blood agar

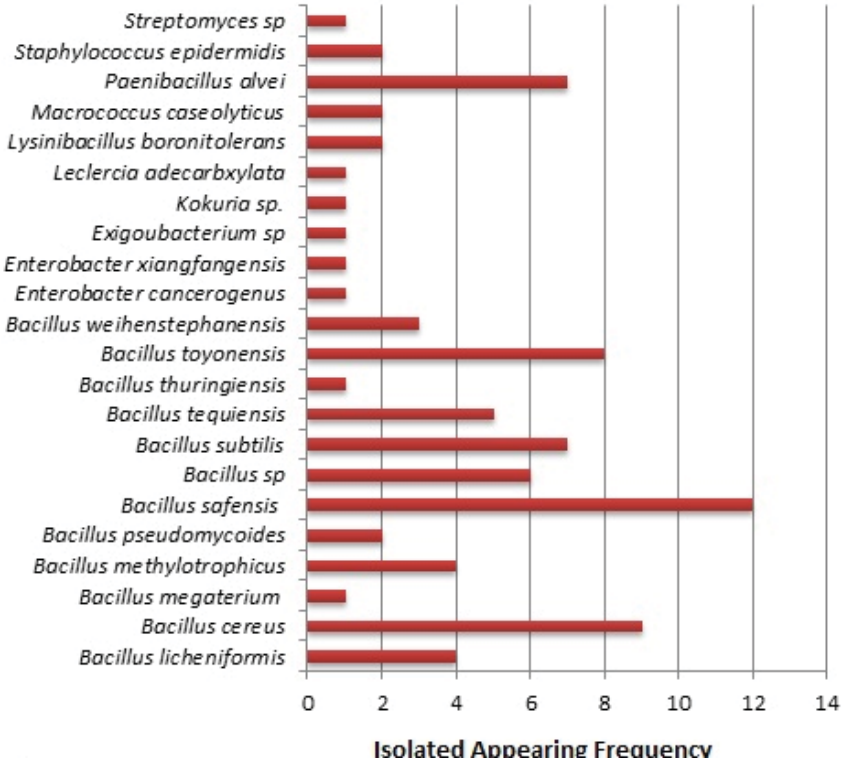

Fig. 4 Diversity approximation of the MED-UNFM station

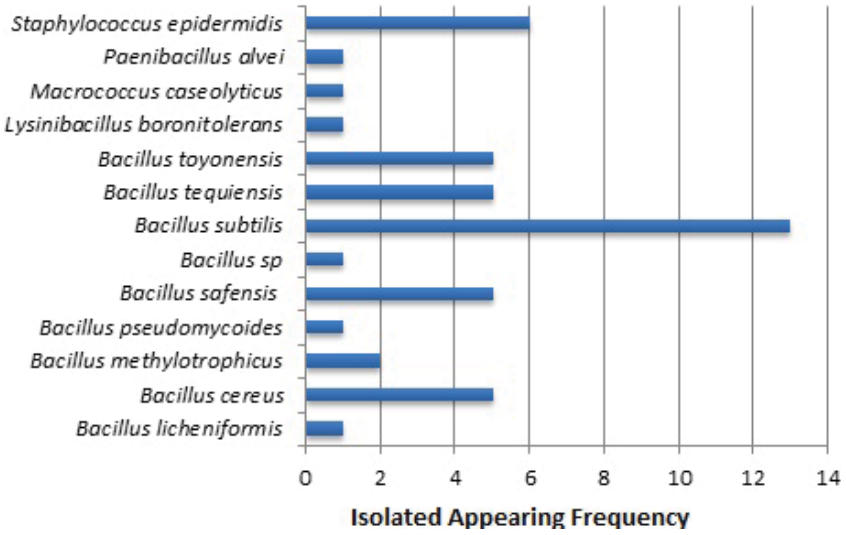

Fig. 5 Diversity approximation of the MED-PJIC station

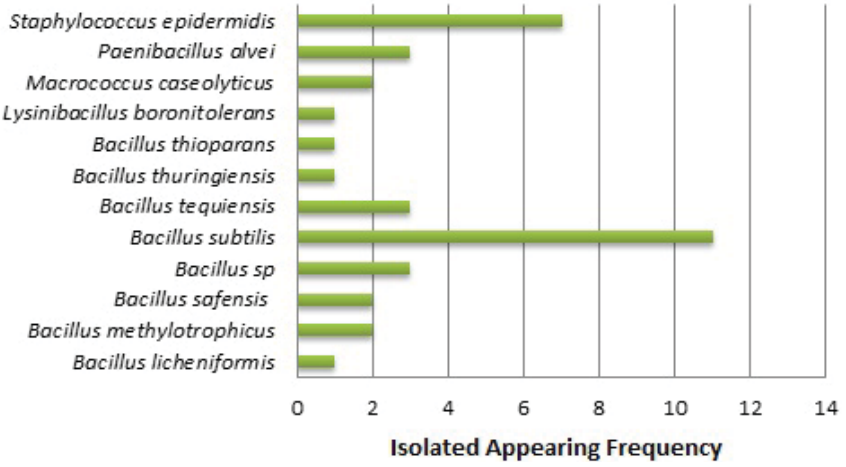

Fig. 6 Diversity approximation of the BAR-PDLA station

The isolated bacteria were classified according to their apparition in each sampling and station, the apparition frequency was analyzed, highlighting the most relevant as risk factor for the human health. The sampling location MED-UNFM was the one with higher diversity of identified isolates with 25 species, followed by MED-PJIC and BAR-PDLA with 13 and 11 species as it is shown in Figs. 4, 5, 6 respectively.

Figs. 4-6 show a higher presence of pathogenic microorganisms in the urban stations MED-UNFM and MED-PJIC compared with the BAR-PDLA station, located in a rural environment; this could be due to the higher contamination degree in these two stations. By one side, *B. subtilis and B. thuringiensis* were found mainly in the MED-UNFM and BAR-PDLA in where is common the presence of trees, grass and other vegetal species, in a manner that their presence is the result of the biofertilizers in these zones.

B. Microbiological Analysis of Fungi

In total, 14 strains were identified and grouped in seven different genres: The first five *Aspergillus, Penicillium, Curvularia, Neurospora, Cladosporium* have been reported in the literature as environmental fungi, in which *Cladosporium* is the most predominant genre [19]-[21].

A colony analysis was made in each station for each one of the samplings, with the objective of determining some differences in the apparition of fungi. It is highlighted that the station located in Polytechnical institution Jaime Isaza Cadavid (MED-PJIC) has the highest colony variety in the six samplings performed, while the Barbosa station (BAR-HSVP) presents a higher variety in three of them. Like this, the National University of Colombia mining faculty station (MED-UNFM) shows the least colony variety for all the samplings as it is shown in Fig. 7.

It is important to highlight that the MED-UNFM and MED-PJIC are urban traffic stations; therefore, we can expect that the observed colonies are more similar among each other than

they are related to the BAR-HSVP station, that is a surburban background station, this occurs due to the complex interrelations between the fungi spores and their environment, and inclusively the human activities, the pollution levels and the surrounding vegetation [22]. The highest species number genres present in their corresponding order *Penicillium* (21%), *Fusarium* (18%) and *Aspergillus* (16%) as it is shown in Fig. 8. All of them are genres where we can find species responsible of illnesses in humans [20] [21].

The Penicillium and Aspergillus genres, besides the *Cladosporium* are responsible of causing from allergic rhinitis to asthma [22]. Particularly *Aspergillus niger* and *Aspergillus fumigatus* can generate invasive pulmonary illnesses as the aspergillosis [22].

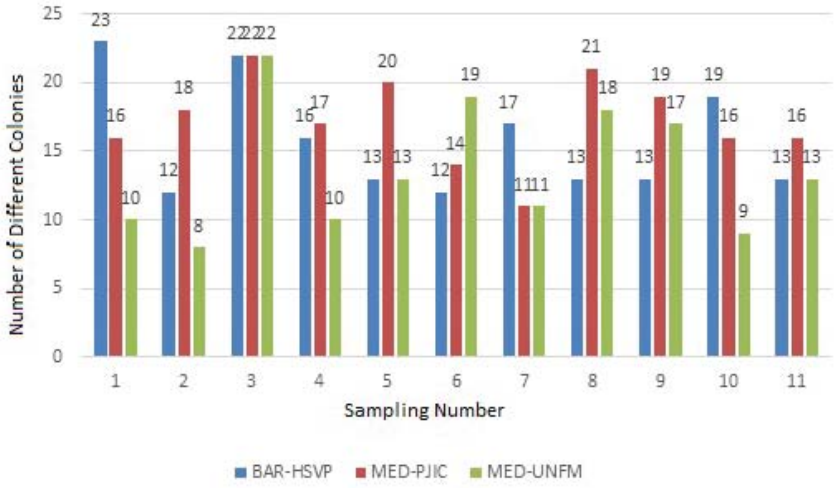

Fig. 7 Diversity approximation of each station

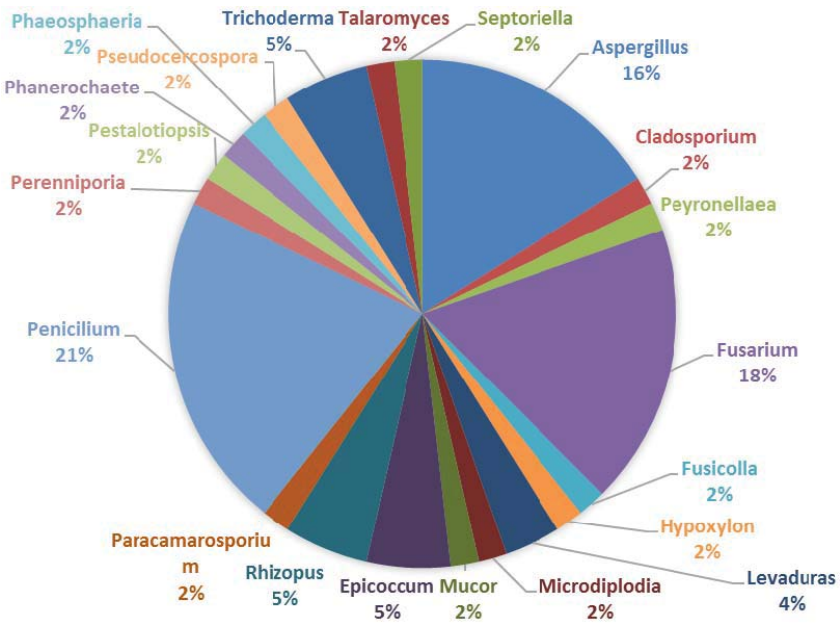

Fig. 8 Fungi types and genres of the found isolates in the three monitoring stations

C. Genotoxic and Cytotoxic Analysis

1) MED-UNFM Station

For the MED-UNFM station, according to the trypan blue dye exclusion test, it is observed that in both cell lines viabilities higher than 80% were obtained, which indicated that at these concentrations there is not damage at a membrane level; like this, a fewer viability was observed in the lower concentration which corresponds to 10 ug/ml, which indicates that at a lower concentration there could be a more cytotoxical effect at the membrane level (Fig. 9).

With base on the MTT test results (in the cell line CHO-K1), more than 90% of the viability was obtained for the negative control and the solvent control, and 50% of the

viability for the positive control; it is possible to say that the collected particulate material in the MED-UNFM station induces a toxic cell response when the concentration increases. After assessing the Jurkat cell line extracts an alteration of the cell proliferation was observed; however, this is not very significant and is necessary to increase the number of treatment to have more points in the viability curve. For the neutral red catchment test, viabilities higher than 90% were obtained for the negative control and lower than 50% for the positive control. This test was only performed the cell line CHO-K1- (Fig. 9).

By means of the neutral red test, it is possible to observe an expected cytotoxical effect; this is a viability loss when the concentration increases as the MTT test indicated; the obtained results support the fact that there is also a response dose effect. This means that at higher concentrations of particulate material, an effect in the cell level is generated, specifically in the lysosomes and endosomes (Fig. 9). Additionally, it was intended to asses the chromosomal, numeric and structural aberrations, however no significant evidence was found in the observed mitosis for each of the assessed treatments (Fig. 9).

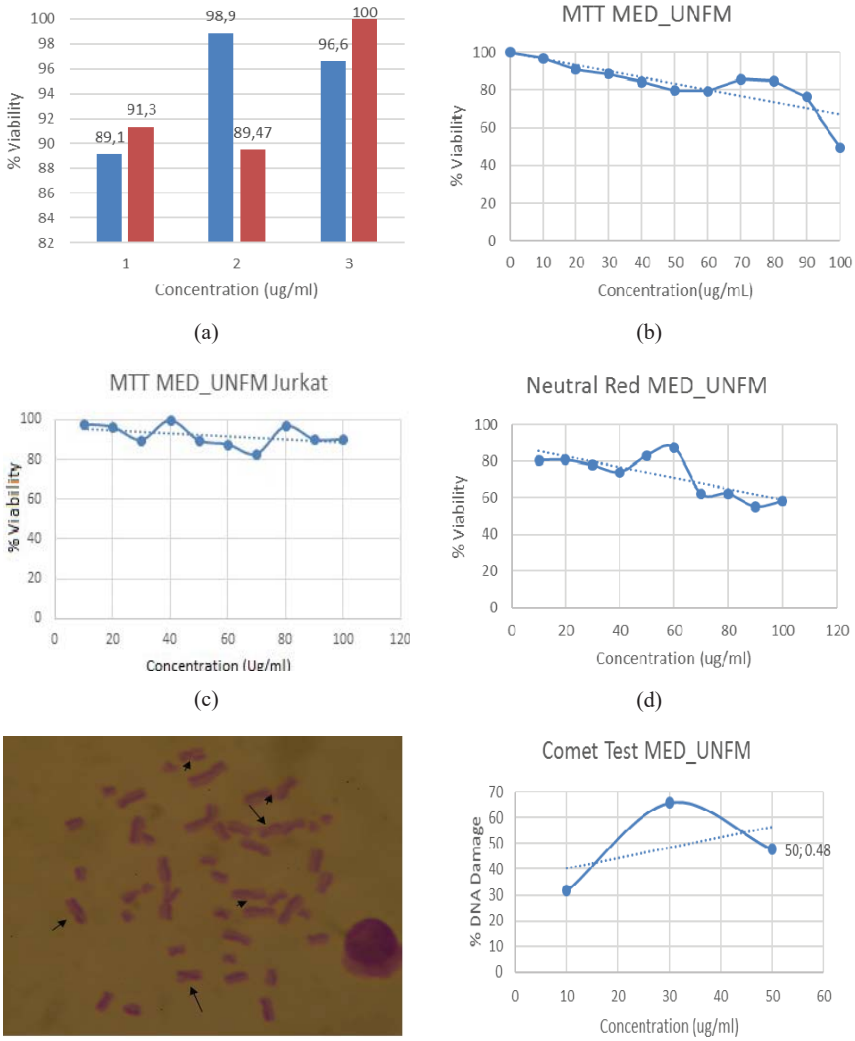

Fig. 9 (a) Trypan blue results (Blue corresponding to the cell line CHO-K1 and orange corresponding to Jurkat) for the MED-UNFM station (b) MTT results for the MED-UNFM station in CHO-K1 (c) MTT for the Jurkar cell line of MED_UNFM filters (d) Neutral red catchment test results in the cell line CHO-K1 (e) SCE 10 µg/ml treatment of PM2.5 of MED-UNFM (6 SCE) (f) comet test results for MED-UNFM PM23.5

The number of sister chromatid exchange (SCE) in a Healthy human being the average SCE per cell is from 5 to 8 for the BrdU concentration used in the developed protocol [23]. The comet test was assessed for the CHO cell line. For the negative control the obtained DNA damage was lower than the 10%, and this same result for the solvent control. For the positive control, the obtained DNA damage was higher than the 60%, a DNA damaging tendency is observed when the

PM2.5 concentration increases. However, the 30 ug/ml concentration was the one that presented the highest damage, even more than the positive control damage percentage. This indicates that in spite of not having chromosomal damage, as the chromosomal aberrations test or SCE indicated, there is DNA damage that implies the rupture of a double or simple chain (Fig. 9).

2) MED-PJIC Station

As it is observed in the trypan blue test, there is a diminishing tendency of the viability when the concentration of the particulate material increases in the Jurkat cell line increases. 24 hours after auditioning the treatment, morphological changes were observed in the cell line. This indicates that there is a cytotoxical effect of the particulate material present in the MED-PJIC monitoring station at the level of the cell membrane (Fig. 10).

For the MTT graphics (cell line CHO-K1), it is observed that for the MED-PJIC station´s filters there is a diminishing tendency of the cell viability when the concentration of the complex mixture of particulate material increases. For the leukemoid cell line, the obtained results also show a tendency to produce a bigger loss of the cell viability when the concentration of particulate material increases. For the Neutral red catchment test the solvent control presented viability above the 80% of the positive control, for the positive control the viability obtained was below 50% (Fig. 10).

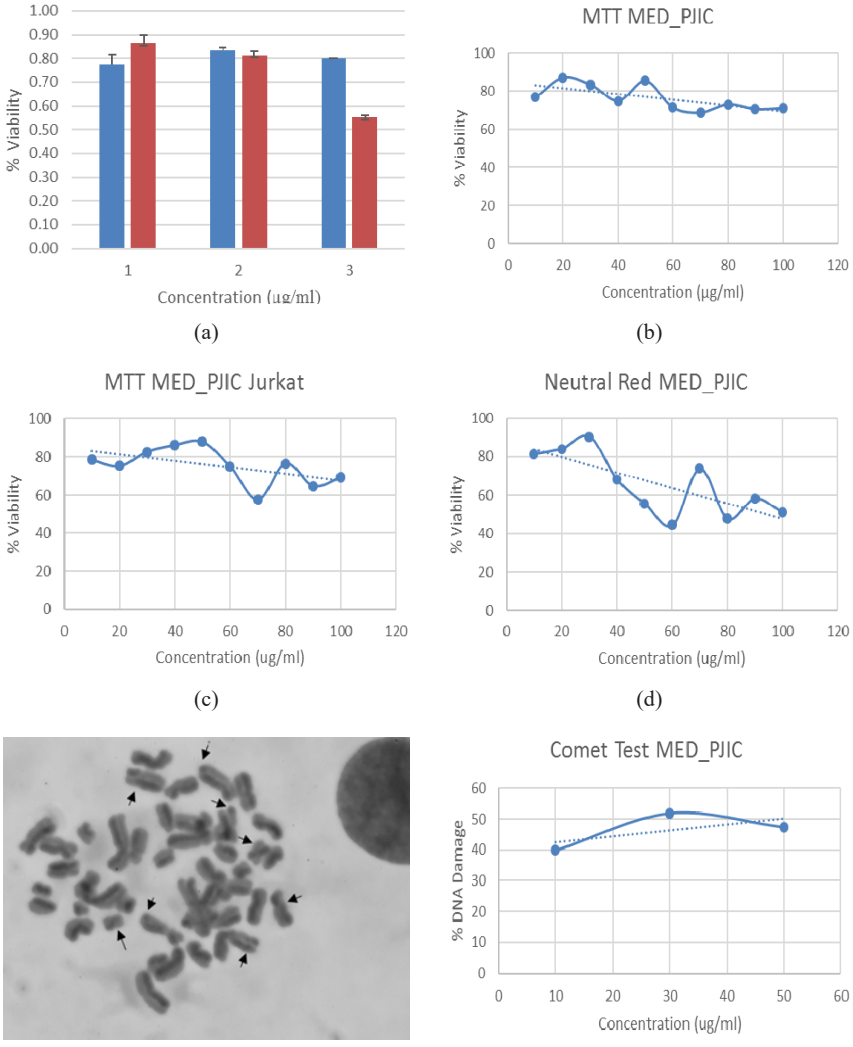

Fig. 10 (a) Trypan blue results (Blue corresponding to the cell line CHO-K1 and orange corresponding to Jurkat) for the MED- PJIC (b) MTT results for the MED- PJIC station in CHO-K1 c) MTT for the Jurkar cell line of MED_ PJIC filters. d) Neutral red catchment test results in the cell line CHO-K1 e) SCE 10 µg/ml treatment of PM2.5 of MED- PJIC (6 SCE f) comet test results for MED- PJIC PM23.5

Regarding the chromosomal aberrations, for the particulate material in the MED-PJIC station, there is no effect over the cell cycle, as the calculation for the mitotic index shows. The same situation happened for the MED_UNFM monitoring station, there was no presence of chromosomal, numerical or structural aberrations. The comet test assessed the CHO cell lines, where the negative control presents damage in the tail of the comet, inferior to 10%. The solvent control presented a damage percentage inferior than 15%, and the positive control superior than 60% (Fig. 10).

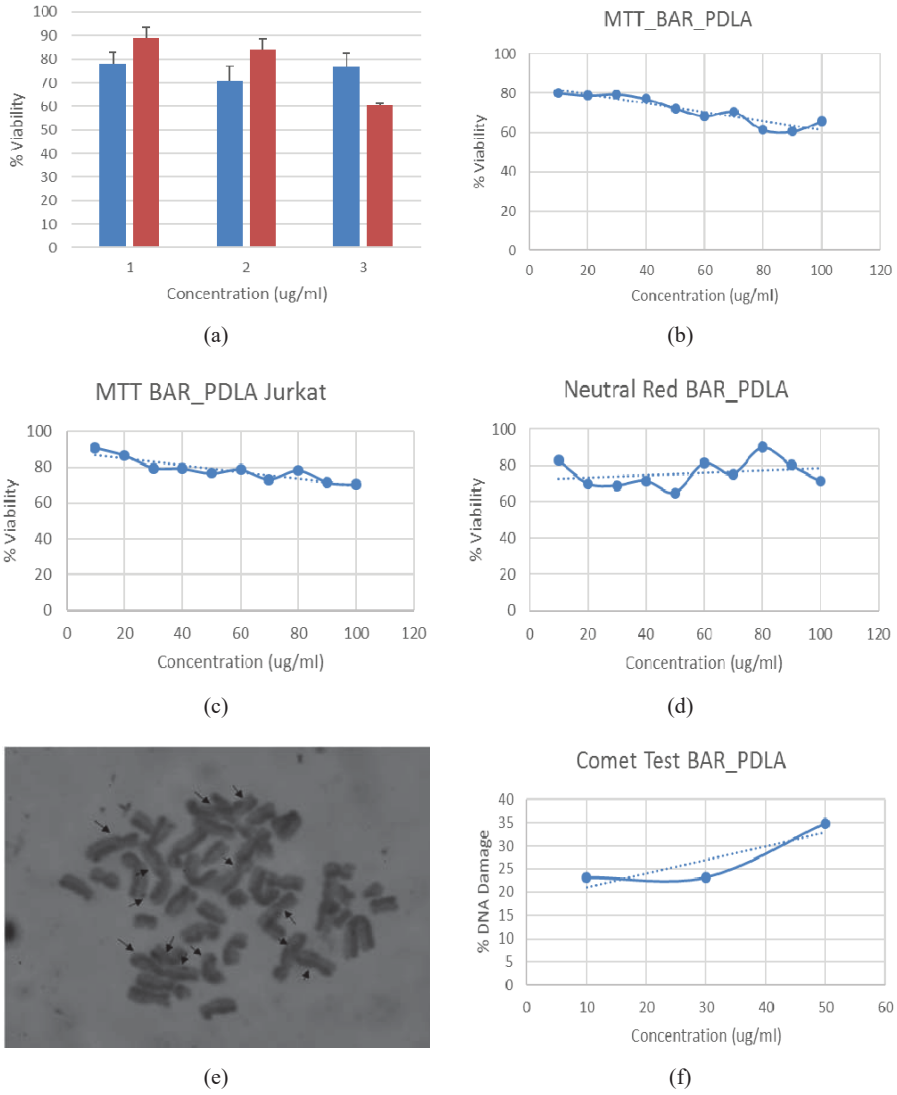

Fig. 11 (a) Trypan blue results (Blue corresponding to the cell line CHO-K1 and orange corresponding to Jurkat) (b) MTT results for the BAR-PDLA station in CHO-K1 (c) MTT for the Jurkar cellline of BAR-PDLA filters (d) Neutral red catchment test results in the cell line CHO-K1 (e) SCE 10 µg/ml treatment of PM2.5 of BAR-PDLA (6 SCE) (f) comet test results for BAR-PDLA PM23.5

3) BAR-PDLA Station

According to the blue trypan exclusion test results, the viabilities for the solvent control and negative presented values above 85% and the positive control below 68%. It is observed a decrease in the viability by the rupture of the cell membrane when the concentration of particulate material increases (Fig. 11). In the MTT (cell line CHO-K1) graphics, it is observed that for the particulate material in this station the is also a dose effect response, which a very defined tendency to decrease the cell viability due to the functionality loss of the succinate dehydrogenase enzyme. The PM2.5 of the BAR_PDLA station has a cytotoxic effect by means of the alteration of the mitochondrial metabolism. For Jurkat, and for the cell line CHO-K1, we have similar effects: a viability loss with the concentration increase. However, the line CHO-K1 seems to be more vulnerable to this particulate material than the Jurkat line. For the Neutral red catchment test, contrary to the results obtained in this particulate material test in the previous stations, in this one we have an alteration of the cell proliferation, but not in the same degree as the others.

According to these results there is a cytotoxical effect over the cell line CHO-K1 in the lysosome and endosome membranes as it is shown in Fig. 11. Additionally, we have a significant increase of the mitotic index. It is possible that the particulate material in this sampling location has some effect over the cell cycle. Regarding the chromosomal aberrations, it was not evidenced mitosis that could present chromosomal, chromatid breakdowns or other type of alterations in the chromosome level. Again, no significant effects were found on the sister chromatid exchange

For the comet test, the negative control presents DNA damage lower than 10%, the same for the solvent. For the positive control the obtained DNA damage was higher than 60%. The results for this monitoring station differ from the previous stations in which the tendency is according to the concentration increments the DNA damage percentage is more marked. Without having the middle point that the MED_UNFM or MED_PJIC stations presented.

IV. Conclusions

A higher frequency of bacteria related to pathogens in the urban environments MED-UNFM y MED-PJIC was found, compared with the BAR-PDLA station, possibly due to the higher concentration of atmospheric pollutants in the urban areas.

One of the most frequent bacteria genres was the Bacillus, possibly because they are low demanding organisms and form spores that act in favor of their survival in atmospheric conditions that are generally hostile for other type of microorganisms.

All along the 2015 it was observed the presence of microorganism colonies belonging to the Aspergillus, Penicillium, Cladosporium and Pestalotiopsis genres. Related to the previous information, the first three mentioned genres can affect the human health, as the literature says, the can cause respiratory illnesses that induce allergic rhinitis, asthma, and other consequences.

With the performed tests it was achieved to put in evidence the cell damage and at DNA level produced by the particulate material PM2.5 from the monitoring stations MED_UNFM, MED_PJIC (both represent traffic stations) and BAR_PDLA (background station)

There was a higher sensitivity on the Jurkat cell line in the cytotoxic tests. This gives insight about the people that present any type of anomaly or illness that would be more susceptible to the PM2.5 particulate material exposure.

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