DETERMINATION OF AIR POLLUTION BY MICROSCOPIC FUNGI IN CENTRAL MARKETS OF THE CITY OF MACAS, ECUADOR

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Abstract

Background: The objective of the present investigation was to determine the air pollution by microscopic fungi in the Central Market and Private Market in the city centerof Macas.

Methodology: This was done using the exposed plate technique, the petri dishes were randomly placed in the Central and Private Market, they were kept unsealed for 30 minutes allowing the sedimentation of fungal spores suspended in the air. Once the exposure time had elapsed, the samples obtained from each study area were sealed and the plates were taken to the laboratory for isolation, placing them in an oven at a temperature of 36°C for 48 hours, resulting in the identification of 6 genera of microscopic fungi in the two markets analyzed.

Conclusion: Through the isolation and taxonomic identification of microscopic fungi obtained in the air analysis of the Central and private markets, it was possible to demonstrate the existence of these

pathogenic microorganisms and to suggest mechanisms to improve the handling of raw and prepared foods in the respective areas of the markets stadied.

Key words: Air Pollution, Omeliansky, Environmental Fungi, Markets, Microorganisms, Air Quality, Bioindicators.

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Introduction

Air pollution generates 2.9 to 8.9 million deaths annually in dense populated areas due to the existence of high levels of pollution, this emphasizes the deaths that are related to the environment in countries with low economic resources according to(Góralska et al. 2022) cited by the WHO. Food markets generally tend to receive large crowds of people, since it is a place that supplies food products (Ccahuana y Escobar 2016). In developing countries, markets do not have an adequate infrastructure, this contributes to problems of insalubrity (FAO 2012).

The insalubrity of the environment of the markets gives relevance to the antigenic conditions with which they commercialize the products, causing health problems and affections to the quality of the environment of urban areas (Canchucaja 2018).

One of the problems presented by food markets is the excess of solid waste and consequently poor waste management, which leads to becoming ideal places for the proliferation and growth of microbial pathogens(GÓMEZ 2021). In indoor environments there are several types of airborne microorganisms known as bioaerosols, among which spore species, fungi, bacteria and viruses can be found (Daza et al., 2015, pp.39). Many of them cause health problems and others cause affectations such as the deterioration of buildings and other materials, which is why the interest in evaluating the quality of these environments has increased (CHUQUILIN y ROJAS 2020).

In the research carried out by Nuñez (2019), in the market of Paucarbamaba – Perú, the existence of environmental fungi is evidence, such as: *Aspergillus Niger - Candica albicans - Microsporumpersicolor - Stachybotrys – Dermatofitos - Aspergillus fumigatus - Penicilliun notatum – fusarium - Trichophyton terrestre – Dermatofitos - Candica albicans - Sporothrixschenkii*, which are responsible for respiratory conditions. Another example is the research conducted in the market in the south of the city of Tunja, Boyacá, which resulted in three different fungal strains: *Saccharomyces, Ascomycota andAbsidiasp,* the latter is known to cause skin and subcutaneous infections.(GÓMEZ 2021).

In the city of Tacna de Perú, a study was conducted in three food markets, where eight genera of fungi were found in the envirnment, such as: se realizó un estudio en tres mercados de abastos, donde se evidencióochogéneros de hongos en elambiente, tales como: la *Penicillium spp*. *Candida sp*, *Cladosporium herbarum*, *Rhodotorula sp*., *Rhizopus stolonifer*, *Aspergillus niger*, *Botrytis sp.*, *Mucor sp*. y *Mucor piriformis* (CALIZAYA, SALAZAR, y SILVA 2010). Another similar case occurred in Modelo market in Tingo María, Peru, where sampling revealed the presence of fungal strains, with the Aspergillus strain being predominant and consequently the Geotrichumstrain, which cause diseases of the respiratory system (Torres 2011).

The lack of research and analysis of air pollution in the province of Morona Santiago could be detrimental to the urban population, as this can be exposed to changes in air quality, due to the high population rate, commercialization activities and poor management of solid waste that is evident in the Central and private markets of the city of Macas, This research is based on the determination of air pollution by microscopic fungi in the Central and Private Market in the central area of the city of Macas, in order to diagnose the air affectation, using techniques and methods that will serve as a basis to know the current situation and for future research on air quality.

This research emphasizes the problem of contamination in food markets, and the most relevant aspects are presented in an understandable conceptual basis. The methodologies and techniques used in this research helped the comprehension and interpretation of the results obtained in the analysis. On the other hand, the analysis and discussion of results, describe what was done in the research, being the basis to determine the problems related to the subject and to present possible solutions.

Methodology

This research is based on an exploratory and diagnostic analysis to identify the microscopic fungi existing in the environment of the supply markets, specifically in the Central Market of the city of Macas, and to diagnose the air pollution caused by the fungal load through the table of permissible values of microorganisms that may be present in the area.

It is important to present in a descriptive and analytical way, the results obtained about the type of each microorganism identified, which by means of the isolation and fungal concentration could demonstrate the air pollution caused by the presence of microscopic fungi in the supply markets of this city.

Sampling points

The selected locations for the determination of air contamination by microscopic fungi, were the establishments of the Central Market and the Private Market, located in the city center of Macas. For the procedure, 2 petri dishes were placed in 5 areas corresponding to the Central Market (meat, vegetables, food court, groceries and shopping center) and 5 random samples in the Private Market as there is no distribution by specific areas.

Analysis of data according to temperature and humidity

The parameters taken into account for the data analysis in the two samplings were the following: temperature, humidity, climate and exposure time, these were described according to the distribution of each selected area of both the Central Market and the Private Market. The process started with the measurement of both temperature and humidity with the digital Thermo-Hygrometer in each area of the Central Market, as well as, the humidity and temperature of the Private Market. It should be noted that the first sampling was done on a day with precipitation and the second sampling was done on a sunny day.

Exposure plate method

To obtain this information, a passive microbiological method known as the exposed plate technique was used, where the sedimentation of air particles occurs. This technique consists of exposing a number of 90 mm diameter Petri dishes containing Sabouraud Agar and chloramphenicol to the environment for 30 minutes in different areas of the Central and Private Market. This method is called non-volumetric since a fixed volume of air is not evaluated(CEPEDA 2019).

The procedure was developed in first instance by placing two Petri boxes with sabouraud agar + chloramphenicol, in the different areas of the Central Market of Macas. The exposure time was 30 minutes, where the temperature, humidity and climate with precipitation were also taken into account, these are parameters obtained from the digital thermo hygrometer. In addition, the respective sealing and labeling was performed to avoid contamination or possible modifications of the samples; furthermore, for conservation until reaching the laboratory, the samples were stored in a thermal box, these were placed in the incubator at 36°C for 72 hours

Staining method

Using the technique of staining fungal structures with water-soluble vegetable dyes according to GONZÁLEZ (2011), a solution composed of 1 ml of vegetables dye was prepared, followed by 10 ml of acetic acid, in addition to 15 ml of glycerin that will serve as a humectant and 75 ml of sterilized distilled water in a flask.

Based on the good practice guide NTP 488, which corresponds to Indoor air quality and identification of fungi, the following procedure was carried out for sample preparation by means of cultivation on microscope slides. To carry out this procedure, a small 2 cm block of the culture medium was cut with the help of a sterile scalpel, and then placed on the slide with the agar block on a Petri dish; with the help of a bacterial inoculation loop, the four sections of the block were inoculated with the selected strain of fungi to be identified.A sterile coverslip was placed on the surface of the block of culture medium, then the Petri dish containing the block was then closed and incubated at 30°C. At the end of the incubation period, the block with the sample was placed on a slide containing lactophenol blue or aniline blue (in this case the stain prepared with vegetable dye was used), by microscopic observation it was possible to distinguish the shape and arrangement of the spores of each genera isolated, through the use of the microscope and the PROVIEW program, it was also possible to observe the structures of the fungal colonies for their subsequent taxonomic identification.

Quantification of microscopic fungi

To perform this procedure, daily observations of each plate were made to detect the growth of microorganisms, and a record of the observed colony count was kept to facilitate the count; with the reference of MONTALUISA (2018),it was possible to identify the total number of colonies in each Petri dish, after the application of the Omeliansky formula, to obtain the result of CFU/m3.

Identification of microscopic fungi in the Central Market environment.

In the taxonomic analysis of microscopic fungi, 6 fungal genera could be identified, among which the genera*Aspergillus, Penicillium, Cladosporium, Rhizopus, Mucor y Fusarium*. According to SORIA (2017) this type of fungi are commonly found in vegetables, meat, poultry, fruits, among other foods, since these microorganisms usually feed on decaying organic matter.

The Central Market, as it has a wide infrastructuredivided by areas, different genera of fungi were identified, which are generally found in environments with high humidity, in warm areas and dark areas,(SORIA 2017) says that, these conditions allow them to survive and proliferate. Microscopic fungi are found in food markets due to the variety of products offered in these places, such as (meats, vegetables, grains and fruits) and because of the environmental conditions that are suitable and favor their fungal proliferation.

The first sampling showed an excessive growth of the samples analyzed, while the second sampling showed low values compared to the previous concentrations, this was reflected 48 hours after the fungal isolation. The result of these analyses was the existence of a high concentration of microscopic fungi in the different areas of the Central Market. The factors that allowed the development of these microorganisms were the environmental conditions, such as temperature and humidity; from these observations it was possible to analyze that the concentrations had significant variations due to climatic changes, referring to warm days and humid days. In this regards Nuñez (2019), mentions that the study of the relationship of microorganisms present in the air of a market, in the food area, is obtained less amount of fungal concentration, unlike the data obtained in the samplings of the Central Market, whose showed that the conditions of the food court are the most essential for fungal growth.

In all the distribution areas of the Central Market of Macas, the existence of environmental contamination was demonstrated by fungal concentration. Each of the areas showed that fungal concentrations exceeded the permissible limits. The highest contamination in the Central Market is evidenced in the food court with a maximum value of 802 CFU/m3, followed by the grocery area with 760 CFU/m3 and the commercial center with 750 CFU/m3, also with a high level of fungal concentrations.

Identification of microscopic fungi in the private market environment.

The Private Market has a small infrastructure and does not have a distribution of areas, it is separated only by units for each stall, where vegetables and fruits are the most commercialized, the meat stalls (especially chicken) are within the units, of which there are only 4 stalls where this type of animal products are commercialized.

Despite their structure and distribution, several fungal genera were identified. Among the fungal genera analyzed, the following were found:*Aspergillus, Penicillium, Cladosporium, Rhizopus, Mucor, and Candida sp.*

In the Private Market, a concentration of 1000 CFU/m3 was obtained in the first sampling taken in May, as opposed to the second sampling taken in July, where a maximum value of 958 CFU/m3 and a minimum value of 583 CFU/m3 was obtained. The results show significant variations in concentrations between the two samples taken, this is due to environmental conditions, such as rainfall, that promotes adequate levels of temperature and humidity that collaborate with the proliferation and fungal growth.

Central Market temperature and humidity ratio

In the Central Market there is a high fungal concentration, in a temperature range that fluctuates between 26°C and 28°C, from this temperature microscopic fungi tend to proliferate more easily. The temperature values are in the optimal range for fungal growth.

The temperature between the days of precipitation and the days without rain did not show significant differences, which means that the temperatures in the samples taken were similar.

Private Market Temperature and Humidity Ratio

In the private market, the temperature was

25.18°C and the humidity was 68%, although the conditions remained constant, there was a high level of fungal concentration, resulting in a higher level of contamination by microscopic fungi. This result may also be due to the fact that the private market has an open structure, which means that microorganisms from the outside environment have more opportunity to become involved.

Determination of air pollution levels by fungal concentrations obtained from central and private markets.

It was possible to determine the air pollution levels by means of the general average of the concentrations obtained in the Central Market and in the Private Market. The results showed thar there is high contamination in the air of both markets, according to the permissible values of microorganisms present in the environment in UFC/m3 levels established by the (WHO) in conjunction with the Commission of the European Communities (ECA 1994), which mention that if the concentration levels are less than 25 CFU/m3 or have a range between 25 to 100 CFU/m3 are considered low contamination environments, also, if the levels are in a range of 100 to 500 CFU/m3 are considered environments with intermediate contamination levels, however, if the range increases to 500-2000 CFU/m3 or exceeds this value are considered high and very high contamination environments.

Fungal genera present in the air of central and private markets.

According to GUTIÉRREZ (2020), Fungi can become an atmospheric bioindicator, since by increasing their proliferation they can cause changes in the environment or health problems. This is required to assess the environmental conditions affecting the population or ecosystems, these microorganisms would become an essential tool to evaluate air quality in specific locations.

Conclusions

Through the isolation and taxonomic identification of the microscopic fungi obtained in the air analysis of the Central and private markets, it was possible to demonstrate the existence of these pathogenic microorganisms. The fungal concentrations of the Central Market were 706 CFU/m3, and of the Private Market were 913 CFU/m3, demonstrating that there is a high level of air contamination by these microorganisms, when compared with the permissible values of the (WHO) of fungi in a given environment. The Private Market obtained a higher level of contamination compared to the Central Market, where temperatures and humidity contributed to the proliferation and dispersion of fungi in the environment. This is due to the fact that the conditions in which the Private Market is located with respect to the organization of the stalls, the combination of products, and the high humidity level in the area, produce a higher concentration of fungi in the air. The identification of fungal genera was documented and characterized using reliable sources, such as the Studies in Mycology database, which provided several studies that analyzed the taxonomic characteristics of each fungal genus. Using the INEN 2687 standard, it was determined that the central and private markets are not in adequate conditions; the matrix for evaluating the requirements of INEN 2687 was used to consider and propose measures to improve on-site distribution, food handling, disinfection and cleaning programswith objective improving the management and organization of the markets and, at the same time, contribute to reducing atmospheric contamination caused by pathogenic microorganisms in these markets, given their importance for the health and nutrition of the population.

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