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“Temporal analysis of indoor bioaerosols by epifluorescence microscopy: the implications of biochar during monitoring”

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sobre la Tesis intitulada:

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que se desarrolló bajo la dirección de

Dra. Sonia Lorena Arriaga García

El Jurado, después de deliberar, determinó

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Abbreviations

AMS	Andersen Microbial Sampler
BA	Bioaerosol
BAs	Bioaerosols
BC	Biochar
BET	Brunauer - Emmett - Teller Adsorption Isotherm
EU	Endotoxins Levels
EFM	Epifluorescence Microscopy
FTIR	Fourier-Transform Infrared Specrtroscopy
LAL	Kinetic chromogenic Limulus assay
NOx	Nitrogen Oxides
PM	Particulate Matter
PHAs	Polycyclic Aromatic Hydrocarbons
SEM	Scaning Electro Microscopy
SINAICA	Sintema Nacional de Información de la Calidad del Aire
SRSV	Small Round Structured Viruses or Noroviruses
SAM	Spores And Mold
SD	Standard Deviation
SOx	Sulphuric Oxides
TGA	Thermogravimetric Analysis
UV	Ultraviolet
USEPA	United States Environmental Protection Agency
VOCs	Volatile Organic Compounds
WHO	World Health Organization
XPS	X-ray Photoelectron Spectroscopy

Abstract

The air pollution represents a threat to society. Bad air quality can lead to consequences to ecosystems and human health. Since humans spent more than 90% of the time at indoor spaces it is necessary to warrant good indoor air quality. Bioaerosols, a predominant indoor pollutant, are biological particulate matter like fungi, bacteria, and viruses. These agents cause several diseases like influenza, allergies, and aspergillosis. Its proper control and inactivation are mandatory to ensure better human health.

Residual biomasses (RB) are sources of air pollution when these are improperly managed. Thermochemical transformations are a reliable alternative to obtain value-added products from RB like biochar; a solid rich in carbon structures and great potential to apply in different fields like the control of outdoor and indoor air quality. Also, there is a possibility to improve this material via functionalization as this is a cost-effective option to generate a material useful as a tool to improve air quality.

The aim of the present work is to study new alternatives to obtain functional biochar to be used to remove bioaerosols. To archive this, the biochar was functionalized with inorganic acids and the surface characteristics were explored. The acid treatment made the surface more negative, increasing the acid groups. The concentration of acids was found to be determinant in the final characteristics of the treated biochar. With this, it was determined that functionalized biochar has the potential for its use in the monitoring of bioaerosols and filtration of air polluted with this type of contaminant.

This biochar was later used in bioaerosols monitoring in a pediatric care center for three months, using liquid impingement as a sampling method and epifluorescence microscopy as an analytical method to count bioparticles. The results show that bioaerosols concentration follows seasonal patterns with coincidences with epidemical data reported by health authorities. Temperature, relative humidity, and human density had an important role in indoor bioaerosol dynamics. The biochar was found to be a good media to retain spores, mold, and bacteria, improving the number of bioparticles retained in comparison with traditional buffer. The viruses where inactivated when entering in contact with biochar.

CHAPTER I Monitoring and Control of Indoor and Outdoor Air Pollution.

1. The Atmosphere and Air Pollution

The atmosphere, soil, and water are environmental matrixes where life develops. The atmosphere is the gaseous layer that covers the Earth [1]. It is important for multiple processes of ecosystems. It regulates the temperature of the planet and filtrates UV radiation from the Sun. Also biogeochemical cycles, substances transport, and climatologic phenomena are regulated by this matrix.

Human activities have caused air pollution. Multiple pollutants can be present in the atmosphere and trigger adverse effects on ecosystems and human health. Air pollution can cause detrimental effects to ecosystems since they can cause toxicity to flora and fauna as well as climatic change [2], [3]. Atmospheric pollution can cause diverse respiratory illnesses like asthma and certain types of allergies to humans. This causes an increase in hospital admissions from vulnerable sectors of the population like children[4], [5]. Air pollution can occur both in outdoor and indoor spaces entailing environmental problems, then air pollution control is facing several challenges on a global scale.

2. Outdoor air pollution

2.1. Criteria pollutants

Outdoor air pollution comes principally from incomplete combustion of organic matter, vehicles, extraction of soil material and industrial activities. The United States Environmental Protection Agency (USEPA) has established 6 pollutants considered harmful to public health and the environment as “criteria air pollutants”. These pollutants are: a) suspended particles (PM_{10} and $PM_{2.5}$), b) ozone, c) volatile organic compounds (VOCs), c) nitrogen oxides (NOx), d) sulphuric oxides (SOx) and c) carbon monoxide (CO) [6].

a) Suspended particles (PM) are solid or liquid particles that remain in the air. These particle matter are classified according to its size as PM_{10} (Particles

from 10 to 2.5 μm of diameter) and $PM_{2.5.}$ (Particles bellow to 2.5 μm of diameter). The first type causes a high impact in atmospheric visibility and the latest can penetrate the upper respiratory system causing adverse effects on human life. Health issues of particulate matter include asthma, acute respiratory illnesses and cardiovascular illnesses [7]. On the other hand, particles can be beneficial for ecosystems since it serves as nuclei for raindrops and ice crystals influencing the hydrologic cycle and natural albedo of the Earth [8].

b) Ozone (O_3) is a potent oxidant and a greenhouse gas that is continually produced and destroyed in the atmosphere by chemical reactions. This gas filters in the stratosphere the UV radiation that comes from the Sun. However, tropospheric ozone causes adverse effects on human health as its highly oxidative properties to cause irritation in the respiratory tract [9].

c) Volatile Organic Compounds (VOCs) are molecules of C, H, and O with high partial pressure (higher than 10 Pa) and boiling point (80 – 100 ºC). VOCs are organic compounds containing one or more carbon atoms that evaporate rapidly to the atmosphere and react photochemically to form ground-level ozone. Also, VOCs may condense in the atmosphere to contribute to ambient PM formation. Besides biogenic sources (e.g. vegetation), other major sources include the petroleum industry, mobile sources, and solvent use. Some VOCs, such as formaldehyde and benzene, are carcinogenic [10].

d) Nitrogen Oxides (NOx) can be directly emitted to the atmosphere through combustion processes at 100 ºC or formed in the atmosphere via oxidation of atmospheric nitrogen. This gas reacts with VOCs and forms photochemical smog which forms a brown haze over the cities. Exposure to photochemical smog causes irritation of the respiratory tract, bronchitis, and pneumonia. This gas is also known as an inhibitor of plant growth [7], [11], [12].

e) Sulfur oxides (SOx) are a family of gases that consist mostly of sulfur dioxide (SO_2), a colorless gas. It can be chemically transformed into acidic pollutants, such as sulphuric acid and sulfates. SO_2 is generally a by-product of industrial processes and also comes from the burning of fossil fuels, such as ore

smelting, coal-fired power generators, and natural gas processing. SO_2 transformed into sulphuric acid is the main ingredient of acid rain, which can damage crops, forests, and ecosystems [10].

f) Carbon monoxide (CO) is produced from incomplete combustion of biomass or organic compounds. This gas is also emitted from car exhausts. This gas can be transported into the bloodline reacting with hemoglobin and forming carboxyhemoglobin which reduces the oxygen-carry capacity of the vascular system highly affecting the nervous system of humans [7].

Other less common pollutants of outdoor air include polycyclic aromatic hydrocarbons (PAHs), hexachlorobenzene, ammonium and heavy metals like mercury and lead. These substances are usually emitted in trace concentrations but the emission of these pollutants is highly dependent on the activities developed in different places of the earth [10].

2.2. GHG

Greenhouse Gases (GHG) are important for the Earth since they regulate the temperature of the planet by reflecting the radiation emitted from the Earth's surface back to keep it at a warm temperature of around 14.5 ºC. Without GHG, the temperature of the planet would be -4 ºC. However, since the industrial era, the concentration of GHG in the atmosphere has raised and with it the temperature of the planet. The temperature has raised 0.6 °C and with the current tendencies it is expected that it increases another 5.8 °C to 2100. This has caused climatic change which can be catastrophic for the life in the Earth [13].

The principal GHG are CO_2, CH_4, and N_2O. These molecules are non-polar with low water solubility and high stability. Table 1 presents a comparison between these three gases. Although CO_2 emissions are highest among the three gases, the global warming potential (GWP) is much higher for methane and nitrous oxide. Since CO_2 contributes mostly to global warming, mitigation strategies are principally focused on this gas.

Table 1 Emissions of GHG and mitigation strategies [14]–[23].

Gas	Carbon Dioxide	Methane	Nitrous Oxide
GWP	1	28-36	265-298
Estimated emissions (Mt of eq. CO_2)	37 520 (71%)	7504 (20%)	2853 (8%)
Sources of emission	Fossil fuels combustion especially in power plants.	Livestock, Creation of Dams, Exploitation of fossil fuels, rice production and disposition of waste.	Agricultural soils (nitrification process of fertilizers). Production of nitric acid, adipic acid, and caprolactam.
Mitigation strategies	Membrane technologies, absorption, and adsorption	Catalysis, biofiltration, and adsorption	Prior-emission action, catalytic reduction

2.2.1 Mitigation Strategies of GHG

Currently, several strategies for mitigation of GHG have been reported. Mitigation technologies for Carbon Dioxide include membrane separation, absorption, and adsorption; for methane, it can be mentioned catalysis, biofiltration, and adsorption; and for nitrous oxide catalytic reduction.

Carbon Dioxide

Carbon Dioxide (CO_2) is a molecule composed of two atoms of oxygen and one of carbon. Membrane separation of CO_2 considers the differences in the relative transfer rates or permeation of various gases through a membrane barrier to achieve

gas separation. This process is influenced by both the relative diffusivity and surface adsorption of the various gases present. The exact mechanisms for transport and adsorption vary for different membrane materials. The most common materials to capture CO_2 include polymers, ceramics, metals, and zeolites. [24].

The phases included in the absorption of CO_2 are the diffusion of CO_2 from the bulk-gas phase to the interface, the reaction at the interface and the diffusion of aqueous CO_2 species into the bulk liquid. The materials typically used to accomplish this are amines, non-aqueous solvents and carbonate-based solvents [24].

Adsorption processes to capture CO_2 are not usually chosen over membrane separation or absorption in the industrial sector. The principal problem with such technologies is that they have low cost-effectiveness. According to the Energy Department of the United States of America, the ideal sorbent for adsorption of CO_2 has to be cheap, stable (chemically and mechanically) and highly selective. It is also necessary a high CO_2 uptake at low pressure, a high adsorption capacity, and low requirements of energy during the regeneration process [24].

None of the materials currently available for the adsorption process achieves all the characteristics of the ideal sorbent. The most common conventional CO_2 adsorbents are natural zeolites, activated carbon and alkali-based metal materials. However, these present limitations on the CO_2 retention capabilities per adsorbent mass [19]. For example, the adsorption capacities of zeolites range from 4 to 216 mg of CO_2 per gram of adsorbent and for traditional carbonaceous materials these values range between 100 and 1130 mg of CO_2 per gram of adsorbent [22].

Methane

Methane (CH_4) is an organic molecule composed of one atom of carbon and four hydrogens. The aim of catalysis is the oxidation of methane to break the C−H bonds, transforming it into methanol and ultimately in CO_2 and water. The noble metals are particularly appropriate to catalyzing CH_4 oxidation because they are stable over a wide range of conditions and are reasonably resistant to fouling by interfering compounds in the gas stream, such as sulfur gases [18].

Biofiltration is a biological oxidation method performed by methanotrophic bacteria. As catalysis, the aim of this process is the ultimate oxidation of CH_4 into CO_2. This technology has been successfully tested with emissions from landfills, piggery and ventilation coal mine air with maximum removal rates of 102 g of CH_4 per cubic meter per hour [18].

Adsorption of CH_4 to reduce the emissions of this gas to the atmosphere has been tested. Some of the adsorbents typically used to capture CH_4 include activated carbon and zeolites. In addition to this, some new materials have been studied seeking to increase the cost-effectiveness of the process which includes MOF's, modified activated carbons, and porous polymers [19]. This technology takes advantage of the pressure resultant from industrial processes.

Nitrous oxide

Nitrous oxide (N_2O) is an inorganic molecule with 2 nitrogens and one oxygen. It is a potent GHG (see Table 1). Thus, the efforts for N_2O mitigation are prior-emission. For example, agricultural N_2O emissions can be reduced by increasing nitrogen use efficiency in crop and animal production with proper use of fertilizers and manure. Also, it has been proposed the modification in diets and the use of nitrification inhibitors in crop production [23].

Compared to agriculture, N_2O emissions from combustion and industrial sources are small, thus the mitigation technologies are limited. Catalysis has been widely used in the nitric acid industry in the United States (US). This industry uses both selective and non-selective catalytic reduction. Non-selective catalysis reduction is more effective than selective catalysis reduction at controlling N_2O. However, these units are generally not used in current facilities because of their high energy costs. Another type of catalyst is the iron supported by zeolites which either decomposes N_2O into N_2 and O_2 or reduces N_2O using various reducing agents such as hydrocarbons [23].

It is worth to notice that most industrial applications of these technologies consume high amounts of energy since most of the GHG are emitted at standard conditions. The development of new mitigation of GHG strategies is needed in order to have

cost-effective processes and available to all parts involved and responsible for climatic change.

3. Indoor Air Pollutants

The human being spends most of its time indoors (e.g. working or resting). For this reason, a high concern for indoor air quality has raised over the years. Indoor air quality (IAQ) aims to improve the environment of buildings and structures for the occupants. Usually, is caused by combustion products (e.g. VOC's, CO and black carbon) and particles [25]. Improper ventilation, presence of sources of pollution (both from inside and outside the buildings) and microbes are the main factors that contribute to indoor air pollution [26].

Global public health is threatened by bad IAQ. It demands higher research efforts for those in charge of public policies. These efforts should be focused on exposure doses and health effects of air pollutants [27]. IAQ has been widely studied in rural zones in Mexico where wood stoves are still normally used. There are also studies about radon, especially for the Northern/Central parts of the country. VOCs and particulate matter are occupational matters and have been also studied [28]–[32]. The biological fraction of particulate matter has been a specific attention focus because of the possible adverse effects and the dominance of this type of pollutant.

3.1. Bioaerosols

From 5 to 34% of total PM in indoor air is composed of bioaerosols (BAs). BAs are also called bioparticles and they are referred to the biological part of airborne particles. These airborne particles are living or originate from living organisms and came in different sizes which go from pollen particles (1 mm) up to small protein chains, allergens and endotoxins of 10 nm (see figure 1) [3], [33], [34] Bioaerosols are responsible for some epidemic illnesses and rot of certain types of food [35].

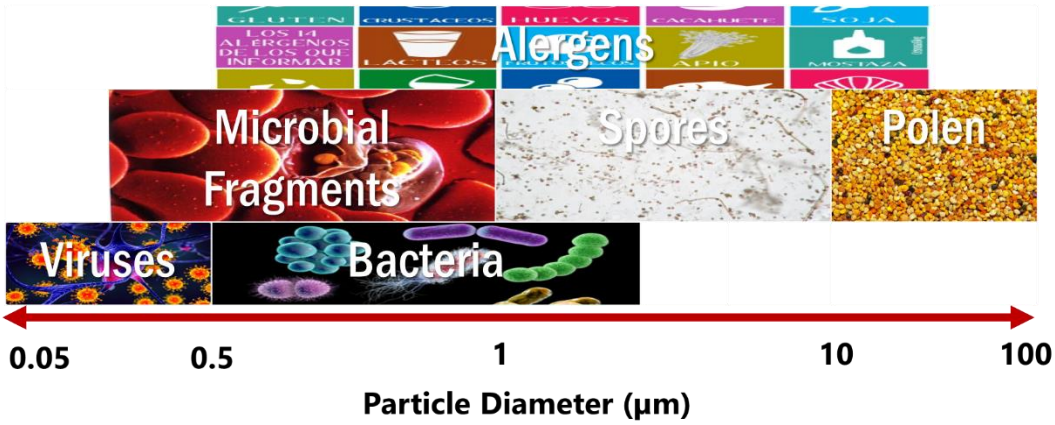

Figure 1 Size and types of bioaerosols

The indoor BAs may possibly come from Heat Ventilation and Air Conditioning (HVAC) systems, areas with high moisture, porous surfaces, pets, plants, food, and outdoor air [26] [36]. Also, human activities like mopping and sweeping can be sources or biological particles [37]. Humans act as vectors for bioaerosols due to the possibility of transportation from the outside via hair, skin, clothes or belongings [38].

High concentrations of BA (around 10^2 – 10^4 CFU/m^3) can threaten human health and cause several adverse effects [39]. Several studies of occupational health point that concentration of bioparticles in indoor air is correlated with the incidence of illnesses of workers [35], [39]. Waste management, food processing, and agriculture workers are the most exposed to BAs pollution [33].

The possible damages of BAs include infectious diseases, respiratory illnesses, and cancer. The main infectious illnesses are aspergillosis, histoplasmosis, and blastomycosis. In the case of respiratory illnesses, they can cause allergic and non-allergic responses as well as asthma, Chronic Obstructive Pulmonary Diseases (COPD) and alveolitis [3]. Cancer can be triggered via oncogenic viruses, pesticides and inductive mycotoxins like aflatoxin. Lung cancer is not the only type of cancer associated with BA since lip, liver, stomach, and brain cancer have been also reported [3].

3.1.1. Bioaerosols monitoring

The monitoring of BAs refers to all efforts to determine the concentration of these. National Institute for Occupational Safety and Health (NIOSH) establishes three techniques widely developed for bioaerosols sampling: a) solid impactation; b) membrane filtration and c) Liquid impingement [3], [40]–[42].

Solid impactation

This method collects microorganisms and airborne particles by the separation from air current via inertia. Microorganisms are collected onto agar or glass plates by a direct crash with the surface of particles with dynamic energy [40]. Impactors can have different sizes, shapes of the input and number of collection chambers. [39], [42]. After collection, microorganisms are cultivated in plate dishes and the colonies grown are later counted [42], [44], and [45].

This method has a collection efficiency of 50% (especially for spores) and also affects the viability of stress-sensitive microorganisms. Another disadvantage of the impact methods is the overload by microorganisms in the agar plates that may make the counting of colonies difficult. Also, problems of humidity or desiccation of the impact media can occur, which reduces the available area of impact and limits the number of bioaerosols sampled [43], [44].

Membrane filtration

This type of sampler is relatively simple and less expensive compared to impact and impingers. Filters are the most commonly used retention media when resistant cells such as spores and pollen are sampled, the particles retained in the filters tend to represent the interaction patterns to which people are exposed to bioaerosols.

The air is sucked through a vacuum line through a membrane filter made of fiberglass, PVC, polycarbonate, cellulose acetate, polyester fibers or soluble gelatin. The forces responsible for the collection or capture of particles are inertial, diffusive and electrostatic [45], [46]. The retention of bioaerosols depends on three parameters: a) aerodynamic diameter, b) pore size of the filter, and c) airflow through the filter [47].

When filters are used in environments with a high level of contamination, the enumeration of bioaerosols becomes very difficult or impossible with dependent culture methods due to the overload of filters with microorganisms [42]. This method is recommended only for the analysis of microorganisms that are not sensitive to the desiccation of the medium [40]. Dehydration stress depends on the sampling time. Therefore, it has been shown that this method has a recovery efficiency of bioaerosols of around 50% [39].

Liquid impingement

This technique uses an isotonic liquid or a buffer solution to avoid osmotic stress that may occur to the collected microorganisms that collide inside the impinger. Liquid impactation is one of the most used methods for its easy handling, high representativeness, and reduction of stress towards microorganisms and better conservation of bioaerosols [3]. In addition to their design, the impactors are especially useful for the study of airborne microorganisms that cause infectious diseases by having the ability to separate them into breathable (those that remain within the liquid) and non-breathable (those that remain in the nozzle) [3], [39].

When the air collides with the liquid it does tangentially, that is, the bioaerosols disaggregate by inertia and the bubbling caused when they collide with the impactation liquid and the air current penetrates the capillary tube inside the impinger and is forced to change direction being suspended and trapped in the liquid. This process occurs under sonic flow [48].

This sampling method has been evaluated and compared with others and has proved to cause less cellular stress, maintains a percentage greater than 75% of viable fragile cells or 93% of viable resistant cells. In addition, cells retained in the impaction fluid can be subsequently analyzed by independent methods like epifluorescence microscopy (EM) and dependent culture methods such as pate counting dishes [47], [48].

Table 2 shows the state of art for Indoor Air BA. Hospitals and educational centers are the most studied places due to the implication of bad IAQ in this environments. There is a marked interest in the occupational effects of BA and several facilities

have been studied. Molds and Bacteria are the most studied type of BA via plaque counting (PC). This method is the easiest way to determine the concentration BA but has problems of underestimation of total BA (viable and non-viable. The three methods proposed by USEPA are widely used. The viable BA concentration are close to the threshold established by the WHO (10^2 CFU/m^3).

Table 2 Monitoring of Indoor Air Bioaerosols

Monitoring Site	Monitored Bas	BAs concentration.	Techniques used		Remarkable findings	Reference
			Sampling	Counting		
Primary School	Mold and bacteria	Bacteria: $2.94x10^2$ CFU/m^3 Mold: $5.53x10^2$ CFU/m^3	Solid Impactation (SI) (single-stage AMS)	Plate Counting (PC)	Total microbial cell concentrations measured by using non-culture-based methods were 100 to 1000 times higher than those by using culture-based method.	[49]
Primary School	Mold and bacteria	Total: $8.17x10^5$ cells/m^3	Liquid Impactation (LI) (AGI-30)	Eprifluorescence Microscopy (EMF)		[49]
Biofilter	Mold and bacteria	Mold: $9.45x10^6$ Bacteria: $2.55x10^7$	LI (AGI-30)	EMF	Values 8400 times greater than the reported value for culture-based method.	[2]
Daycare and	Bacteria and Viruses	Bacteria: $3.40\pm1.60x10^5$ BLP/m^3 Viruses:	Membrane Filtration (MF)	EMF		[50]

Monitoring Site	Monitored Bas	BAs concentration.	Techniques used		Remarkable findings	Reference
			Sampling	Counting		
Healthcare center		$2.90\pm2.30x10^5$ VLP/m^3				
Cancer Treatment Center	Mold	30 530 CFU/m^3	Cyclonic Air Sampler	Plate counting (PC)	Highest values found in summer due to high humidity levels.	[51]
Office complex	Bacteria and endotoxins	277 CFU/ m^3 and 20.3 EU/mg	SI (Merck100 Air Sampler)	Bacteria: PC Endotoxins: LAL	Human-related and pet-related species. There were also differences between offices and nursery.	[52]
Hospitals	Mold and bacteria	From 32 to 342 CFU/m^3 and from 90 to 548 CFU/m^3	MF	PC	Dermatophyte agents and respiratory illnesses-related agents were identified. Infer bad IAQ.	[53]
Biometanization plant	Mold, bacteria, and endotoxins	$3.7x10^3$ CFU/m^3 during summer and $3.2x10^2$	SI (six-stage AMS)	Bacteria and mold: qPCR Endotoxins: LAL	High recommendations for the use of PPE.	[54]

Monitoring Site	Monitored Bas	BAs concentration.	Techniques used		Remarkable findings	Reference
			Sampling	Counting		
		CFU/m3 during winter				
Campus	Endotoxins	72 endotoxin units (EU)/m^3	SI	LAL	Other environmental pollutants like $PM_{2.5}$ and COV's positively correlate with endotoxins concentration.	[55]
Residencial	Mold and Bacteria	From 1.38×10^6 to 4.48×10^6 cells/m3 (total)	LI	EFM	The fluorescent method detects between 630 y 5200 more BA than traditional plaque counting methods.	[56]
Hospitals	Mold	47.68 CFU/m^3 for rainy season and 49.57 CFU/m^3 for dry season	SI	PC	The *Aspergillus* genera were dominant and maybe causing aspergillosis.	[57]

Monitoring Site	Monitored Bas	BAs concentration.	Techniques used		Remarkable findings	Reference
			Sampling	Counting		
Campus libraries	Mold	360/1230 CFU/m^3	SI	PC	Environmental factors (e.g. RH and temperature) have significatan influence on BA concentrations.	[58]
Hospital Dental Departament	Bacteria	From 247.97 to 4058.67 CFU/m^3	SI	PC	Humans influence the concentration of BA.	[59]
Tanneries	Mold and Bacteria	From 30 up to 9760 CFU/m^3	SI	PC	Low IAQ causes a detriment in workers' health	[60]
Residential	Mold	1653 CFU/m^3	SI	PC	RH and temperature influence spore concentration. Because of this, there is a seasonal pattern.	[61]

Monitoring Site	Monitored Bas	BAs concentration.	Techniques used		Remarkable findings	Reference
			Sampling	Counting		
Pediatric Cares Center	SAM, Bacteria and Viruses	SAM: $5.05\pm4.93x10^5$ BA/m^3 Bacteria: $4.45\pm2.35x10^5$ BA/m^3 Virus: $4.37\pm1.66x10^6$ VLP/m^3	LI	EM		This work

Characterization of bioaerosols

Figure 1 shows the characterization techniques for BA. They can be divided into culture-dependent and non-culture dependant. The culture-dependent techniques identify microorganisms based on isolates and the analysis of their metabolic properties, expressing themselves as colony-forming units after an adequate incubation. This allows the study of physiology and biochemistry of microorganisms to confirm ecological field processes and allows them to evaluate or confirm the hypotheses of molecular studies (for each strain). However it is not possible to obtain an accurate view of the biodiversity of complex microsystems and only about 10% of bioaerosols present in the ambient air can be quantified and identified since it is not possible to recreate natural conditions for the growth of all microorganisms [42].

Independent culture techniques can be separated into molecular, immunological and microscopic techniques. Molecular techniques focus on the study of genomics and metagenomics with the help of molecular techniques, allowing phylogenetic comparisons [62]. With this technique, it is possible to archive an increased knowledge of microbial diversity and allow comparisons with different resolution levels. By studying the DNA, it is possible to define the type and quantity of microbial species present in a specific sample, while the study of RNA allows us to understand what is the metabolically active part of the population. However, this technique is highly cost base on the equipment and reagents used. Also, the overestimation of bioaerosols can be attained as fragments of DNA can be overquantified.

Microscopy techniques are based on the study of microorganisms directly from the sample, making the microscopy observation for the human eye, facilitating this process by the use of contrasts and dyes. With this is possible to count the entire cell population. Also, the use of specific dyes allows a more detailed classification of viability, morphology and cell separation. However, this method is time demanding.

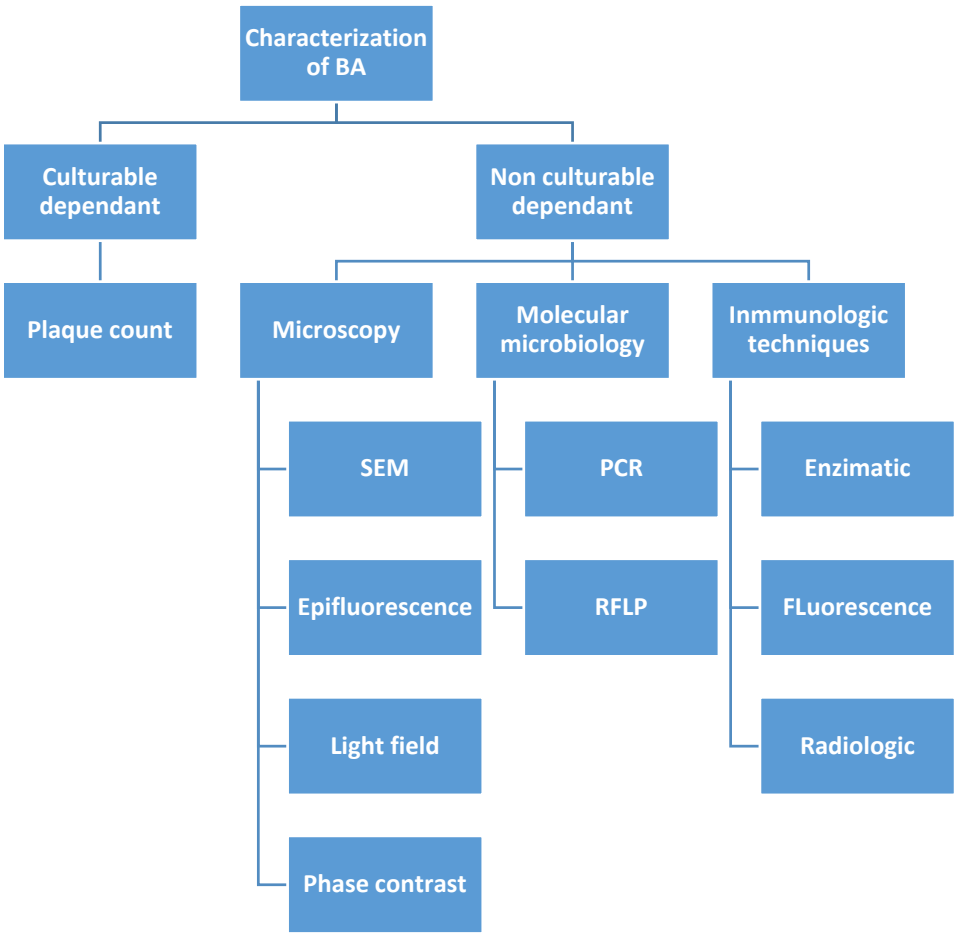

Figure 2 Characterization of BA

3.1.2. Bioaerosols Legislation

The World Health Organization (WHO) stablished a limit exposuere value to BAs from indoor air less than 300 CFU/m^3 . This value only considers culturable microorganisms, however as explained earlier, this underestimates the total concentrations of BAs. Also, microbial fragments and substances (e.g. edotoxins and β-glucans) can cause adverse effects to human health and thus these type of BAs must be taken in account in any legislation attempt [40], [42], [63].

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CHAPTER II Valorization of Residual Biomass: Biochar Production and Applications

1. Valorization of Residual Biomass

Residual biomasses (RB) are those waste materials that decompose under the action of microorganisms [1]. These come from different sources like wood and food processing industries and municipalities (e.g. restaurants, gardens, and food markets). The main problem with RB is that they are generated in high volumes which difficult their management and proper disposal, leading to several environmental problems [2]. The bad management of RB causes soil, water, and air pollution. Also, RB attracts pests and they are a source of diverse infections and illnesses since they may contain pathogen agents [3], [4].

In 2016, the worlds' cities generated 2.01 billion tons of solid wastes. Waste generation is expected to increase to 3.40 billion tons in 2050, corresponding to an increase of 70% from 2016 levels [5]. North America generated around 260 million tons of waste in 2012, with an individual contribution of 7.1 % for Canada, 77.7% for the USA and 15.2% for Mexico.

RB is the second most abundant and inexpensive source of carbon on the planet, just after atmospheric CO_2 [6]. This characteristic makes it a promising source of alternative by-products via valorization. Valorization of RB is an alternative that allows the production of value-added products avoiding the problems of its disposal and contributing to the reduction of environmental impact.

There are several technologies available to valorize RB like animal rendering and aerobic and anaerobic processing. Animal rendering is the process of cooking and drying livestock and poultry that are not intended for human consumption. These results in edible and inedible by-products that can be used as food supplements, health and beauty goods, fertilizers, and pet food [7]. During anaerobic processing, the organic matter breaks down in the absence of oxygen into biogas which consists of CO_2 and CH_4 principally which can be later used with energetic proposes. This process also leaves an organic solid residue called digestate that can be used as a

biofertilizer. The advantage of this process is that the natural gas obtained from RB has a negative life-cycle carbon footprint (-23 gCO2e/Mj) [7].

Aerobic processing and treatment, also known as composting, refers to the transformation of organic materials by aerobic microorganisms into a natural fertilizer. The uses of compost are dictated by its quality and not all RB are suitable for this process due to humidity content [4], [7].

2. Thermochemical Transformation of Residual Biomasses

2.1. Thermochemical transformation definition

Another alternative for the valorization of RB is thermochemical transformation. As the name may suggest, thermochemical transformation consisting of the application of thermal treatment of biomass to produce solid, liquid or gaseous products, which can be upgraded to synthetic biofuels, catalysts, and fertilizers [6].

When thermal treatment is applied the first element being degraded is hemicellulose. This process takes place between 195 y 350 ºC. Later, lignin is degraded between 280 y 500 ºC [8]. As a result, the liberation of volatile compounds and gases happens, remaining a carbon-rich solid phase [9].

Thermochemical transformation stabilizes the carbon present in the biomass since most of the organic forms are converted into inorganic structures. The by-products obtained possess specific properties that give added value to them.

2.2. Processes for thermochemical transformation.

The technologies used for the thermochemical conversion of RB include gasification, pyrolysis, roasting (also called torrefaction), carbonization, combustion, and hydrothermal processes. The main differences between these processes are shown in Table 1. The temperatures used in thermochemical treatments range from 150 to 2000 ºC. It can be noticed that the lower the temperatures, the higher the yields of solid-phase produced. Also, there is a tendency to increase pressure conditions within the diminution of temperature.

Table 3 Strategies used in the thermochemical transformation of RB [10]–[12].

Process	Temperature (ºC)	Conditions	Yields (%)		
			Gas	Oil	Char
Gasification	900 – 2000		85	5	10
Pyrolysis	300 – 900	Inert or low-oxygen atmosphere	20	70	10
Roasting/Torrefaction	200- 350	Inert or low-oxygen atmosphere	35	30	35
Carbonization		Pressure higher than atmospheric	30	25	45
Hydrothermal Carbonization	150 - 350	Applicable to slurry	35	20	45

The value-added products obtained with the different thermal processes are Syngas (gas phase), Pyrolytic Oil (volatile compounds), Biochar (solid phase) and energy in the case of combustion. Syngas consists mainly in CO and hydrogen-rich molecules (mainly H_2S). Pyrolytic oil is a mix of volatile molecules of high molecular weight and high viscosity values [10].

3. Biochar

3.1. Biochar definition

Biochar (BC) is a solid carbonaceous material obtained from the thermochemical transformation of biomass [13], performed under oxygen-limited atmosphere. The

material is a black, carbon-rich, stable, refractory and highly aromatic solid [14], [15]. Biochar differs from charcoal (a fossil fuel) since it is produced from organic carbon.

3.2. Characteristics of biochar

In general terms, BC is a material with a high content of carbon and with negative electrostatic surface charges caused by the presence of epoxide, hydroxide and carboxylic groups [16]. BC composition and structure are not uniform. On its surface, it is possible to find inorganic salts, amine groups and metalorganic structures [8].

Biochar properties and characteristics depend mainly on the feedstock characteristics and pyrolysis conditions causing that the physical, chemical, and biological characteristics of material change [8], [11], [13], [17]. For example, high salt and ash content is expected in wheat straw derived BC but not in wood-derived biochar. C and N content should be higher in pence chips derived BC than in poultry litter derived BC [9].

Characterization methods developed for biochar include proximate analysis (moisture, ash content, fixed carbon), ultimate analysis (C, H, O and N content), physical and chemical analysis (pH, conductivity, particle size distribution), surface analyses (morphology, FT-IR, acid/basic functional groups), and molecular and structural analysis (thermal stability, Raman Spectroscopy, TEM). The specific determinations to be performed to the material depends on the final use of this [11], [18].

3.3. Application of biochar

Biochar has plenty of potential applications [13], [17]. The roles and influence of biochar in different applications have been deeply studied and yet have not been fully understood [11]. These applications include, but are not limited to, soil amendment, reduction, and capture of GHG emissions, adsorption processes, and energy production [11], [17].

3.3.1. Soil amendment.

The BC has the capacity to improve soil quality and release nutrients conserving its carbon structure [10], [19]. BC contains a large proportion of stable carbon that is

very resistant to decomposition and can stay in the soil for several hundred years [20]. For this reason, this material can be considered as a method to sequester carbon in the soil.

The advantages in the utilization of BC in soil amendment are the low cost of the process, high retention of water and nutrients, and reduction in the utilization of fertilizers. However, it is possible to unintentionally add heavy metals and PAHs to the soil [10].

3.3.2. Sequestration of Greenhouse Gases

Biochar is a promising material that can help with this task either in a passive way or in an active way. The use and production of biochar is intrinsically an important mechanism to capture CO_2 as the atmospheric CO_2 is captured by the plants via photosynthesis and subsequently transformed to char which is a more stable form of C [10], [13]. This way the emissions of CH_4 are reduced since organic matter does not enter the process of anaerobic decomposition in landfills or open terrains [21]. N_2O emissions can be attained when biochar is applied to the soil since fewer amounts of fertilizers are needed, reducing the conversion of these into the GHG [19]. Moreover, this material has proved to have capabilities of CH_4 and N_2O adsorption even though more research is still needed to fully understand the mechanisms involved and possible improvements to the processes [2], [22].

3.3.3. Adsorption

The abundance of oxygenated groups on biochar facilitates adsorption of multiple pollutants [10]. The pollutants successfully attached to the biochar surface are heavy metals[23]–[29], dyes [12], [30], [31], nitrogen compounds, microorganisms [32] and greenhouse gases (GHG) [2], [16], [22], [33]. Other compounds like enzymes have been successfully attached to indirectly treat other pollutants like medicines in wastewater [34].

The advantage of this material over other types of adsorbents is the low cost of productions. However, the efficiency of adsorption processes is still uncertain due to the nature of the material [10].

3.3.4. Energy

Biochar is found in electrochemical energy and fuel cell catalysis as electrode materials [35]. This occurs thanks to electron transfer catalysis which involves three types of redox-active structures: phenolic, quinone and condensed aromatic structures [25].

Biochar can also be used as fuel in fuel cells as a substitute for common charcoal [10]. This reduces the utilization rates of mineral coal and GHG emissions are reduced. However, the voltages and the power output is relatively low making it unreliable compared to coal [10].

3.4. Engineered biochar

Engineered biochar (also called, smart biochar or designed biochar) is a special type of biochar obtained through diverse post-production treatments [17], [18]. The key aspect of the engineered biochar is that biochar is produced with specific and controlled properties for particular purposes. These modifications are likely to result in changes in biochar surface properties including surface area, surface charge, functional groups, and pore volume and size distribution [17]. There are two main processes to obtain designed biochar: activation and functionalization.

3.4.1 Production of activated carbon

Biochar can be used as raw material for activated carbon. The activation process can be either chemical or physical. Chemical methods use $ZnCl_2$, KOH, H_3PO_4, and K_2CO_3 as reagents while the physical method uses oxidizing gases like CO_2, steam or air.

Chemical activation can be considered as one-step activation if carbonization and chemical activation taking place simultaneously or two-step activation if are two consecutive steps. Although physical activation is in two-steps, it is clean and easy to control than a chemical one [10].

The interaction of biochar with environmental molecules changes when it is activated. Activated biochar improves covalent bonding of carbon-rich molecules (e.g. CO_2), increases the pore volume and surface area increasing the sites available

to the adsorption process and increases interactions with molecules of water and several heavy metals [2], [12], [16], [29], [30], [36].

3.4.2. Functionalization

Functionalization consists of grafting different functional groups onto the surface of biochar this causes a modification on surface characteristics of the material. Acid/base treatment, carboxylation, and amination, treatment with organic solvents, surfactant modifications, coating, steam activation, gas purging, impregnation with metal oxides and magnetic modification are some of the mechanisms used in the production of engineered biochar [17].

This process is more flexible than the activation process and the results obtained are highly variable depending on the treatment used. For this reason, it is possible to obtain a material capable of treat pollutants of different natures like dyes, heavy metals, organic compounds and nitrogenated agents [26]–[28], [31], [37], [38].

Table 2 shows the state of art for biochar functionalization. Multiple organic feedstocks are used, mostly consisting of RB form industrial processes. Pyrolysis is the production process most recurrent since, as stated before, the highest yields of the solid byproduct can be obtained with this thermochemical transformation. We can notice that there's is a high variety of functionalization agents like acids, amines, and metal oxides. Oxidation with acids is the functionalization type most recurrent among them. The principal reason to preform post-production processes is to improve interaction between biochar and a target pollutant. This transformation is principally focused on heavy metals and organic compounds. Almost all transformation leads to an increased affinity between the sorbent and biochar proving the high versatility and success of this process.

Table 4 Modifications of biochar with different aims

Biochar feedstock	Treatment conditions	Type of treatment	Modification	Characterization	Findings	Ref.
Silvergrass fibers	Pyrolysis Temperature (T) =497 °C	Acid functionalization with H_2SO_4 and HNO_3 with biochar: agent rate of 1g:100 ml during 6h	Production of composites with a polymer matrix	FTIR, Raman Spectroscopy, SEM, Elemental Analysis and TGA.	The mix of HNO_3–$H2SO_4$ treatment revealed to be the most effective at modifying the biochar providing the best degree of functionalization.	[39]
Hazelnut shells	Hydrothermal carbonization T= 220-260 °C Time (t) =4-6 h	Acid functionalization with HCl and H_2SO_4 with biochar: agent rate of 1g:100 ml during 4 h at 60 °C. Basic functionalization with NaOH and NH_3. Coating with $(NH_4)_2SO_4$.	Adsorption of cationic and anionic dyes (methyl orange, methylene blue)	SEM, BET, X-ray Photoelectron q Spectroscopy (XPS), FTIR, Boehm Titration and Zeta Potential.	Surface functionalization leads to an increase in oxygen-containing functional groups on the surface. .	[31]

Biochar feedstock	Treatment conditions	Type of treatment	Modification	Characterization	Findings	Ref.
Luffa cylindrica	Pyrolysis T=650 °C t= 1h	Acid functionalization with HNO_3 at 80 °C for 3 h.	Rare lands binding	Boehm Titration, FTIR, XPS, and SEM-EDX	The biochar presented a strong bind with trivalent lanthanides attributed to the vascular structure of the biochar fibers. Also, the chemical affinity for U (VI) was increased.	[40], [41]
Pinewood	Pyrolysis T= 525 °C t= 2 min	Acid functionalization with HCl, H_2SO_4, HNO_3 and mixtures of them with biochar: agent rate of 1 g:250 ml during 48 h	Immobilization of laccase for removal of carbamazepine from water.	Boehm Titration, SEM, FTIR, BET, and Particle Size	The treatment increased the laccase binding to biochar. Thus the material exhibited 83% and 86% of carbamazepine removal.	[34]
Wheat straw	Pyrolysis T= 450 °C	Acid functionalization with mixtures of HNO_3 and H_2SO_4 with biochar: agent rate of 1	Simulation of the aging process of biochar to determinate	Elemental Analysis, FTIR, XPS, SEM-EDS, BET, and TGA	The oxidation process augmented the quantity of COO- causing an increase in the active sites for Cd adsorption.	[26], [27]

Biochar feedstock	Treatment conditions	Type of treatment	Modification	Characterization	Findings	Ref.
		g:80 ml during 6 h at 70 °C Alkaline-oxidative functionalization with NaOH and H_2O_2 using a biochar: agent rate of 1 g:3 ml during 24 h	modifications in Cd adsorption capacities.			
Bamboo	Pyrolysis T= 550 °C	Acid functionalization with HNO_3 with biochar:agent rate of 1 g:10 mL at 99°C for 24 h. Alkaline functionalization with NaOH under the same conditions as above.	Furfural removal	Elemental Analysis, BET, FTIR, and Boehm Titration.	Chemical functionalization reduced the capacity of furfural adsorption. However, the heat treatment resulted in a hydrophobic matrix with more affinity to furfural.	[37]

Biochar feedstock	Treatment conditions	Type of treatment	Modification	Characterization	Findings	Ref.
		Oxidative functionalization with $KMnO_4$ under the same conditions as above.				
		Heat treatment with an N_2 atmosphere at 800 °C for 2 h.				
Rice Straw	Pyrolysis T= 800 °C t= 1 h	Acid/Oxidative functionalization with H_2O_2 and HNO_3 with a bichar:agent rate of 1 g:25 mL at 25°C for 24 h	Cd^{2+} adsorption	FTIR, Boehm Titration, BET and SEM-EDS	The acidic sites formed by the agents played a major role in cadmium adsorption. The electrostatic forces between positive Cd^{2+} and the negative surface of biochar augmented the adsorption capacity.	[28]

Biochar feedstock	Treatment conditions	Type of treatment	Modification	Characterization	Findings	Ref.
Cow dung	Pyrolysis T=700 °C t= 2 h	Coating with $FeCl_3$ with biochar: chloride rate of 2g:1g at 60 °C during 4 h	Improve the adsorption of perchlorate ion	SEM, BET, Boehm Titration, Elemental Analysis, FTIR, XPS and Z-Potential	The impregnation increased the biochar adsorption capability almost 6 times. The adsorption is mainly due to electrostatic interactions, hydrophilic conditions, larger surface area, and a larger amount of oxygenated groups.	[14]
Rice Husk	Pyrolysis T= 500 °C t= 2 h	Surfactant modification with CTAB and SDBS using biochar: surfactant solution rate of 1g: 20 ml	Improve the adsorption of nitrates and ammonium	BET, Z-Potential and FTIR	The modification with CTAB successfully improved the adsorption of nitrate.	[38]
Cardboard	Torrefaction T= 250 and 300 °C	Chemical activation with KOH for 1 h	Improve the adsorption of methane	XRD, BET, SEM, FTIR, Elemental Analysis and TGA	The chemical treatment decreased the adsorption capacity due to an increase in biochar moisture caused	[2]

Biochar feedstock	Treatment conditions	Type of treatment	Modification	Characterization	Findings	Ref.
	t= 1, 1.5 and 2 h				by stronger interaction between water molecules and biochar.	
Municipal Solid Waste	Pyrolysis T= 400, 500 and 600 °C t= 0.5 h	Chemical activation with KOH using biochar: solution rate of 1g:250 mL for 1h under constant agitation	Improve the adsorption of As (V)	BET, FTIR, SEM, Elemental Analysis	The activated biochar enhanced its adsorption capacity to As (V) due to an increase in surface area and pore volume and the changes of functional groups on the surface of activated biochar.	[29]
Pinewood	Pyrolysis T= 550-600 °C	Amination with N- (3-dimethyl aminopropyl-N′-ethyl carbodiimide hydrochloride (EDC), tetraethylenepentamine (TEPA), methanol and hydroxy benzotriazole	Improve the adsorption of CO2	Raman spectroscopy, FTIR, Elemental Analyze, SEM	The biochar was successfully activated using ultrasound leading to an effective amination used to covalent-bind CO_2 improving the adsorption	[16]

Biochar feedstock	Treatment conditions	Type of treatment	Modification	Characterization	Findings	Ref.
		(HOBt) during 24 h at 35 °C			capacity of almost ten times compared to raw biochar.	
Cassia fistula	Pyrolysis T= 800 °C t= 4 h Hydrothermal Carbonization T= 190 °C T= 24 h	Chemical activation with K_2CO_3 Physical activation with temperature = 800 °C during 4 h	Improve the adsorption of iodine and methylene blue.	TGA, density, hardness, BET, SEM, XRD, FTIR Boehm Titration,	The high superficial area (598m^2/g) and total acidic groups (0.72 mmol/g) along with high fixed carbon (84%) and percentage of carbon (77%) contributed in good adsorption of iodine (794 mg/g) and methylene blue (33 mg/g).	[12], [30]
Phragmites australis	Pyrolysis T= 450 °C t= 1h	Acid functionalization with H_3PO_4 with a mass ratio of 2:1 for 10 h at 25 °C (before pyrolysis).	Improve the adsorption of Pb (II) in humic acids	SEM, Boehm titration, Elemental Analysis, FTIR, XPS.	The adsorption of Pb was successfully archived via strong π-π interactions.	[42]

4. Conclusions

The by-products obtained through the valorization of residual biomass contribute to the solution of multiple environmental problems. The use of these products can help to mitigate the emissions of GHG by the reduction of the utilization of fossil fuels and other materials with higher environmental impacts (like metallic catalyzers). Thermochemical transformation produces syngas, pyrolytic oil, and biochar. All of these by-products can positively contribute to the amendment of the environment both directly and indirectly.

There is an important field of research in current and possible applications for biochar. However, more information is needed in order to make this material cost-effective in processes like catalysts and energy generation. Other processes, like adsorption, need deeper insights into the mechanisms involved in them for a later application in bigger scales.

Engineered biochar opens a new world of uses for this material since the properties can be purposely changed to archive diverse aims. The optimization of the processes is vaguely explored due to the high variations on the properties of raw material. However, current studies show high potential for its future application in adsorption, catalysis and air treatment.

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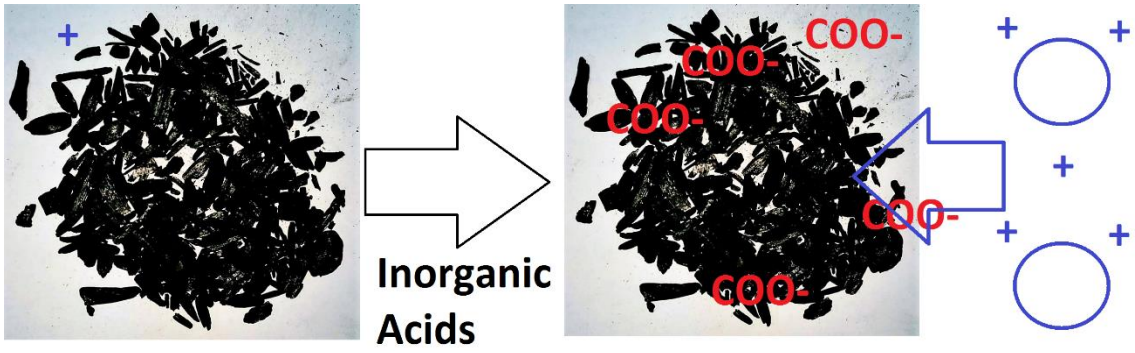
CHAPTER III Biochar Functionalization with Inorganic Acids and Cationic Surfactants

GRAPHICAL ABSTRACT



ABSTRACT

Biochar is a carbonaceous material produced using organic matter by thermochemical transformations in the absence of oxygen. The principal use of this material is the soil amendment but recently has been explored its use in other fields. The modification of biochar has been archived trough the processes of functionalization and activation with different agents. The final properties of the material highly depend on the feedstock and the agent used.

The aim of this work was to functionalize two different types of biochar using Cetyl Trimethyl Ammonium Bromide (CTAB) and inorganic acids (H_2SO_4 and HNO_3) in different concentrations. Alterations in the final surface properties were evaluated. The results suggest successful functionalization processes with the formation of new functional groups and the modification in the surface charge. This biochar can later be used in applications of air pollution monitoring and treatment.

1. Introduction

Biochar is a material produced with the thermochemical transformation of lignocellulosic biomass. These thermochemical transformation processes include torrefaction and slow pyrolysis [1]. In general terms, the biochar is rich in diverse carbonaceous forms (aromatic, aliphatic and amorphous) with negative surface

charges caused by the presence of oxygen groups like epoxide, hydroxide and carboxylic [2]. It is also possible to find onto its surface inorganic salts, amine groups and metalorganic structures[3]. The characteristics of the final product may vary depending on the raw material used and the time and temperature of production [4], [5].

Biochar has been widely used in multiple areas like catalysis, soil amendment, energy production, water treatment and adsorption [4]. Biochar has been tested as adsorbent of different pollutants like heavy metals[6]–[12], dyes[13]–[15], greenhouse gases (GHG) [2], [16]–[18] and biosolids[19], [20]. Table 5 shows the works that used this solid as adsorbent. Since this material is mostly applied to soil amendment most works focus on the role of adsorption in the reduction of GHG emissions have been attained. Heavy metals are also the target of these studies, especially to treat wastewater. The adsorption of biosolids is also an emerging field in the study of possible sorbates able to attach onto the surface of biochar.

Table 5 Studies reported for the adsorption of pollutants using biochar

Biomass	Temperature (T) and time (t) of production	Target	Reference
Sawdust	T = 250, 350 and 500 °C, t= 8, 24 and 72 h	N_2O	[17]
Wood	T= 350 and 700 °C	*Escherichia coli*	[20]
Tree bark	T= 550°C, t= 0.75 h	N_2O	[18]
Dew melon peel	T= 450 °C, t = 4 h	Cr (VI)	[6]
Floristery	T= 300 – 600 °C, t= 2 h	Sb	[8]
Rush	T= 350, 450 and 550 °C, t= 4 h	Zn	[7]

The sorption capabilities of biochar can be improved by applying post-production processes to the raw material [21]. As, an example, functionalization, and activation processes have been used. Functionalization implies the grafting of functional groups to improve the biochar interaction with specific particles. The activation process aims to produce a material with exceptionally large specific surface area, high pore volume, well-developed internal porous structure, and abundant surface functional groups [14]. The functionalization process has advantages over the activation process since it is more cost-effective and flexible than activated biochar [15].

Table 6 shows several processes used to improved biochar physicochemical characteristics to adsorb pollutants and using different raw materials. Surface area, functional groups, and surface charge are the principal modifications present after the functionalization/activation process. It is noticeable that there are both positive and negative results in terms of adsorption capacity. Surface area, functional groups, and surface charge are the principal modifications present after the functionalization/activation process.

Table 6 Post-production processes used for biochar modification from different raw materials in order to improve the adsorption of pollutants.

Biomass	Production conditions	Agent	Target	Adsorption Capacities (mg/g)	Findings	Ref.
Municipal Solid Waste	T= 400, 500 and 600 °C t= 0.5 h	KOH	As (V)	Raw: 24.49 Modified: 30.98	Pore volume and surface area increased	[12]
Cardboard	T= 250 and 300 °C t= 1, 1.5 y 2 h	KOH	CH_4	Raw: 5.17 Modified: 3.16	Humidity retained and pore blocking	[16]

Biomass	Production conditions	Agent	Target	Adsorption Capacities (mg/g)	Findings	Ref.
Cow dung	T=700 °C, t= 2 h	$FeCl_3$	Perchlorate	Raw: 0.03 Modified: 0.6	Surface area increased and surface charge modification.	[22]
Wood	T= 550-600 °C	EDC (amine)	CO_2	Raw: 13.2 Modified: 122.76	Adsorption capacity increased 10 times	[2]
Flowers	T= 800 °C t= 4 h	K_2CO_3	Methylene blue	Raw: 291 Modified: 632	Increased surface area and carboxylic groups	[14]
Rice husk	T= 500 °C, t= 2 h	CTAB	Nitrates and Ammonium	Raw: 44 Modified: 213	Increased adsorption capacity	[23]
Wood	T= 350 °C, t= 6 h	CTAB	Cr (IV)	Raw: 2.65 Modified: 52.63	Increased adsorption capacity	[24]
Bamboo	T= 550 °C	HNO_3	Furfural	Raw: 25.03 Modified: 55.56	Reduced adsorption capacity	[25]
Hazelnut	T= 220-260 °C t =4-6 h	HCl and H_2SO_4	Methyl Orange	Raw: N.A. Modified: 306.13	Increase of oxygen groups	[15]

Biomass	Production conditions	Agent	Target	Adsorption Capacities (mg/g)	Findings	Ref.
Rice Straw	T= 800 °C t= 1 h	H_2O_2 and HNO_3	Cd^{2+}	Raw: 69.3 Modified: 93.2	Increase of electrostatic forces	[11]

Different agents can cause different responses in the material, but the conditions of post-production processes influence the final characteristics of biochar even while using the same functionalizing agent. It is necessary to deeply explore all the variables involved in the process of improving biochar adsorption capabilities. The present work research aim was to analyze the effect of the functionalization of biochar on its surface characteristics. To do this, two different types of biochar were used as well as two different functionalizing agents: CTAB and Inorganic Acids. The final product (functionalized biochar) is meant to be used in the air pollution treatment of GHG and bioaerosols.

2. Materials and Methods

2.1. Biochar origin

Two different types of biochar where used in this work: 1) cabbage-derived biochar (CB) produced at the University of Quebec in Trois-Rivières (UQTR, and 2) wood-derived biochar (WB) produced by the Innovation Center of Lignocelulsic Products (Innofibre). The first biochar was produced by slow pyrolysis using residual cabbage leaves that came from the salad industry as raw material. The second was produced using wood from the demolition process of old houses from the province of Quebec, Canada. Both types of biochar were molten until visible homogeneity and dried at 105 ^{o}C for 18 hours before functionalization.

2.2. Functionalization

2.2.1. Functionalization with cationic surfactants

The CB was functionalized with an aqueous solution of CTAB, a cationic surfactant of 17 carbons. The objective of this was to replace the cationic ions of the biochar's surface and increase the aliphatic groups exposed on it in order to improve its capacity of non-polar molecules adsorption like CO_2 and CH_4 [26], [27].

In brief, one gram of pretreated biochar was mixed with 20 mL of CTAB. The mix was kept under constant agitation at 200 rpm. The material was later recovered using filtration and rinsed with deionized water. Afterwards, the material was dried at 60 ºC overnight.

Table 7 shows the experimental design used for the functionalization considering previous findings [23], [24]. It considers two factors: 1) CTAB concentration and, 2) contact time. Each variable consisted of three different levels plus a blank.

Table 7. Experimental design for functionalization with surfactants

Variable	Low	Middle	High
Concentration (mM)	60	90	120
Contact time (h)	6	12	24

The response variables were the surface charge and the presence of aliphatic groups. These were measured using Z potential and Fourier-transform infrared spectroscopy (FTIR).

2.2.2. Functionalization with inorganic acids

The WB was functionalized with an aqueous solution mixture with a ratio of 3:1 (v/v) consisting of H_2SO_4 98% (v/v) and HNO_3 70% (v/v). The objective of this was to increase the number of carboxylic groups present on the biochar's surface in order to improve the attraction of protein particles [19].

The functionalization was performed using slight modifications to methods previously reported [9], [10], [19], [28]. Briefly, a certain amount (see Table 4) of pretreated biochar was mixed with 100 mL of aqueous solution and kept under reflux

for 6 hours at atmospheric conditions of temperature and pressure. The material was later recovered by filtration and rinsed with deionized water until rinse water reached pH values from 5-6. After that, the material was dried at 105 ºC overnight.

Table 8 shows the experimental design for biochar functionalization Considering previous findings. Two variables were explored: 1) The proportion biochar: aqueous solution and, 2) the concentration of the acid mixture. There were used 3 and 2 levels respectively.

Table 8 Experimental design for acid functionalization

Variable	Low	Middle	High
Concentration (% v/v)	**28**	**-**	**60**
Ratio Biochar:Solution (g:mL)	**1:200**	**1:100**	**1:33.3**

The response variables were the surface charge and the presence of carboxylic acids. These were measured using Z potential, FTIR and Böhem Titration.

2.3. Biosolids adsorption

In order to evaluate adsorption capacities of functionalized WB adsorption assays were performed using a coliforms bacteriophage (MS2) as a model of biosolids. The virus was propagated using as host *Escherichia coli* (ATCC 15597). 1.5 mL of the propagated virus was diluted in 28.5 mL of peptonized water. 5 mL of diluted phages were mixed in tubes with 20 mg of biochar. For these experiments, there was considered only the biochar functionalized with the ratio 1:33.3.

The mixture was agitated for 30 min at 20 rpm. After time passed, sedimentation of the material was allowed, and the supernatant was filtrated using membrane filters (0.22 µm). The viral concentration of the filtrate was estimated using plate dishes counting using Triptych Soy Agar culture medium.

2.4. Analytical methods

Surface charge was determined using a Zetasizer nano series from Malvern™ using the provider's instructions [29]. Briefly, biochar samples were sieved through a 53 µm sieve and, afterwards, an aqueous solution was prepared using these particles (concentration = 0.3% (w/v)). The solution was sonicated in an ultrasonic bath for 1 h ans then it was read fivefold in the Zetasizer. pH was measured after the lecture and adjusted to 7 (if necessary) for a second read. The results are reported at pH= 7.

Infrared spectra were obtained using a Nicolet iS5 spectrum equipped with the accessory iD7 ATR. Twenty scans per lecture were made, each with a resolution of 0.482 cm^{-1} from 500 until 3500 cm^{-1}. The results were reported in terms of absorbance.

Total acidity of biochar was determined using the original Böhem's protocol for black carbon considering the specifications for this material [30]–[32]. The results are shown in terms of mmol of acidity per gram of biochar.

The viral count was made in terms of Plaque Forming Units (PFU) per milliliter using *E. coli* as host and tripithic soy agar following the ISO 10705-1 standard and the recommendation of previous works [33]. The results are shown in terms of Mean ± one SD.

2.5. Statistical Methods

Significant differences between the variables explored during both functionalizations were established using two-ANOVA.

3. Results and discussion

3.1. CTAB functionalization

3.1.1. Z potential

Figure 3 shows the response curve obtained after functionalizing the biochar with surfactants. ANOVA revealed significant differences in all treatments. Z potential becomes less negative with the increase of surfactant concentration from 0 to 90

mM. After 90 mM the surface charge becomes more negative again. This coincident with Mathurasa and Damrongsiri whose findings stated that 90 mM ensures full saturation of the biochar surface [23].On the other hand, it is observed that with the increase of functionalization time the surface charge also does it. This also coincides with the same authors' conclusions. They report a change from -36.57 to -27.33 mV in the surface charge of biochar after its functionalization with CTAB[23].

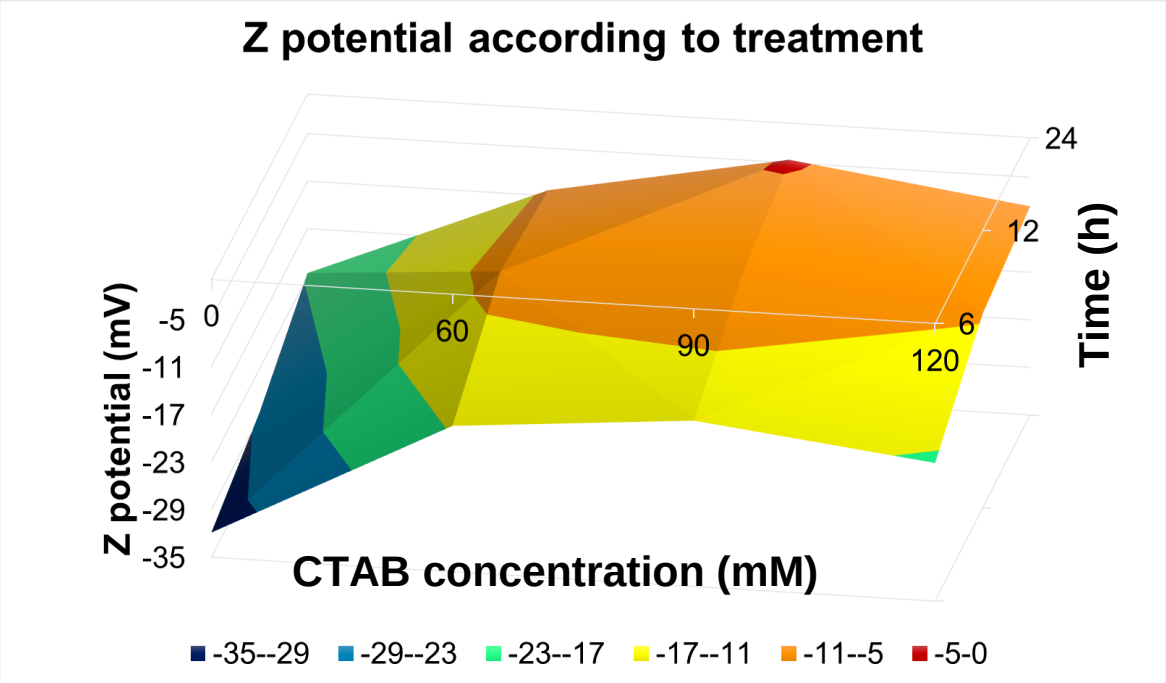


Figure 3 Z potential of biochar functionalized with CTAB

Raw biochar changed its surface charge from de -48 ± 2.48 mV to -4.62 ± 0.45 mV after functionalization (90mM, 24 h). The cationic surfactant attaches to the biochar's surface by electrostatic interactions with the negatively charged groups present in the surface and by cationic exchange with Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+}. This causes an increase in the values of Z potential and in the material's hydrophobicity facilitating the adsorption of non-polar molecules [27].

3.1.2. FTIR

Figure 4 shows the spectral sign obtained for raw biochar and functionalized biochar. The spectra of different treatments did not show major differences within them, for

that reason, the figure shows only the biochar functionalized with CTAB 90 mM for 24 h since it had the best values of Z potential.

Raw biochar presented 3 main functional groups: 1) aliphatic hydrocarbons present with peaks at 1560 cm^{-1} and between 2800 cm^{-1} and 3000 cm^{-1}, 2) carboxylic acids salts with a peak at 1550 cm^{-1}, and 3) inorganic sulfates generating peaks at 600 cm^{-1} and 1100 cm^{-1}. This corresponds with the general characteristic peaks of biochar reported in literature [34].

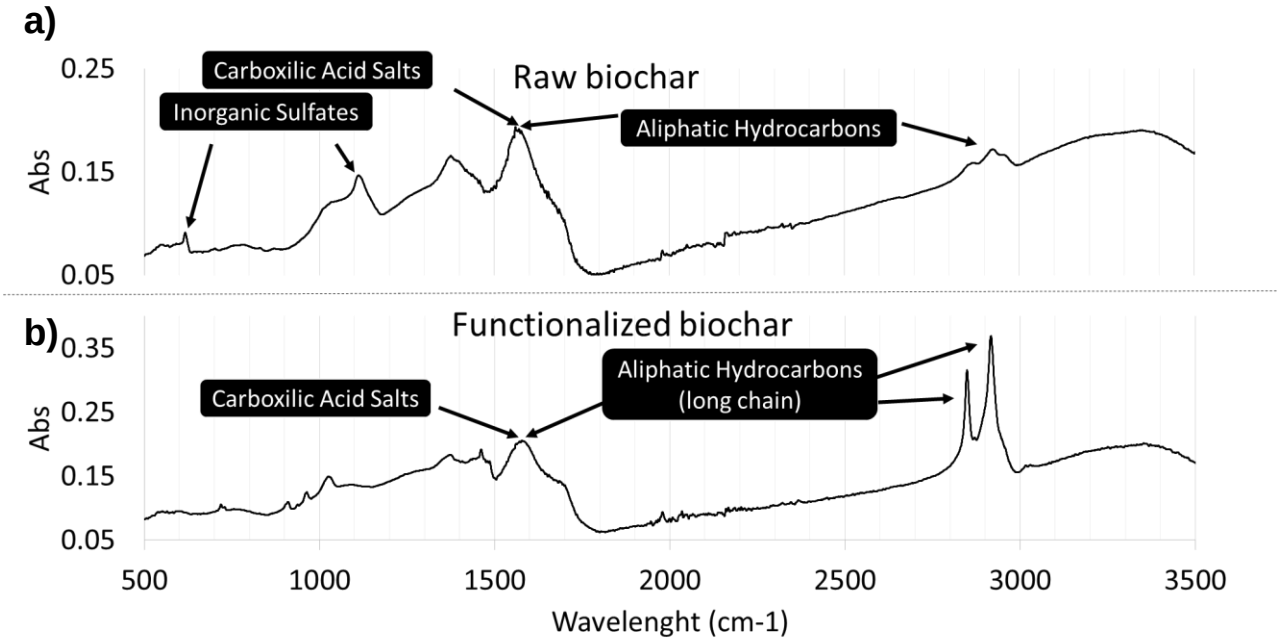


Figure 4. Infrared spectra obtained with FTIR were a) Biochar without functionalization and b) Biochar functionalized with CTAB (90mM, 24 h)

All of the characteristic peaks of CTAB (see *Supporting Information*) were present in the functionalized biochar spectrum indicating a successful functionalization. The two main differences between functionalized and raw biochar spectra are: 1) the increase of the peaks at 2900 cm^{-1} y 2850 cm^{-1} which indicates the presence of long-chain aliphatic hydrocarbons, and 2) the banishment of inorganic sulfates peaks.

These results suggest that the functionalized material is suitable for GHG adsorption since they are non-polar molecules. GHG emissions occur mostly at environmental conditions. At these conditions, adsorption processes are governed by molecular

interactions between adsorbent and adsorbate and pore density and surface area become less relevant [35]. It is presumable that the high amount of aliphatic groups present on the surface of biochar will help in the adsorption of GHG.

3.2. Inorganic acids functionalization

Figure 5 shows the results for the Z potential determination. Raw biochar used for inorganic acids functionalization had Z potential values equals to -31.4±1.41 mV. That value became more negative after functionalization reaching a minimum value of -54.1 ± 1.27 mV (treatment 28%, 1:100).

ANOVA revealed that there were no significant differences ($p < 0.001$) between the ratios used while functionalizing biochar. However, there were significant differences when different concentrations of acid solution were used. For that reason, from this point the results will only referred to the biochar treated with the least cuantity of acid that correspond with the biochar:aqueous solution proportion of 1:33.3 (g of BC: mL of aqueous solution). From this point B0% will be raw biochar, B28% biochar treated with the aqueous solution at 28% of concentration and B60% biochar treated with the aqueous solution at 60% of concentration.

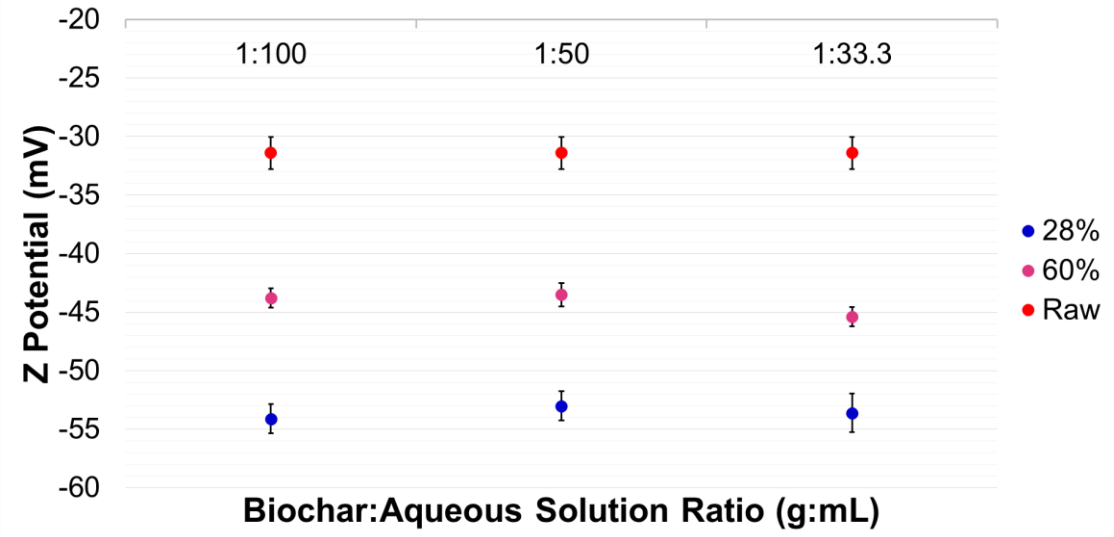


Figure 5. Effect of functionalization condition of wood-dervied biochar in Z potential

3.2.2. FTIR

Figure 6 shows spectral signs of raw and functionalized biochar with inorganic acids. B0% had carboxylic acid groups demonstrated by the peak at 1550 cm^{-1}. After the functionalization, a shoulder and a peak raised at 1705 cm^{-1} for B28% and B60% respectively and there is also a peak at 1535 cm^{-1}. The first is caused by the formation of new carboxylic acids due to the oxidation of the carbon groups present in the biochar's surface. The later was caused by the formation of new nitrated aromatic rings generated by the presence of nitric acid [10], [19], [28].

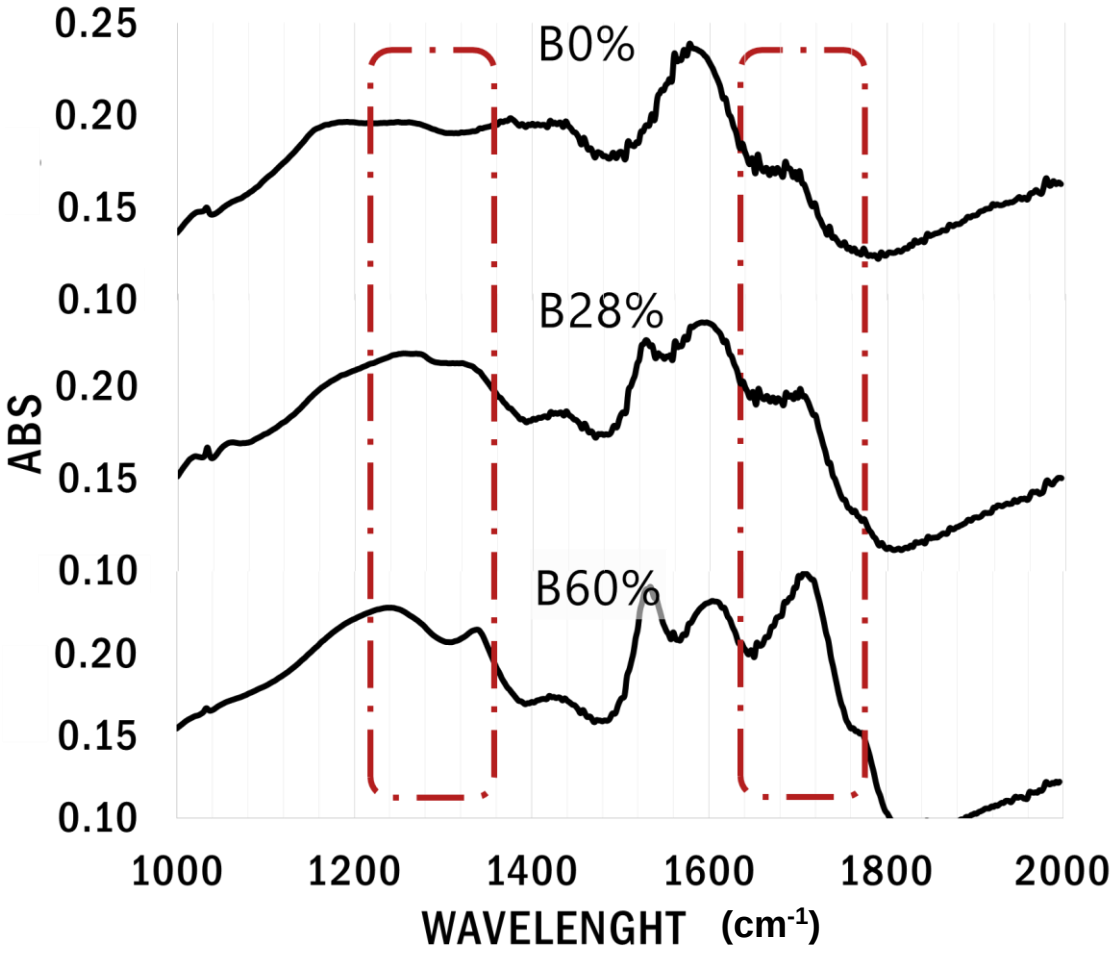


Figure 6 Infrared spectra for raw and functionalized biochar with inorganic acids.

3.2.3. Bohem Titration

The carboxylic acids were quantified using Böhem titration. The results are shown in Figure 7. Total acidity was higher in B28% and was coincident with Z potential

results however, the amount of carboxylic acids was higher in B60% corroborating the results for FTIR. B28% had a higher amount of phenolic and lactonic groups that cause more electronegativity on the surface of biochar.

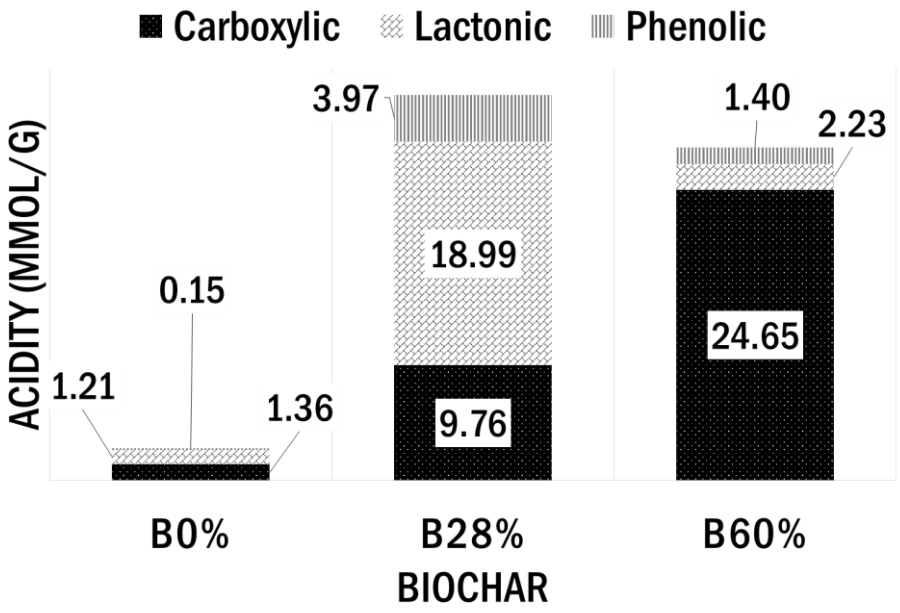


Figure 7 Contributions of each functional group to total acidity of biochar

These results suggest that B28% and B60% are suitable for biosolids bonding since the acidic groups have an affinity for amine and nitric groups present in these biosolids. Some viruses have proteic envelopes of capsids that protect the genetic material, bacteria have structural and functional proteins on the cell membrane and cell wall of fungi is rich in chitin that carries electrical charges [36], [37]. All of these biosolids are susceptible to be attached to the surface of functionalized biochar.

3.3. Adsorption assays

Finally, Table 9 shows the results of plate dishes counting after adsorption assays with raw and functionalized biochar. Results show that when the biochar was functionalized there was a reduction of 2.48 logs which implies retention of 99% of original phages in suspension.

Table 9 Viral removal with biochar

Biochar	Phages (PFU/mL)	LOG removal
Blank	$4.62\pm3.3x10^{9}$	-
B0%	$1.43\pm0.0x10^{9}$	0.51
B28%	$1.52\pm0.4x10^{7}$	2.48
B60%	$1.48\pm0.8x10^{7}$	2.49

Sasidharan and collaborators neglected virus retention to biochar when the material was activated. They suggest that a higher surface charge will increase the attachment of these particles to the surface of biochar which is coincident with the results found in this work [38]. To the best of our knowledge, there are no other research work about virus attachment to the surface of biochar so further investigations have to be made in order to discard virus inactivation since it is well known that enveloped viruses can be inactivated due to protein damage [37].

4. Conclusions

Biochar's surface characteristics can be modified successfully with different agents. CTAB attaches to the surface by electrostatic forces and cationic exchange and reduces the negativity of the surface. This effect varies according to the configuration of time of reaction and concentration of surfactant used in the process. In this work the maximum value of Z potential was reached when the biochar was functionalized with CTAB 90 mM for 12 h. Aliphatic groups of CTAB were exposed in the surface of biochar causing that non-polar molecules become more affine to the material. This can be used as a cost-effective GHG adsorbent.

Inorganic acids increase the acidity of the material by carbon oxidation generating functional groups that make the surface electrically more negative. This effect is

mostly influenced by the concentration of the acids used. The functionalization caused that the material was able to retain viral particles and it is suspected that it will be capable of retaining different types of biosolids like bacteria and fungi.

5. Supporting Information

1) Spectral sign of CTAB

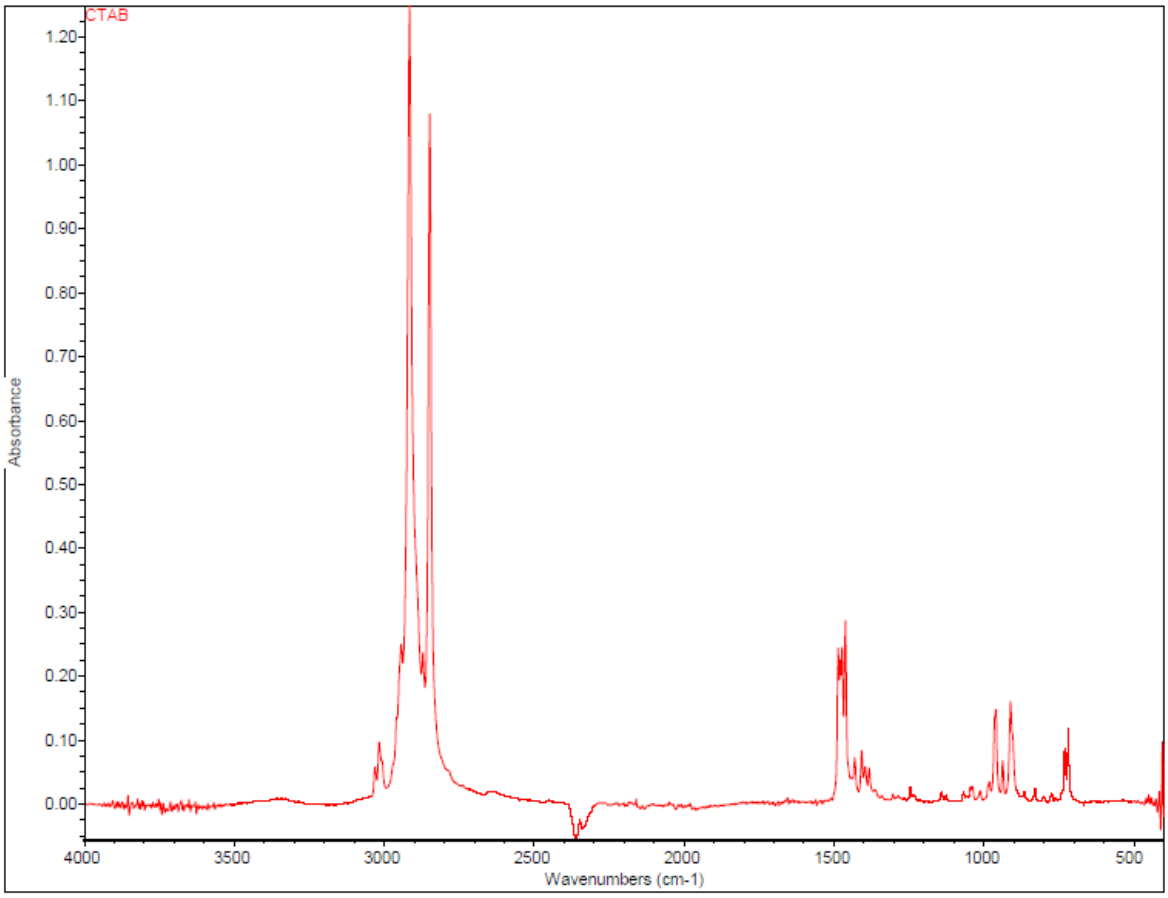


Figure 8 Characteristic Spectral Sign pf CTAB

The spectral sign of CTAB presents 6 characteristic peaks. At 3000 cm^{-1} exists a peak caused by the extension of N-H bonds. There are two strong and sharp peaks at 2900 cm^{-1} and 2850 cm^{-1} corresponding with the extension of C-H alkyl bonds. The peaks at 1487 cm^{-1} and 1462 cm^{-1} correspond with C-N bonds and the peak at 1473 cm^{-1} is caused by the excitation of methylene groups [39].

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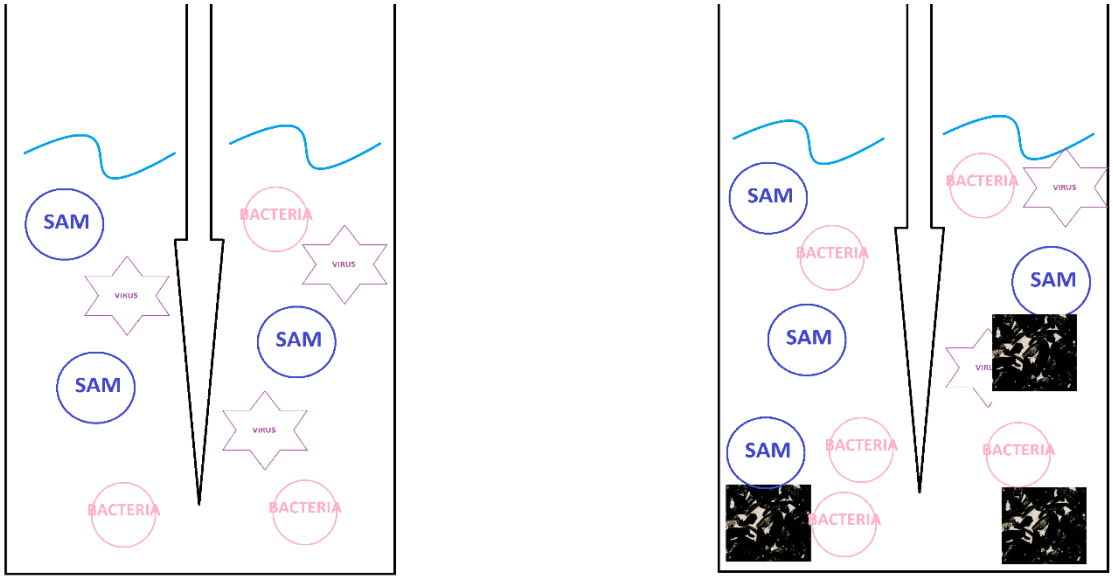
CHAPTER IV Bioaerosols Monitoring in a Pediatric Cares Center

GRAPHICAL ABSTRACT



ABSTRACT

Bioaerosols (BAs) are the biological fraction of suspended particulate matter. These air pollutants can cause adverse effects on human health like asthma, infectious diseases, and cancer. For this reason, it is necessary to constant monitoring of their concentrations in order to prevent outbreaks and public health problems. Atmospheric parameters like temperature or relative humidity (RH) influence the concentration of BAs but the mechanisms are not yet fully understood.

Diverse sampling and counting methods are available to monitor BAs. Liquid impingement and epifluorescence microscopy (EFM) are techniques widely used to enumerate the concentration of this pollutant.

The aim of this work was to study the BAs concentration in a Pediatric Cares Center (PCC) for three months and how is this influenced by climatic factors and sampling method. The results show that viruses are the microorganisms with the highest concentrations in time. It is presumable that these viruses correspond with influenza

virus given the seasonal patterns and the behavior of concentrations with atmospheric variables with temperature and RH as the most influential parameters in the concentration of BAs at the inside of the PCC.

1. Introduction

Indoor Air Quality (IAQ) refers to the air quality within and around buildings and structures, especially as it relates to the health and comfort of building occupants. Understanding and controlling common pollutants indoors can help reduce your risk of indoor health concerns [1]. Indoor air pollutants include VOCs, radon and particulate matter. Of these the biological fraction is a major concern since high concentrations of bioparticles cause adverse effects to human health and infectious illnesses like influenza and pneumonia [2], [3].

Bioaerosols (BAs) monitoring is the count of both viable and unviable airborne microorganisms found in outdoor and indoor air. Outdoor air monitoring helps allergists to effectively treat allergic reactions to biological agents[4]. Indoor air monitoring helps with the prevention of occupational diseases caused by infectious agents like the common cold, pneumonia, chicken-pox, flu, and some gastrointestinal illnesses.

Usually, indoor BA concentration is greater than outdoor's concentrations in the same place (e.g. students' dorm rooms and office buildings) [5]. Also, it is worth considering that humans spent >90% of their time in indoor spaces [6] making indoor BA monitoring more relevant than in the outsides. The principal sources of BA at the inside of buildings are construction materials, furniture, pets, plants, air from the outside and occupants [7].

Atmospheric variables (e.g. rainfall, wind direction, and UV incidence) influence BA concentration of the air from the outside [8]. Of these variables, temperature and RH are reported as the most determinant in BA's variation [7]–[11]. However, the tendencies are not yet clear nor conclusive and more studies have to be performed considering how these variables influence different types of microbes. About the occupants, it is known that humans are a source of airborne bacteria and fungi via shedding and acting as vectors for microbes to enter the built environment through

tracking on clothing [7], [12]. Also, sick humans can spread infectious agents into the air via sneezes, cough and barf [7].

The variation of the concentration of indoor air bacteria and fungi has been largely studied because of the illnesses caused by these agents (e.g. legionellosis and aspergillosis) [13]. However, there are fewer studies about indoor air viruses since there are many challenges associated with studying viral BA including sample collection, low biomass, and lack of a conserved gene among all types of viruses [14]. The monitoring of these microbes is essential since they are related to some healthcare-associated respiratory infections like common cold, pneumonia, and influenza [2], [3].

Regardless of the type of BA, the monitoring of aerial microbes needs to be accurate and feasible in order to enlighten the real situation of indoor air quality. Monitoring includes two phases: sampling and counting. Different strategies have been used in both stages of BA monitoring. Liquid impingers, filters and solid agars have been typically used in the first stage [4].

Liquid impingers method has been compared with others and has proved to cause less cellular stress, maintains a percentage greater than 75% of viable fragile cells or 93% of viable resistant cells. [15], [16]. It is also possible to add new elements to the samplers that can improve the preservation and representatively of samples and hence give more accurate and close-to-reality results [4]

Cells retained in the impaction fluid can be subsequently analyzed by independent methods like microscopy. EFM is rising as a primer choice to BA counting due to its easiness and the ability to count both viable and unviable microorganisms. Different types of microorganisms can be studied at the same time if the right fluorophore(s) is(are) used (e.g. calcofluor for spores and molds (SAM), Syto 13 for Gram + and Gram – Bacteria) [17].

EFM has been reported in several studies for counting bacteria and fungi but there is low literature about viruses counting. These microbes have been studied with this technique in soil and water [18]–[20] and in recent years in air [8], [17], [21], [22].

The aim of this work was to monitor bioaerosols at the inside of a PCC by using liquid impingement method using Phosphate Buffer Saline (PBS) as the liquid of impaction and EFM coupled with fluorochromes. BA were counted in terms of SAM, live and dead bacteria cells and virus particles contined in air using specific fluorochromes.

2. Materials and Methods

2.1. Bioaerosols Sampling

2.1.1. Sampling site

The samples of bioaerosols (BA) were taken at a Pediatric Cares Center (PCC) specialized in respiratory illnesses. It is located in the city of San Luis Potosí, San Luis Potosí (SLP), Mexico. In this place, from 100 to 160 kids of 0 -14 years old are consulted daily from 2 pm to 8 pm from Monday to Friday. These patients came from different municipalities around the capital city of SLP. There are 3 full-time workers at the center: a receptionist, a doctor's assistant, and the doctor himself.

A diagram of the center is shown in Figure 9. It has two main areas: 1) the doctors' room where patients are diagnosed and treated.; and 2)a lobby with a capacity around 50 people where all the patients and companions are received and wait until the doctor calls them (usually they last around 30 to 90 minutes there). The doctor's room has a big window to ventilate the area and a ventilation system for climate control (heating and air conditioning (HVAC)). Normally, 6-10 kids are diagnosed at the same time by the doctor and his assistant every half hour.

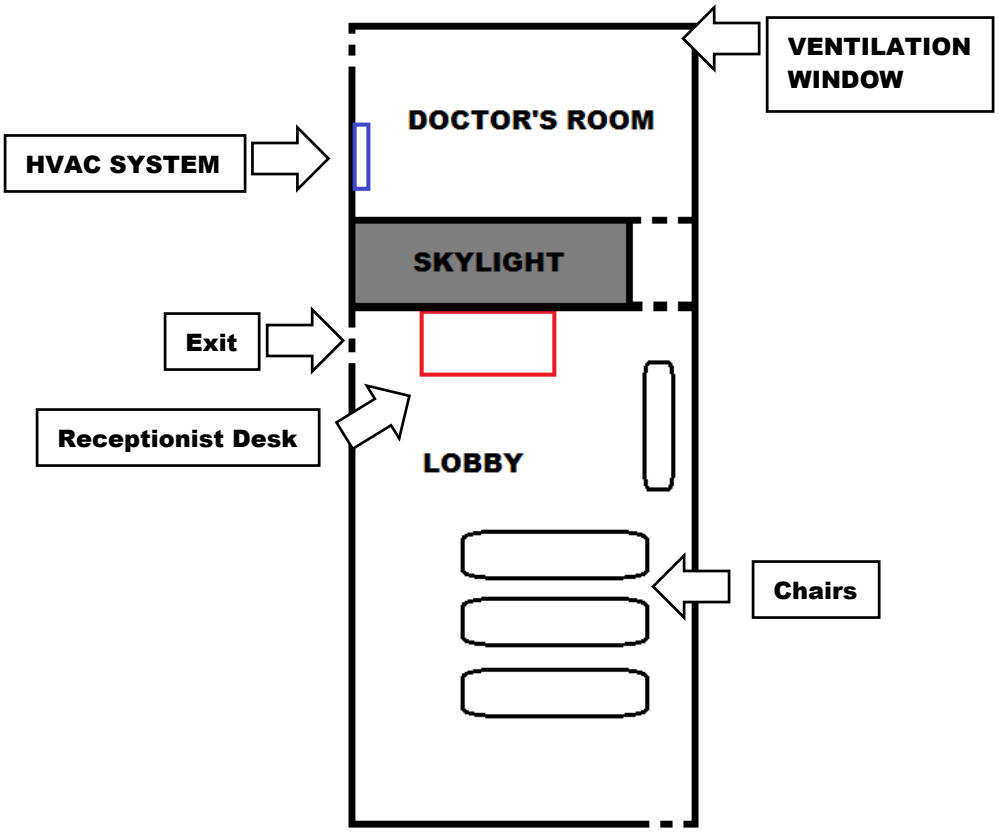


Figure 9 Pediatric Cares Center Diagram

2.1.2. Sampling materials and method

The BA were sampled using a liquid impinger (AGI-30, Ace Glass, Inc.). Two different impact media were used separately to collect BA: 1) 20 mL of PBS pH=7.4, and 2) 20 mL of PBS pH=7.4 added with 20 mg of functionalized biochar. This biochar was produced using wood from the demolition of old cabins and functionalized with a mix of nitric and sulfuric acids and sieved at particle size between 250 and 90 µm.

The sampling system is shown in Figure 10. It consists of 1) the impinger nozzle, 2) the sampler, 3) PBS to impact BA, 4) a humidity tramp of dendrite and 5) a vacuum pump. The air enters the nozzle and it is drawn through a very small capillary tube and bubbled through the liquid. BA gets trapped in the liquid medium due to the sonic flow of 12.5 L/min applied. The humidity tramp is used to prevent malfunctioning of the vacuum pump.

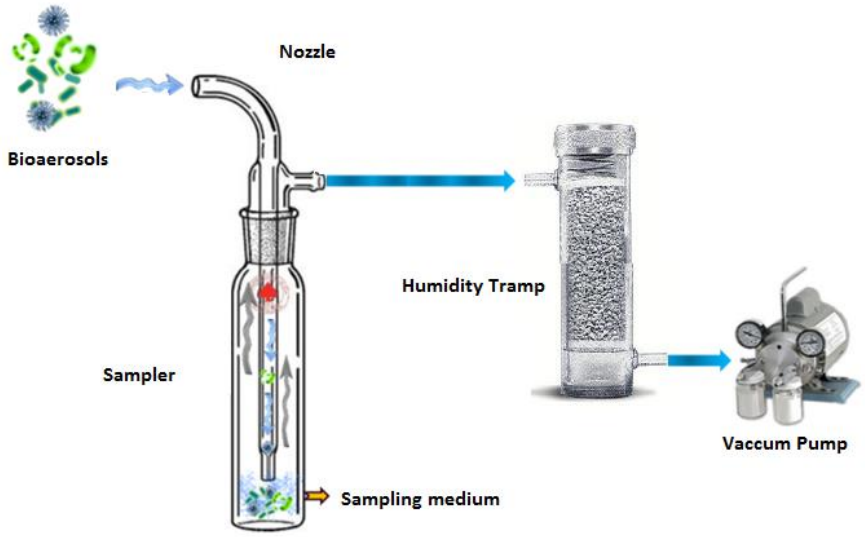


Figure 10 BA's sampling System

The nozzle was located at 1.65 m height. Sampling lasted 20 minutes according to NIOSH standards [4]. After this time samples were identified, transported to IPICYT and frozen until analysis. One or two samples per day were taken in both areas randomly. Also, the different media used to collect BA was intercalated between samples.

Temperature and RH were recorded using data from the National Air Quality System (Sistema SINAICA) of Mexico. The monitoring station used was SLP-IPAC which is located at 1 Km from the PCC [23]. At the sampling time, patients (considering these as the kids between 0 and 14 years old) and companion (considering adults with the children) were counted and registered.

2.2. Bioaerosols Counting

The samples of BAs collected using PBS + biochar were treated into an ultrasonic bath for 30 minutes in order to liberate the particles attached to the material.

An epifluorescence microscope ZEIZZ (Zeizz Inc, Modelo AXON Imager M2) equipped with a mercury lamp (HBO-100W Hg) and a camera of 1.4 megapixels (AxionCam MRc Rev.3-FireWire, Zeizz Inc.) were used to count BA.

Four fluorophores were used to identify different types of BA: Calcofluor White Stain (CALC), Propidium Iodide (PI), Thiazole Orange (TO) and SYBR® Gold (GOLD).

CALC is used to detect spores and mold (SAM) by bonding with cellulose and chitin present in the cell wall[24]. PI binds to DNA but cannot pass through cell membrane which makes it useful for counting dead cells[25]. TO is used in conjunction with PI as viability kit because TO passes through the cell membrane[25]. GOLD bonds to DNA as well as single and double chain RNA, being it is used to count total microorganisms [26].

All samples were stained using the instructions of the manufacturers [24]–[26]. 10 µL of the stained sample was injected into a Neubauer's chamber (Brand-Blau Brand, Germany) and then counted in the microscope. SAM were identified using CALC, live bacteria and dead bacteria were identified using TO and PI respectively and viruses were identified with GOLD using size discrimination (fluorescent particles between 0.02 and 0.50 µm were counted as Virus-Like Particles (VLPs)) [22]. The equations used to determine BA concentration are displayed in supporting information based on the study of Esquivel-Gonzalez et. al. [17].

2.3. Statistical Methods

To verify the existence of a correlation between BA's concentration and the different factors registered (Temperature, RH, number of patients and number of companions) simple and multiple linear models were created using software R (v. 3.5.2) [11], [27], [28]. The models created were selected and discussed according to their significance level using AIC criteria, a technique based on in-sample fit to estimate the probability of a model to predict/estimate the future values. A proper model is the one that has minimum AIC among all the other models[29].

Differences between the two areas of the PCC (lobby and doctor's room) as well as differences between both media used to sampling BA (PBS without biochar and PBS with biochar) were estimated using an analysis of variance ANOVA. ANOVA collects the statistical models and their associated estimation procedures to analyze the differences among group means in each sample.

3. Results and discussion

3.1. Variation of bioaerosols over time

Figure 10 shows the variation in time of the different types of BAs counted. The results are presented using the logarithmic concentration (base 10) of BAs. BAs' monitoring was carried out from February to April 2019 which covered winter and spring season. Dead bacteria, live bacteria, SAM and viruses concentration is shown in pink, green, blue and purple respectively. The mean concentration of total BA during all the monitoring time was $4.6x10^6$ total BA per m^3 of air. Also in Figure 11 is shown the relative composition and total concentrations of BA during the monitoring months.

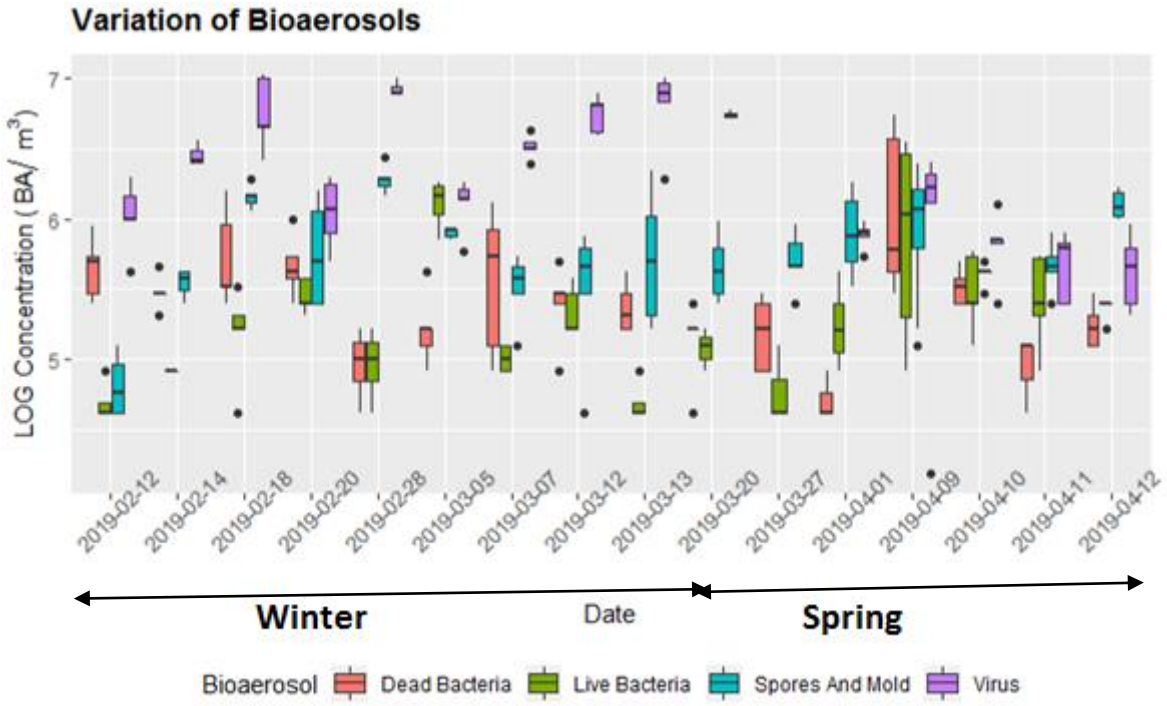


Figure 11 Variation in time of bioaerosols.

Viruses present the highest concentrations among the different types of BA. The mean concentration of viruses in BAs samples was $2.76x10^6$ VLPs per m^3 of air representing 60% of the mean relative concentration of total BAs. The highest virus concentrations in air ($8.41\pm1.3 \times10^6$ VLP per m^3 of air) were found around March 15th with a marked decrease after this date.

SAM concentrations oscillated between 6.94±4.8 $x10^4$ and 1.96±0.5 $x10^6$ SAM per m^3 of air. The peak concentration values are present at the beginning of March and the midterms of April. This type of BA represented 19% of the mean relative concentration of total BA with 8.74 $x10^5$ SAM per m^3 of air.

The concentration of both live and dead bacteria fluctuates around March 15th. After the end of February, the concentration of bacteria (live and dead) remained with the lowest values in comparison to the rest of bioaerosols. The concentration values ranged between $1.67x10^4$ and $3.01x10^6$ cells per m^3 of air for live bacteria and between $3.33x10^4$ and $4.56x10^6$ cells per m^3 of air for dead bacteria. The mean concentration was $4.60x10^5$ cells per m^3 of air and $5.06x10^5$ cells per m^3 of air for live and dead bacteria respectively representing 10% and 11% of the mean relative concentration of total BA.

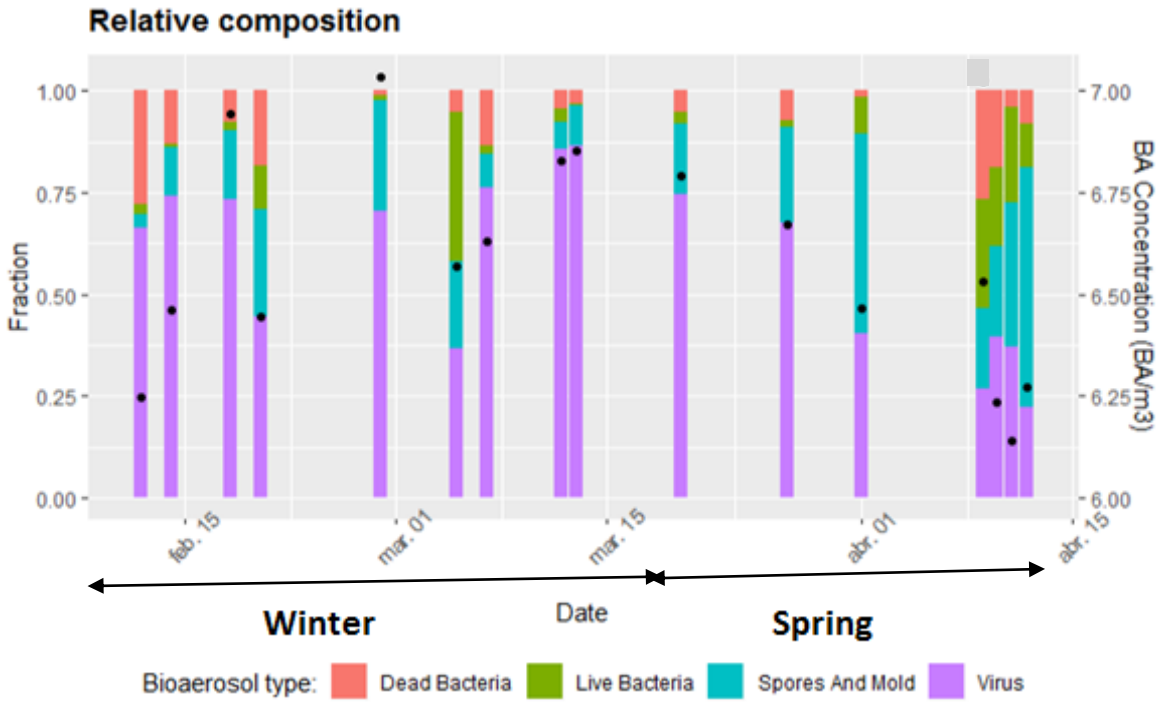


Figure 12 Relative composition of bioaerosols

Viruses´ relative abundance is higher during February and March, and dimished in April. SAM became more important for the composition of total BA with this viral diminution. As seen in Figure 12, SAM concentration remained relatively constant

during the last months of monitoring impacting directly in relative composition. Of all three BA (bacteria, spores and mold, and viruses), bacteria keep lower relative abundances indicating their minor relevance in air contamination by bioparticles during the analysed period of time.

According with total BA, it was observed that the highest concentration of total BA was around $1.08x10^{-7}$ BAs per m^3 of air and it is mostly attibuted to the high concentration of viruses and SAM during that date (February 28th) while the lowest concentration of total BA was observed on April 12th with $4.92x10^5$ meaning a total BA concentration diminished in two magnitud orders. International regulation only takes into account CFU (Coliny Forming Units) and with EFM is possible to consider both viable and unviable microorganisms. To take in consideration only viable microorganisms underestimates the real values of total BA because not all microorganisms are culturable in laboratory conditions [17]. Furthermore, non-viable microorganisms are also harmful to human health, since dead microorganisms liberate mycotoxins, endotoxins, and glucans [5]. International institutions have tried to determine acceptable values of BA concentrations for example, Healthy Buildings International (HBI) considers <750 CFU/m3 and Indoor Air Quality Agency (IAQA) < 300 CFU/m^3 [5].

The total concentrations of BA are fluctuant over time. The highest concentration values are found in February. This fluctuations in the concentration of BA followed similar trends that data reportedand by Esquivel et. al. [17], [30]. The decrease of viral relative concentration and the total concentration of BA can be explained by seasonal epidemiological events of respiratory and gastrointestinal illnesses. The PCC especiallices in respiratory illnesses since viral infections are the principal etiology in childs [31]. Influenza survives better in cold months since the rise of temperatures and humidity favors other type of microorgnisms and reduces the infection capacity of the first. This information suggests that viral concentrations in the air became lower at the PCC by the disminution of infectious agents survival when the months pass.

In general, infectious respiratory illnesses caused by viruses present seasonal patterns keeping the highest values in winter [2]. It is believed that influenza is the dominant virus present in PCC's air because this virus (especially Influenza A) follows seasonal patterns caused by the low temperature and RH values registered in winter [9], [32]. In Mexico, and specifically in SLP, the seasonal pattern can be corroborated with epidemiological data retrieved from the Ministry of Public Health from Mexico (see *Supporting Information*). This report concluded that in the months from January-March, influenza has the highest morbidity values coincident with the pattern found in this work. Furthermore, in the local newspaper there was also evidence that an influenza outbreak around the date where the highest viral concentrations were found [33]. Influenza sickness has high morbidity rates in SLP, especially in group ages of 1-4 and 65+ years suggesting that the appearance of this virus in the PCC is highly viable.

Respiratory Sincital Virus (RSV) is also reported in winter and spring seasons [30]. However, this microbe escapes the human body via droplets that quickly settle in the floor and its airborne occurrence is very unlikely to happen [32]. According to the literature, the principal infectious viruses airborne-transmitted in Human Healthcare Centers are enteroviruses (cold and mengitis), noroviruses (cold), zoster virus (chickenpox), and influenza because of its morphology and composition. According to literature, enteroviruses outbreaks happen mainly in autumn with rare occurrences in winter and spring. Noroviruses cause gastrointestinal illnesses and aerosolize when an infected person throws up [32]. This event was recorded only once during the monitoring (March 26th) but it was not virus-related according to doctors' diagnosis. Zoster virus causes chickenpox and none of the children were diagnosed with this illness during the monitoring time since the main consulting reasons from patients were respiratory problems.

SAM are suggested to come from the outdoors via vents and air flows caused by sporulation of fungi. The mechanisms of sporulation vary between fungal species and they are directly influenced by atmospheric variables like wind speed, temperature, and RH which change with seasons. Fungal sporulation is predominant in summer when the temperatures are higher than 30 ºC and RH is around 60%.

Higher values of RH decreases the concentrations of spores since they precipitate to the ground, while lower values reduce the growth rate of the fungi and the production of spores [34]. According to the data from SINAICA, the humidity values in the monitoring months were enough to promote the fungal sporulation during the monitoring time despite the fact that sporulation rate is not the highest possible of the year (see *Supporting Information*). Indoor airborne molds sources are wet and dark places like bathrooms, old furniture and mopping stations [7]. Based on the characteristics of the studied areas of the PCC, we can stand that the mold growth is not feasible.

Bacteria represent the lowest concentration among the BA evaluated and its presence can be attributed to the occupation of the space. In most indoor spaces skin-related bacteria (*Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria*) are dominant in the air [30]. In healthcare centers, it has been reported the presence of *Staphylococcus, Bacillus, and Micrococcus* [35]. *Klebsiella pneumoniae* has also been reported as the origin of some respiratory infections associated with workers of human healthcare (formerly known as nosocomial illnesses) [36]. All of these agents may be present in the air of the PCC. However, molecular studies are yet to be performed to determine the species present in the air of the center.

3.2. Concentration differences between seasons

Table 10 shows the concentration of BAs in the different seasons evaluated. According to ANOVA (*see Supporting Information*) there were significant differences ($p<0.001$) in the total concentrations of BAs. The concentration of viruses in winter was significantly different ($p<0.001$) to all the rest of bioaerosols in the same season and to the viruses concentration in spring. The variables influencing this behaviours are analysed below.

Table 10 Concentration of BAs in summer and spring

Season	SAM (BA/m^3)	Live Bacteria (BA/m^3)	Dead Bacteria (BA/m^3)	Virus (BA/m^3)
Winter	7.67±6.8x10^5	2.65±4.3x10^5	3.84±3.3 x10^5	4.03±3.1x10^6

Spring	$8.18\pm5.7x10^5$	$5.54\pm9.6x10^5$	$5.76\pm12x10^5$	$1.40\pm1.4x10^6$

3.3. Factors influencing the concentration of bioaerosols

Table 12 shows the statistic values of the temperature (T), relative humidity (RH), number of patients (P) and number companion (C) present at the monitoring moment. Regarding atmospheric conditions, room temperature swings between 22.42 and 27.58 °C and RH between 9 and 50 %. There were higher mean values of these variables in winter than in spring.

The patient's affluence was between 2 and 15 with a mean value of 8 all present during the 20 minutes of monitoring. There was always at least one companion per patient, increasing sometimes depending specially on the age. When the child was 0-5 years old (visual approximation) was assisted by their parents and/or their grandparents. There was a mean of 14 companions oscillating between 5 and 28. The affluence diminished by the end of monitoring coincident with the morbidity of respiratory illnesses.

Table 11 Statistic data of external parameters

Factor	Mean		Min.		Max.		Median	
	Winter	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**
Temperature (°C)	25.94	23.32	22.42	22.56	27.58	25.60	26.41	22.79
RH (%)	22.63	18.48	10	9	50	41	20	13
Companion (#)	19	12	7	5	28	19	23	9
Patients (#)	8	6	2	2	15	10	7	7

Table 13 summarizes the influence of the increase of these variables in the concentration of virus, SAM, and live and dead Bacteria using the data from models' adjustments (see *supporting information*). The adjusted models were linear and

selected with the base on AIC criteria. Those with better predictive capacity are represented for equations (Eq.) 1, 2 and 3 and correspond with virus (in spring), SAM (in winter), and live bacteria (in winter) concentrations respectively.

According to Eq. 1 temperature is the variable with highest impact in virus concentrations with a change of 10^6 per increased unit. RH and patients are two magnitude orders below the influence of T. In the case of Eq. 2 the SAM concentration is also highly influenced by T but it is joined by P with changes of 10^5 per increased unit. C and RH also influence the concentration but 10 times less that T and P do. Finally Eq. 3 shows again the high influences of T in concentration of BAs (Live Bacteria for this case) with values of 10^5 per °C. C and P also influence the concentration of live bacteria with minor impact than T.

The concentration of dead bacteria could not be properly explained using the considered variables (T, RH, Patients, and companion). This concentration pattern is coincident with the results reported in literature [8], where the only significant variable was rainfall, and which was not considered in this study.

Table 12 Influence of the increase of metrological and occupancy variables (summary).

Increasing factor	Virus		SAM		Live Bacteria		Dead Bacteria	
	Winter	Spring	Winter	Spring	Winter	Spring	Winter	Spring
Temperature	+	+	-	-	-	N.I.	N.I.	N.I.
RH	-	-	-	N.I.	N.I.	N.I.	N.I.	N.I.
Companion	-	N.I.	-	+	+	-	+	-
Patients	+	-	+	-	+	N.I.	N.I.	N.I.
p value	0.000	0.000	0.000	0.045	0.000	0.002	0.062	0.027
r^2	0.4244	0.761	0.597	0.199	0.783	0.218	0.080	0.135

N.I. = No Influence.

$$[\mathbf{Virus}]_{\mathbf{spring}} = -\mathbf{4.78x10^7} + \mathbf{2.21x10^6(T)} - \mathbf{8.63x10^4(RH)} - \mathbf{6.40x10^4(P)} \quad \text{Eq. 1}$$

$$[\mathbf{SAM}]_{\mathbf{winter}} = \mathbf{3.59x10^6} - \mathbf{1.14x10^5}(\mathbf{T}) - \mathbf{3.66x10^4}(\mathbf{RH}) - \mathbf{2.98x10^4}(\mathbf{C}) + \mathbf{1.63x10^5}(\mathbf{P}) \quad \text{Eq. 2}$$

$$[\mathbf{Live\ Bacteria}]_{\mathbf{winter}} = \mathbf{8.34x10^6} - \mathbf{3.32x10^5}(\mathbf{T}) + \mathbf{1.39x10^4}(\mathbf{C}) + \mathbf{3.13x10^4}(\mathbf{P}) \quad \text{Eq. 3}$$

The morphological differences between SAM, bacteria and viruses caused a differentiation in the influence of temperature over the concentration of these BA. The temperature has been reported as one of the main factors that influence BA' concentration[9]. The literature reports that high temperatures can damage and kill bioaerosols[8]. Temperatures over 24°C decrease survival of microorganisms including bacteria and molds [7]. In this work, the temperature raised above this value causing a decline in the concentration of these microbes.

The temperature has also an important effect on viral activity, particularly in the case of enveloped viruses[3]. One study used aerosolized particles of MS2 (a bacteriophage often used as airborne virus model) to evaluate the effect of temperature on its activity. They concluded that this enveloped virus was inactivated faster when the temperature increased above 35 °C [37]. Another study evaluated adenovirus surveillance and proved that it was stable between 4 and 36 °C but its infectivity drops above 29°C [37]. The temperature ranges of this work did not reach these critical temperatures (24.5 - 27.5 °C) and these temperature effects on airborne viruses were not seen. However, the results about temperature effect on BA concentration are not yet conclusive and it will be necessary to evaluate the effects of it in a wider range of values for this parameter.

Different viruses react differently to RH changes[37]. Enveloped viruses (most respiratory viruses, especially influenza) survive longer at lower RH values (20%-30%) [7], [9]. In this work, the mean value of RH was 20.36 % which supports our previous statement of influenza being the dominant virus in the air.

The correlation between relative humidity and SAM coincides with previous findings [10]. Low humidity values lead to higher rates of conidia release due to dehydration causing a weaker bonding with fungi. These low humidity values also triggered the mechanism of sporulation and the spores produced were later propagated by the

wind [10], [38]. Some authors report a positive correlation with humidity since it is important for the development of mycelial structures that produce spores [7], [11].

Our results suggest that companion act as sinks of SAM that can be retained in their clothes, their belongings or their skin. Hoseinzadeh and collaborators evaluated the concentration of BAs in different hospital wards. Their results suggest that a high concentration of BAs in those places can be attributed to personal belongings and overcrowding [35]. It is possible that companions can act as sources of bacteria a through skin shedding or resuspension of microorganisms from inanimate surfaces [12]. However, people in the PCC remained sitting together. This activity has minimal influence on the generation of BA communities [12] .

Patients follow a similar pattern as companion acting as contributors of bacteria and, in this case, also fungi. Fungi can be dragged from outdoor air with belonging related to kids or specifically on their skin. It is assumed that even though cough and sneezes (common symptoms of infectious respiratory illnesses) spread viral particles to the air [2], temperature ultimately regulate its permanence in air.

3.4. Concentration differences between rooms

Table 13 shows the main concentrations of BA between different rooms of the PCC. ANOVA revealed significant differences in the concentration of live and death bacteria ($p<0.05$ and $p<0.1$ respectively) in both rooms and in both seasons. Viruses show significant differences among the different rooms only in spring ($p<0.5$). SAM did not show significant differences. The ANOVA tables are presented in *supporting information.*

Table 13 Concentration of BA in lobby and doctor's room

	SAM (BA/m^3)		Live Bacteria (BA/m^3)		Dead Bacteria (BA/m^3)		Virus (BA/m^3)	
	Winter	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**
LO	8.35±7.5x10^5	6.90±4.3x10^5	1.58±1.1x10^5	1.75±1.6x10^5	4.54±3.8x10^5	2.15±1.1x10^5	4.08±3.1x10^6	1.78±2.1x10^6

DR	6.3±5.2 x10^5	9.45±6.7x10^5	4.72±6.9x10^5	9.34±12 x10^5	2.50±1.1x10^5	1.03±1.8x10^6	3.91±2.3x10^6	1.13±0.7x10^6

LO= Lobby DR= Doctor's Room

The three main differences between the doctor's room and lobby were the size, HVAC system, and light incidence. Since the doctor's room is a smaller place than the lobby it presents lower occupancy density and thus, BAs are more concentrated. There are also fewer patients in this room at the same time which increases the survival and latency of bacteria. HVAC controls temperature refreshing the air and keeping it in low temperatures that influence the concentration of live bacteria. HVAC systems can act as sources of BA if not cleaned properly since the inner filtration methods provide conditions for its growth and later release to the room's air after forming a biofilm inside the HVAC [39], [40]. The UV light is harmful to BA but its effects may be mitigated by RH since water coat protects aerosolized particles [7].

3.5. Influence of biochar

Table 5 shows the main concentrations of airborne microorganisms when counted using PBS with and without biochar. According to ANOVA, the significant ($p<0.5$) changes in concentration are found in the same cases as the proposed models in section 3.3: virus in spring and SAM live bacteria in winter. SAM and bacteria concentrations were higher when the biochar was used but viral concentration decreased.

Table 14 Concentration of BA when using different media

	SAM (BA/m^3)		**Live Bacteria (BA/m^3)**		**Dead Bacteria (BA/m^3)**		**Virus (BA/m^3)**	
	Winter	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**	**Winter**	**Spring**
PBS	2.83±2.2x10^5	7.89±5.9x10^5	9.34±10x10^4	7.23±11x10^5	3.31±2.0x10^5	7.51±16x10^5	4.15±3.0x10^6	1.77±1.7x10^6
PBS +BC	1.15±0.7x10^6	8.67±5.6x10^5	4.08±5.4x10^5	2.75±1.5x10^5	4.60±3.7x10^5	3.31±1.6x10^5	3.59±3.5x10^6	9.29±7.3x10^5

It is speculated that the cell wall and membrane had a higher affinity to the surface of functionalized biochar than viruses. Adsorption to biochar may occur via different processes including hydrophobic attraction or electrostatic forces [41]. The ability of biochars to retain bacteria will vary greatly depending on the biochar properties including the ash content, pore size, and volatile content that are highly variable [41]. The acid functionalization was not beneficial for viruses' adsorption because of the surface characteristics (charge and elemental composition) of these microorganisms. There is a possibility of capsid damage of the viruses. Suggesting that the viruses cannot be retained into the surface of biochar but can be inactivated by it.

4. Conclusions

Viruses were the predominant microorganisms present in the air of the PCC during the monitoring of bioaerosols seconded by spores and mold. The concentration of bacteria remained relatively low during the monitoring time. There was a decline in the total BA concentration as time passed presumably caused by the seasonal changes in the occurrence of respiratory viruses. It is believed that Influenza is the main virus present in the air of the PCC. The use of personal protective equipment in order to avoid illnesses is highly recommended.

Temperature and RH strongly influence the concentration of indoor air BA. The activities of the people present in the PCC caused that they acted more as sinks than sources of BA. This reinforces the necessity of protection methods for the patients and companions.

The two different rooms in the PCC have different microbial ecosystems due to their physical characteristics. Functionalized biochar can be used to enhance the collect of spores, mold and bacteria present in the air, but it is not recommended its use to collect viruses.

5. Supporting Information

a) Equations

$$\mathbf{CBA} = \frac{\mathbf{BA}}{\mathbf{A} * \mathbf{P}} \ldots\ldots\ldots\ldots \mathbf{Ec\ 1}.$$

$$\frac{\mathbf{Bioaerosoles(BA)}}{\mathbf{m^3\ de\ aire}} = \frac{\mathbf{CBA}}{\mathbf{v_m} * \mathbf{t_m} * \mathbf{Q}} \ldots\ldots\ldots\ldots\ldots \mathbf{Ec\ 2}.$$

where:

CBA=Bioaerosols concentration

BA= Observed bioaerosols

A= Observation Area

P= Chamber deepness

Vm= Sampling volume

Tm = Samping time

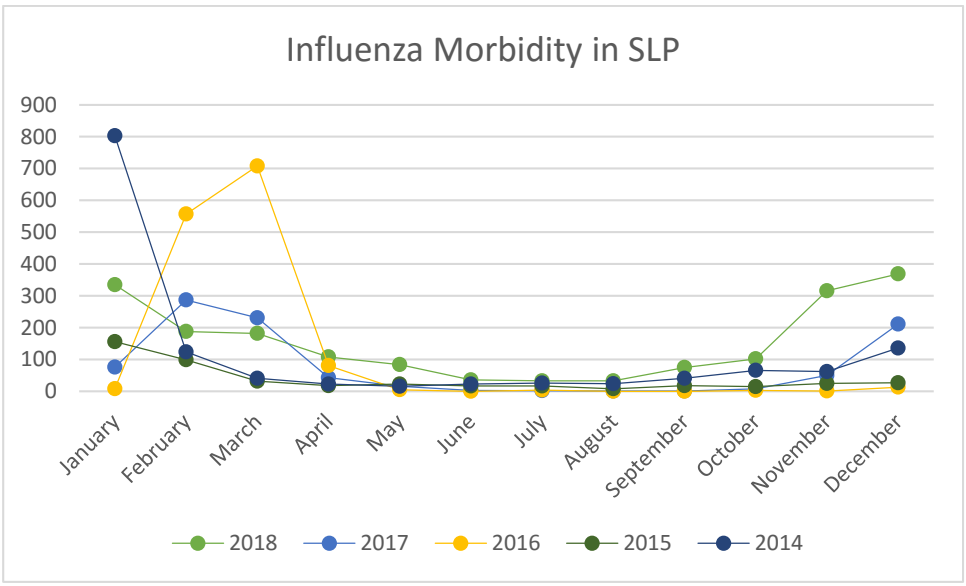
Q= Sampling flux

b) Influenza data in SLP

Incidencia de Influenza (J09-J11) por grupos de edad
Estados Unidos Mexicanos 2018
Población General

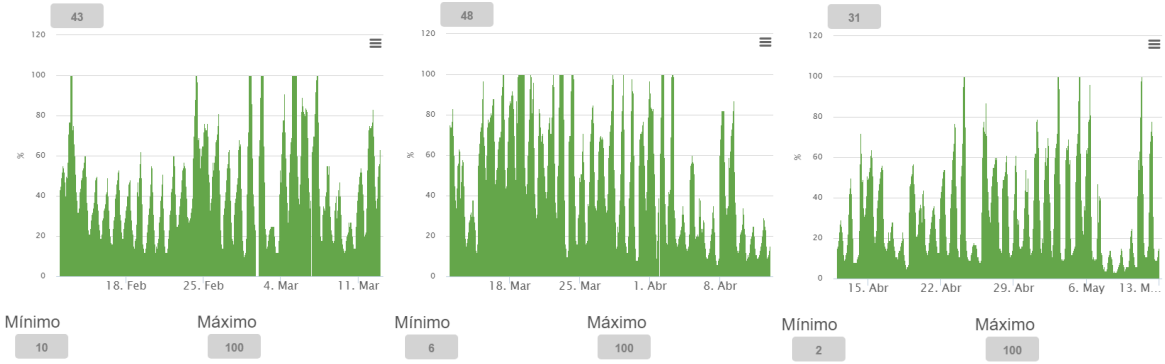
Estado	Grupos de edad											*Incidencia**
	< 1	1 - 4	5 - 9	10 - 14	15 - 19	20 - 24	25 - 44	45 - 49	50 - 59	60 - 64	65 y +	
Aguascalientes	331.47	207.87	84.09	29.94	45.92	56.53	58.13	58.49	67.47	85.64	151.29	**79.60**
Baja California	78.65	49.23	36.40	14.36	12.26	24.74	34.97	45.86	39.35	38.22	27.83	**32.94**
Baja California Sur	327.58	103.16	38.74	30.42	31.86	47.19	53.46	77.85	81.79	117.83	376.57	**80.90**
Campeche	389.50	164.02	84.60	55.41	58.31	91.87	116.64	141.64	148.09	120.09	143.82	**115.78**
Coahuila	87.15	61.58	33.04	17.88	14.15	26.45	29.00	38.63	48.17	39.16	74.03	**36.30**
Colima	409.04	319.34	155.62	133.14	121.36	131.73	125.18	140.59	133.00	114.83	124.30	**148.88**
Chiapas	44.07	39.91	18.14	11.38	9.49	11.58	15.79	18.26	26.17	26.32	20.07	**18.87**
Chihuahua	108.30	88.01	43.16	20.58	14.05	10.49	24.83	29.30	32.87	25.73	32.42	**31.30**
Ciudad de México	123.06	217.09	136.52	73.36	36.42	33.32	53.91	50.73	48.56	43.28	47.96	**64.04**
Durango	66.49	34.58	17.98	14.30	19.65	27.91	42.37	57.89	60.19	49.67	140.37	**43.51**
Guanajuato	83.60	75.82	62.59	35.99	31.19	41.57	57.12	56.84	53.07	56.59	67.39	**54.04**
Guerrero	167.93	97.29	36.23	16.55	10.41	21.99	32.33	34.23	40.42	73.23	117.57	**44.44**
Hidalgo	91.19	76.91	46.91	36.00	31.85	37.52	59.98	73.20	64.55	61.15	89.45	**57.46**
Jalisco	106.49	52.78	34.62	31.67	28.23	44.81	61.12	66.98	61.52	54.26	47.36	**51.06**
México	5.78	3.38	2.72	1.76	1.82	3.24	4.54	4.65	4.82	3.68	3.28	**3.66**
Michoacán	98.98	42.22	16.63	6.74	5.39	17.49	19.22	24.64	32.36	42.02	83.11	**26.88**
Morelos	47.30	45.84	32.19	31.13	30.24	30.66	47.19	48.64	47.22	42.37	52.48	**41.94**
Nayarit	52.54	46.22	24.33	21.74	18.22	39.28	53.77	84.62	48.63	55.06	98.42	**47.75**
Nuevo León	152.68	128.00	116.50	51.93	37.23	71.04	69.70	71.37	57.30	56.21	50.72	**71.83**
Oaxaca	133.75	90.27	35.11	18.04	16.03	30.28	48.14	58.46	55.51	78.38	93.71	**50.26**
Puebla	43.35	14.44	10.15	3.89	3.63	6.34	10.25	17.64	14.19	17.38	17.69	**11.10**
Querétaro	87.21	139.62	84.15	49.26	32.74	69.45	110.95	118.43	125.96	163.28	376.12	**112.83**
Quintana Roo	464.60	356.64	117.83	61.55	62.05	97.84	126.24	160.95	148.97	205.95	342.31	**150.07**
San Luis Potosí	116.21	136.37	58.01	40.66	28.10	37.20	56.17	66.84	71.77	65.55	132.86	**65.87**
Sinaloa	129.52	36.63	23.96	15.25	23.48	41.32	56.10	65.64	45.72	46.73	44.78	**43.74**
Sonora	78.23	48.86	31.27	10.13	10.21	12.33	20.87	22.15	18.52	18.43	11.37	**21.10**
Tabasco	136.33	78.66	25.20	17.09	23.15	35.83	52.36	50.66	73.90	82.24	134.36	**54.10**
Tamaulipas	33.27	24.10	19.81	7.87	15.22	20.80	20.91	28.25	21.98	20.37	19.80	**20.08**
Tlaxcala	20.68	27.77	13.04	7.20	4.05	7.49	11.46	16.40	17.00	20.99	44.19	**14.76**
Veracruz	147.85	98.21	60.56	43.36	34.53	56.52	75.26	87.29	84.05	69.77	82.62	**71.01**
Yucatán	416.22	445.41	280.57	159.48	176.90	298.83	378.62	361.13	303.20	256.43	181.25	**304.27**
Zacatecas	307.93	275.64	86.38	51.35	35.28	57.34	80.22	123.74	102.59	87.18	178.48	**104.06**
TOTAL GLOBAL	**107.97**	**83.35**	**47.25**	**27.76**	**23.62**	**36.36**	**49.00**	**54.25**	**51.30**	**51.06**	**68.29**	**49.06**

FUENTE: SUIVE/DGE/Secretaría de Salud/Estados Unidos Mexicanos 2018



Source: National System of Epidemiological Surveillance (Last Checked: June 2019)

c) Humidity



e) Linear Regressions

Concentracion= Concentration

Pacientes = Patients

Temperatura= Temperature

Humedad = RH

Personas = Companion

Invierno = Winter

Primavera=Spring

Organismo= Type of BA

Sala de Espera= Lobby

Consultorio Medico = Doctor’s Room

1. Viruses

WINTER:

```
Call:
lm(formula = Concentracion ~ pacientes + temperatura + humedad +
    personas, data = subset(Invierno, Organismo == "Virus"))

Residuals:
     Min       1Q   Median       3Q      Max
-4396625 -1304816   129148  1436708  5196586

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) -15651730    8792007  -1.780 0.083481 .
pacientes      298183     207134   1.440 0.158629
temperatura    979402     380519   2.574 0.014320 *
humedad       -118110      33488  -3.527 0.001168 **
personas      -303024      77751  -3.897 0.000406 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2471000 on 36 degrees of freedom
Multiple R-squared:  0.4244,      Adjusted R-squared:  0.3605
F-statistic: 6.636 on 4 and 36 DF,  p-value: 0.0004155

Start:  AIC=1211.72
Concentracion ~ pacientes + temperatura + humedad + personas

              Df  Sum of Sq        RSS    AIC
<none>                      2.1983e+14 1211.7
- pacientes    1 1.2655e+13 2.3249e+14 1212.0
- temperatura  1 4.0454e+13 2.6029e+14 1216.7
- humedad      1 7.5962e+13 2.9579e+14 1221.9
- personas     1 9.2753e+13 3.1259e+14 1224.2

Call:
lm(formula = Concentracion ~ pacientes + temperatura + humedad +
    personas, data = subset(Invierno, Organismo == "Virus"))

Coefficients:
(Intercept)    pacientes  temperatura      humedad     personas
  -15651730       298183       979401      -118110      -303024
```

SPRING:

```
Call:
lm(formula = Concentracion ~ pacientes + temperatura + humedad +
    personas, data = subset(Primavera, Organismo == "Virus"))

Residuals:
     Min       1Q   Median       3Q      Max
-2001924  -506936    13689   429666  1263689

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) -43722534    9126607  -4.791 4.54e-05 ***
pacientes     -132044      95392  -1.384    0.177
temperatura   2019505     424982   4.752 5.05e-05 ***
humedad        -70044      42303  -1.656    0.109
```

```
personas        46904      58117   0.807    0.426
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 746100 on 29 degrees of freedom
Multiple R-squared:  0.7664,      Adjusted R-squared:  0.7342
F-statistic: 23.78 on 4 and 29 DF,  p-value: 8.44e-09

Start:  AIC=924.13
Concentracion ~ pacientes + temperatura + humedad + personas

              Df  Sum of Sq        RSS    AIC
- personas     1 3.6262e+11 1.6508e+13 922.89
<none>                      1.6145e+13 924.13
- pacientes    1 1.0667e+12 1.7212e+13 924.31
- humedad      1 1.5262e+12 1.7671e+13 925.20
- temperatura  1 1.2572e+13 2.8717e+13 941.71

Step:  AIC=922.89
Concentracion ~ pacientes + temperatura + humedad

              Df  Sum of Sq        RSS    AIC
<none>                      1.6508e+13 922.89
- pacientes    1 1.1453e+12 1.7653e+13 923.17
+ personas     1 3.6262e+11 1.6145e+13 924.13
- humedad      1 2.9969e+12 1.9505e+13 926.56
- temperatura  1 2.1949e+13 3.8456e+13 949.64

Call:
lm(formula = Concentracion ~ pacientes + temperatura + humedad,
    data = subset(Primavera, Organismo == "Virus"))

Coefficients:
(Intercept)    pacientes  temperatura      humedad
  -47775187       -63997      2211448       -86304
```

2. SAM

WINTER:

```
Call:
lm(formula = Concentracion ~ personas + pacientes + temperatura +
    humedad, data = subset(Invierno, Organismo == "SAM"))

Residuals:
     Min       1Q   Median       3Q      Max
-1028824  -153824     9385   211462  1394408

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  3593756    1529573   2.350   0.0238 *
personas      -29790      14204  -2.097   0.0423 *
pacientes     163246      35916   4.545 4.98e-05 ***
temperatura  -113748      65454  -1.738   0.0899 .
humedad       -36593       5940  -6.160 2.82e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 455400 on 40 degrees of freedom
Multiple R-squared:  0.5971,      Adjusted R-squared:  0.5568
F-statistic: 14.82 on 4 and 40 DF,  p-value: 1.647e-07


Call:
lm(formula = Concentracion ~ pacientes + temperatura + humedad +
    personas, data = subset(Invierno, Organismo == "SAM"))

Residuals:
     Min       1Q   Median       3Q      Max
-1028824  -153824     9385   211462  1394408
```

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  3593756    1529573   2.350   0.0238 *
pacientes     163246      35916   4.545 4.98e-05 ***
temperatura  -113748      65454  -1.738   0.0899 .
humedad       -36593       5940  -6.160 2.82e-07 ***
personas      -29790      14204  -2.097   0.0423 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 455400 on 40 degrees of freedom
Multiple R-squared:  0.5971,      Adjusted R-squared:  0.5568
F-statistic: 14.82 on 4 and 40 DF,  p-value: 1.647e-07

Start:  AIC=1177.3
Concentracion ~ pacientes + temperatura + humedad + personas

              Df  Sum of Sq        RSS    AIC
<none>                      8.2956e+12 1177.3
- temperatura  1 6.2633e+11 8.9219e+12 1178.6
- personas     1 9.1231e+11 9.2079e+12 1180.0
- pacientes    1 4.2844e+12 1.2580e+13 1194.0
- humedad      1 7.8694e+12 1.6165e+13 1205.3

Call:
lm(formula = Concentracion ~ pacientes + temperatura + humedad +
    personas, data = subset(Invierno, Organismo == "SAM"))

Coefficients:
(Intercept)    pacientes  temperatura      humedad     personas
    3593756       163246      -113748       -36593       -29790
```

SPRING:

```
Call:
lm(formula = Concentracion ~ pacientes + temperatura + humedad +
    personas, data = subset(Primavera, Organismo == "SAM"))

Residuals:
    Min      1Q  Median      3Q     Max
-897054 -348842  -45512  248827 1472072

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  8581334    6109058   1.405   0.1689
pacientes    -136408      65075  -2.096   0.0434 *
temperatura  -343291     286036  -1.200   0.2381
humedad        18183      29666   0.613   0.5439
personas       65750      39901   1.648   0.1083
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 536200 on 35 degrees of freedom
Multiple R-squared:  0.207,       Adjusted R-squared:  0.1163
F-statistic: 2.284 on 4 and 35 DF,  p-value: 0.07988

Start:  AIC=1060.05
Concentracion ~ pacientes + temperatura + humedad + personas

              Df  Sum of Sq        RSS    AIC
- humedad      1 1.0803e+11 1.0172e+13 1058.5
- temperatura  1 4.1419e+11 1.0479e+13 1059.7
<none>                      1.0064e+13 1060.0
- personas     1 7.8081e+11 1.0845e+13 1061.0
- pacientes    1 1.2635e+12 1.1328e+13 1062.8
```

```
Step:  AIC=1058.47
Concentracion ~ pacientes + temperatura + personas

             Df  Sum of Sq        RSS    AIC
<none>                     1.0172e+13 1058.5
- personas     1 7.2074e+11 1.0893e+13 1059.2
+ humedad      1 1.0803e+11 1.0064e+13 1060.0
- temperatura  1 1.0961e+12 1.1269e+13 1060.6
- pacientes    1 1.3842e+12 1.1557e+13 1061.6

Call:
lm(formula = Concentracion ~ pacientes + temperatura + personas,
    data = subset(Primavera, Organismo == "SAM"))

Coefficients:
(Intercept)    pacientes  temperatura     personas
    5047024      -111493      -177013        51693
```

3. Live bacteria

WINTER:

```
Call:
lm(formula = Concentracion ~ pacientes + temperatura + humedad +
    personas, data = subset(Invierno, Organismo == "L_Bacteria"))

Residuals:
    Min      1Q  Median      3Q     Max
-522532 -112304  -22751   93184  602468

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) 8338070.3   711211.8  11.724 2.35e-14 ***
pacientes     31929.6    16989.1   1.879   0.0677 .
temperatura -331293.3    30433.6 -10.886 2.19e-13 ***
humedad        -744.5     2807.1  -0.265   0.7922
personas      13510.1     6620.3   2.041   0.0481 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 211700 on 39 degrees of freedom
Multiple R-squared:  0.7838,      Adjusted R-squared:  0.7616
F-statistic: 35.34 on 4 and 39 DF,  p-value: 1.746e-12

Start:  AIC=1083.85
Concentracion ~ pacientes + temperatura + humedad + personas

             Df  Sum of Sq        RSS    AIC
- humedad      1 3.1535e+09 1.7517e+12 1081.9
<none>                     1.7486e+12 1083.8
- pacientes    1 1.5837e+11 1.9069e+12 1085.7
- personas     1 1.8672e+11 1.9353e+12 1086.3
- temperatura  1 5.3130e+12 7.0615e+12 1143.3

Step:  AIC=1081.93
Concentracion ~ pacientes + temperatura + personas

             Df  Sum of Sq        RSS    AIC
<none>                     1.7517e+12 1081.9
- pacientes    1 1.5522e+11 1.9069e+12 1083.7
+ humedad      1 3.1535e+09 1.7486e+12 1083.8
- personas     1 2.0515e+11 1.9569e+12 1084.8
- temperatura  1 5.3626e+12 7.1144e+12 1141.6

Call:
lm(formula = Concentracion ~ pacientes + temperatura + personas,
    data = subset(Invierno, Organismo == "L_Bacteria"))

Coefficients:
(Intercept)    pacientes  temperatura     personas
```

```
    8335895        31319      -331899        13866
```

SPRING:

```
Call:
lm(formula = Concentracion ~ pacientes + temperatura + humedad +
    personas, data = subset(Primavera, Organismo == "L_Bacteria"))

Residuals:
     Min       1Q   Median       3Q      Max
-1117924  -629984   -38224   161720  2442165

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   107658   10149063   0.011    0.992
pacientes     -16012     108110  -0.148    0.883
temperatura    74424     475196   0.157    0.876
humedad       -16656      49285  -0.338    0.737
personas      -73037      66288  -1.102    0.278

Residual standard error: 890900 on 35 degrees of freedom
Multiple R-squared:  0.2292,      Adjusted R-squared:  0.1411
F-statistic: 2.602 on 4 and 35 DF,  p-value: 0.05265

Start:  AIC=1100.65
Concentracion ~ pacientes + temperatura + humedad + personas

              Df  Sum of Sq        RSS    AIC
- pacientes    1 1.7410e+10 2.7795e+13 1098.7
- temperatura  1 1.9467e+10 2.7797e+13 1098.7
- humedad      1 9.0643e+10 2.7868e+13 1098.8
- personas     1 9.6349e+11 2.8741e+13 1100.0
<none>                     2.7777e+13 1100.7

Step:  AIC=1098.68
Concentracion ~ temperatura + humedad + personas

              Df  Sum of Sq        RSS    AIC
- temperatura  1 8.5922e+10 2.7881e+13 1096.8
- humedad      1 2.4118e+11 2.8036e+13 1097.0
<none>                     2.7795e+13 1098.7
+ pacientes    1 1.7410e+10 2.7777e+13 1100.7
- personas     1 5.0493e+12 3.2844e+13 1103.4

Step:  AIC=1096.8
Concentracion ~ humedad + personas

              Df  Sum of Sq        RSS    AIC
- humedad      1 3.0321e+11 2.8184e+13 1095.2
<none>                     2.7881e+13 1096.8
+ temperatura  1 8.5922e+10 2.7795e+13 1098.7
+ pacientes    1 8.3865e+10 2.7797e+13 1098.7
- personas     1 5.0802e+12 3.2961e+13 1101.5

Step:  AIC=1095.24
Concentracion ~ personas

              Df  Sum of Sq        RSS    AIC
<none>                     2.8184e+13 1095.2
+ humedad      1 3.0321e+11 2.7881e+13 1096.8
+ temperatura  1 1.4795e+11 2.8036e+13 1097.0
+ pacientes    1 1.0368e+11 2.8080e+13 1097.1
- personas     1 7.8532e+12 3.6037e+13 1103.1

Call:
lm(formula = Concentracion ~ personas, data = subset(Primavera,
    Organismo == "L_Bacteria"))
```

```
Coefficients:
(Intercept)     personas
    1602503       -87318
```

4. Dead Bacteria

WINTER:

```
Call:
lm(formula = Concentracion ~ pacientes + temperatura + humedad +
    personas, data = subset(Invierno, Organismo == "D_Bacteria"))

Residuals:
    Min      1Q  Median      3Q     Max
-489307 -172326  -54976   55238 1195024

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  -614384    1085905  -0.566    0.575
pacientes     -25089      25940  -0.967    0.339
temperatura    32581      46467   0.701    0.487
humedad         2995       4286   0.699    0.489
personas       16896      10108   1.672    0.103

Residual standard error: 323300 on 39 degrees of freedom
Multiple R-squared:  0.1125,      Adjusted R-squared:  0.02152
F-statistic: 1.236 on 4 and 39 DF,  p-value: 0.3114

Start:  AIC=1121.09
Concentracion ~ pacientes + temperatura + humedad + personas

              Df  Sum of Sq        RSS    AIC
- humedad      1 5.1033e+10 4.1274e+12 1119.6
- temperatura  1 5.1384e+10 4.1277e+12 1119.6
- pacientes    1 9.7782e+10 4.1741e+12 1120.1
<none>                     4.0763e+12 1121.1
- personas     1 2.9205e+11 4.3684e+12 1122.1

Step:  AIC=1119.64
Concentracion ~ pacientes + temperatura + personas

              Df  Sum of Sq        RSS    AIC
- temperatura  1 5.9696e+10 4.1871e+12 1118.3
- pacientes    1 8.1056e+10 4.2084e+12 1118.5
<none>                     4.1274e+12 1119.6
- personas     1 2.5511e+11 4.3825e+12 1120.3
+ humedad      1 5.1033e+10 4.0763e+12 1121.1

Step:  AIC=1118.27
Concentracion ~ pacientes + personas

              Df  Sum of Sq        RSS    AIC
- pacientes    1 3.6842e+10 4.2239e+12 1116.7
<none>                     4.1871e+12 1118.3
- personas     1 2.7726e+11 4.4643e+12 1119.1
+ temperatura  1 5.9696e+10 4.1274e+12 1119.6
+ humedad      1 5.9345e+10 4.1277e+12 1119.6

Step:  AIC=1116.65
Concentracion ~ personas

              Df  Sum of Sq        RSS    AIC
<none>                     4.2239e+12 1116.7
+ humedad      1 4.0935e+10 4.1830e+12 1118.2
+ pacientes    1 3.6842e+10 4.1871e+12 1118.3
- personas     1 3.6937e+11 4.5933e+12 1118.3
+ temperatura  1 1.5483e+10 4.2084e+12 1118.5

Call:
lm(formula = Concentracion ~ personas, data = subset(Invierno,
```

```
    Organismo == "D_Bacteria"))

Coefficients:
(Intercept)     personas
     171613        11464
```

SPRING:

```
Call:
lm(formula = Concentracion ~ pacientes + temperatura + humedad +
    personas, data = subset(Primavera, Organismo == "D_Bacteria"))

Residuals:
     Min       1Q   Median       3Q      Max
-1119182  -589263   -18940   129713  4162444

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -6248701   15750240  -0.397    0.694
pacientes      35533     174219   0.204    0.840
temperatura   378137     741261   0.510    0.614
humedad       -46239      78523  -0.589    0.560
personas     -109070     106271  -1.026    0.313

Residual standard error: 1231000 on 31 degrees of freedom
Multiple R-squared:  0.1478,      Adjusted R-squared:  0.03785
F-statistic: 1.344 on 4 and 31 DF,  p-value: 0.2758

Start:  AIC=1014.31
Concentracion ~ pacientes + temperatura + humedad + personas

              Df  Sum of Sq        RSS    AIC
- pacientes    1 6.3058e+10 4.7057e+13 1012.4
- temperatura  1 3.9449e+11 4.7388e+13 1012.6
- humedad      1 5.2565e+11 4.7519e+13 1012.7
- personas     1 1.5968e+12 4.8590e+13 1013.5
<none>                      4.6993e+13 1014.3

Step:  AIC=1012.36
Concentracion ~ temperatura + humedad + personas

              Df  Sum of Sq        RSS    AIC
- temperatura  1 4.1999e+11 4.7477e+13 1010.7
- humedad      1 6.1369e+11 4.7670e+13 1010.8
<none>                      4.7057e+13 1012.4
+ pacientes    1 6.3058e+10 4.6993e+13 1014.3
- personas     1 5.6494e+12 5.2706e+13 1014.4

Step:  AIC=1010.68
Concentracion ~ humedad + personas

              Df  Sum of Sq        RSS    AIC
- humedad      1 2.2337e+11 4.7700e+13 1008.9
<none>                      4.7477e+13 1010.7
+ temperatura  1 4.1999e+11 4.7057e+13 1012.4
- personas     1 5.2325e+12 5.2709e+13 1012.4
+ pacientes    1 8.8561e+10 4.7388e+13 1012.6

Step:  AIC=1008.85
Concentracion ~ personas

              Df  Sum of Sq        RSS    AIC
<none>                      4.7700e+13 1008.9
+ humedad      1 2.2337e+11 4.7477e+13 1010.7
+ pacientes    1 1.2290e+11 4.7577e+13 1010.8
+ temperatura  1 2.9676e+10 4.7670e+13 1010.8
- personas     1 7.4442e+12 5.5144e+13 1012.1

Call:
lm(formula = Concentracion ~ personas, data = subset(Primavera,
    Organismo == "D_Bacteria"))
```

```
Coefficients:
(Intercept)     personas
    1694139       -89420
```

f) ANOVA

SEASONS:

```
Df    Sum Sq   Mean Sq F value   Pr(>F)
epoca             1 1.941e+13 1.941e+13   10.43  0.00137 **
Organismo         3 3.056e+14 1.019e+14   54.73  < 2e-16 ***
epoca:Organismo   3 1.134e+14 3.780e+13   20.31 4.63e-12 ***
Residuals       316 5.882e+14 1.861e+12
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = Concentracion ~ epoca * Organismo, data = Hoja3)

$epoca
                        diff       lwr       upr     p adj
Primavera-Invierno -490838.6 -789911.1 -191766.1 0.0013725

$Organismo
                             diff       lwr       upr     p adj
L_Bacteria-D_Bacteria  -54952.24 -605417.4  495512.9 0.9939820
SAM-D_Bacteria         330203.54 -218679.8  879086.9 0.4067516
Virus-D_Bacteria      2369018.72 1802671.5 2935365.9 0.0000000
SAM-L_Bacteria         385155.78 -156953.4  927265.0 0.2588707
Virus-L_Bacteria      2423970.96 1864186.5 2983755.4 0.0000000
Virus-SAM             2038815.18 1480586.1 2597044.2 0.0000000

$`epoca:Organismo`
                                                      diff         lwr        upr
p adj
Primavera:D_Bacteria-Invierno:D_Bacteria    191919.19  -743639.12  1127477.5
0.9985064
Invierno:L_Bacteria-Invierno:D_Bacteria    -119318.18 -1006866.73   768230.4
0.9999087
Primavera:L_Bacteria-Invierno:D_Bacteria    170217.80  -739248.82  1079684.4
0.9991756
Invierno:SAM-Invierno:D_Bacteria            382196.97  -500406.98  1264800.9
0.8902819
Primavera:SAM-Invierno:D_Bacteria           433759.47  -475707.16  1343226.1
0.8303232
Invierno:Virus-Invierno:D_Bacteria         3646929.35  2743291.01  4550567.7
0.0000000
Primavera:Virus-Invierno:D_Bacteria        1014907.75    64335.88  1965479.6
0.0270005
Invierno:L_Bacteria-Primavera:D_Bacteria   -311237.37 -1246795.69   624320.9
0.9719596
Primavera:L_Bacteria-Primavera:D_Bacteria   -21701.39  -978078.13   934675.4
1.0000000
Invierno:SAM-Primavera:D_Bacteria           190277.78  -740590.99  1121146.5
0.9985403
Primavera:SAM-Primavera:D_Bacteria          241840.28  -714536.47  1198217.0
0.9944201
Invierno:Virus-Primavera:D_Bacteria        3455010.16  2504174.11  4405846.2
0.0000000
Primavera:Virus-Primavera:D_Bacteria        822988.56  -172558.44  1818535.6
0.1898086
Primavera:L_Bacteria-Invierno:L_Bacteria    289535.98  -619930.64  1199002.6
0.9781136
Invierno:SAM-Invierno:L_Bacteria            501515.15  -381088.80  1384119.1
0.6650560
```

```
Primavera:SAM-Invierno:L_Bacteria           553077.65  -356388.97  1462544.3
0.5824798
Invierno:Virus-Invierno:L_Bacteria         3766247.54  2862609.19  4669885.9
0.0000000
Primavera:Virus-Invierno:L_Bacteria        1134225.94   183654.06  2084797.8
0.0075736
Invierno:SAM-Primavera:L_Bacteria           211979.17  -692662.67  1116621.0
0.9965230
Primavera:SAM-Primavera:L_Bacteria          263541.67  -667327.10  1194410.4
0.9889455
Invierno:Virus-Primavera:L_Bacteria        3476711.55  2551536.22  4401886.9
0.0000000
Primavera:Virus-Primavera:L_Bacteria        844689.95  -126378.54  1815758.4
0.1409282
Primavera:SAM-Invierno:SAM                   51562.50  -853079.33   956204.3
0.9999998
Invierno:Virus-Invierno:SAM                3264732.38  2365950.11  4163514.7
0.0000000
Primavera:Virus-Invierno:SAM                632710.78  -313245.98  1578667.5
0.4560001
Invierno:Virus-Primavera:SAM               3213169.88  2287994.56  4138345.2
0.0000000
Primavera:Virus-Primavera:SAM               581148.28  -389920.21  1552216.8
0.6027304
Primavera:Virus-Invierno:Virus            -2632021.60 -3597633.71 -1666409.5
0.0000000
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FINAL THOUGHTS

This work contributes to knowledge about functionalized biochar as a material that improves the collection efficiency of spores, mold, and bacteria when it is coupled to a liquid impinger. Also, this is the first work in analyse the concentration of bioaerosols in human-health care centers using epifluorescence microscopy.

The functionalized biochar demonstrated to be efficient for its application in control of air pollution. It is necessary to do further investigation about the material properties like changes of the material when this enters in contact with PBS. It is necessary to know the retention capacity of biochar using pure cultures of the different types of microorganisms. This supposes a technical challenge since the concentration of bioparticles may change with time due to metabolism processes.

The presence of viruses in the air must not be underappreciated since they strongly contribute to the conformations of the indoor air micro-ecosystem. A deeper analysis of viruses' behavior under indoor air has to be performed since this seasonally fluctuates.