



## Article

# Microbiological Analysis of the Air in a Popular Fish Processing and Marketing Area

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**Abstract:** Fish are marketed as a food and consumed worldwide. During the production of food, contamination by microorganisms is possible through the air, soil, water, surfaces, food handlers, etc. The air does not have a natural microbial composition, but it is a vehicle for the transmission of microorganisms of economic and health interest because they are associated with food spoilage and human diseases. The objective of this study was the microbiological analysis of the air in an area popular for the processing and marketing of fish products in the city of Tepic Nayarit. Using the passive or sedimentation method to collect microorganisms present in the air, the proportion of aerobic mesophile bacteria, coliform bacteria, fungi and yeast was determined at different locations in the fish processing and marketing area for four weeks. The results indicated that the aerobic mesophiles had the highest counts among all the microbial groups analyzed at the twelve different sampling points during the four weeks of the study; their numbers ranged from 2.44 to 2.95 log CFU/m<sup>3</sup>/h, followed by molds with counts from 1.44 to 2.75 log CFU/m<sup>3</sup>/h, yeasts with counts from 0.7 to 2.01 log CFU/m<sup>3</sup>/h and coliforms with counts that ranged from 0.7 to 1.68 log CFU/m<sup>3</sup>/h. We determined the proportion of the viable microbiological population present in the air at the different sampling points of the study area; several of these sampling points presented values above those recommended by various agencies around the world. Knowledge of the biological hazards transported through the air is important to establish and reduce the risk to the health of occupants and the contamination pathways of processed and marketed fishery products that may be associated with spoilage and foodborne diseases.

**Keywords:** food safety; food quality; air pollution; airborne biohazard; environmental monitoring

## 1. Introduction

Any food extracted from oceanic or continental waters intended for human or animal consumption is designated by the name of “fish”, which is a generic term that describes fish, crustaceans, mollusks, algae, etc. [1]. Through aquaculture and fishing activities, fish destined mainly for human consumption represented an estimated world production (for both activities) of 178.5 million tons live weight for 2018, with Asian countries such as China among the main producers [2].

Fish is considered a widely produced and commercialized food and an important element of the human diet, as it is of high nutritional quality due to its content of biologically

valuable and digestible proteins, as well as lipids, minerals, and vitamins [1,3]; the reported annual per capita consumption worldwide is 20.5 kg [2].

Throughout the different stages of the human food chain, fish are highly susceptible to contamination and deterioration, which can compromise their quality, safety, and nutritional value. These characteristics are related to factors such as species, age, environmental conditions, feeding, capture conditions, handling conditions, processing, transportation, distribution, storage, and marketing, as well as their intrinsic characteristics, such as a pH close to neutrality, high water content, and the content of nutrients available for microbial activity and growth, oxidation, and enzymatic activity (autolysis) [1,3,4].

The contamination of fish throughout the food chain can be physical, chemical, and biological; the latter regularly involves different bacteria (*Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., and *Vibrio* sp.), parasites (*Anisakis simplex*, *Cryptosporidium parvum*, and *Giardia lamblia*), viruses (Norwalk-like virus and Rotavirus), and fungi that compromise fish quality and safety and lead to cases or outbreaks of foodborne illnesses, which are considered a major public health problem around the world [1,4,5].

In the fishery products industry, the analysis of microbiological risk in various aspects of food safety highlights the postcapture stages from processing to marketing, considering storage, handling, and hygiene conditions [1]. The routes of contamination identified in the food industry are varied and include those of natural origin or reservoirs through the air, dust or soil, utensils, surfaces, water or other contaminated food, handlers, insects, rodents, etc. [4,5].

The air (atmosphere) comprises gases of varying concentrations (nitrogen, oxygen, inert gases such as argon and traces of carbon dioxide). Aerosols, which are particles that include liquids and solids whose sizes typically range between approximately 1 nm and 100 µm, are found in suspension in the atmosphere [5–7]. Aerosols include bioaerosols, which consist of living substances such as pollen, plant debris, insect excretions, algae, viruses, spores, toxins, peptidoglycans, parasites, and vegetative cells of bacteria, mold, and yeast; microbes are particularly relevant in the food industry [5,7–10].

The air does not have indigenous microorganisms but collects microorganisms (spores, bacteria, parasites, viruses, and fungi) present in various natural environments, such as soil, water, plants, animals, and human beings. These microorganisms are transported through the air in the form of bioaerosols and are able to survive in this environment, where many of these microorganisms are responsible for the contamination and deterioration of food, as well as diseases in plants, animals, and humans [5,7,9,11–13].

The sources of aerosols and bioaerosols in the Earth's atmosphere can be natural (rain, rivers, and seas) or anthropogenic; the latter are derived from pollution caused by humans that is released into the atmosphere [7,11]. The rise of industrialization has led to greater exposure to a wide variety of polluting aerosols/bioaerosols from industry, livestock, food processing, dust mobilized in areas with agricultural activity, the combustion of fossil fuels, waste sorting, and composting [7].

Microorganisms can be carried by the outside air to the interior of buildings through ventilation and the personnel that inhabit buildings [8,14]. These biological agents influence the quality of the air inside buildings such as industrial and manufacturing areas and air quality related to the health of the people who occupy them, their activities, and the hygiene conditions within such constructions [8,9,15].

Bioaerosols in indoor environments are mainly related to human activities [7,8,16]. It is estimated that human beings project and release between 1000 and 10,000 microorganisms per minute into the atmosphere, with considerable variation related to conditions and activity (type of clothing, skin hygiene, sneezing, coughing, washing, cleaning, combing hair, walking, talking, etc.); the microorganisms present are those that inhabit skin, feces, and hair [7,8,16,17]. Microorganisms in the air in the food industry can settle and colonize food products, furniture, equipment, containers, and other surfaces in contact with food during handling, and they represent a source of indoor air pollution [5,16].

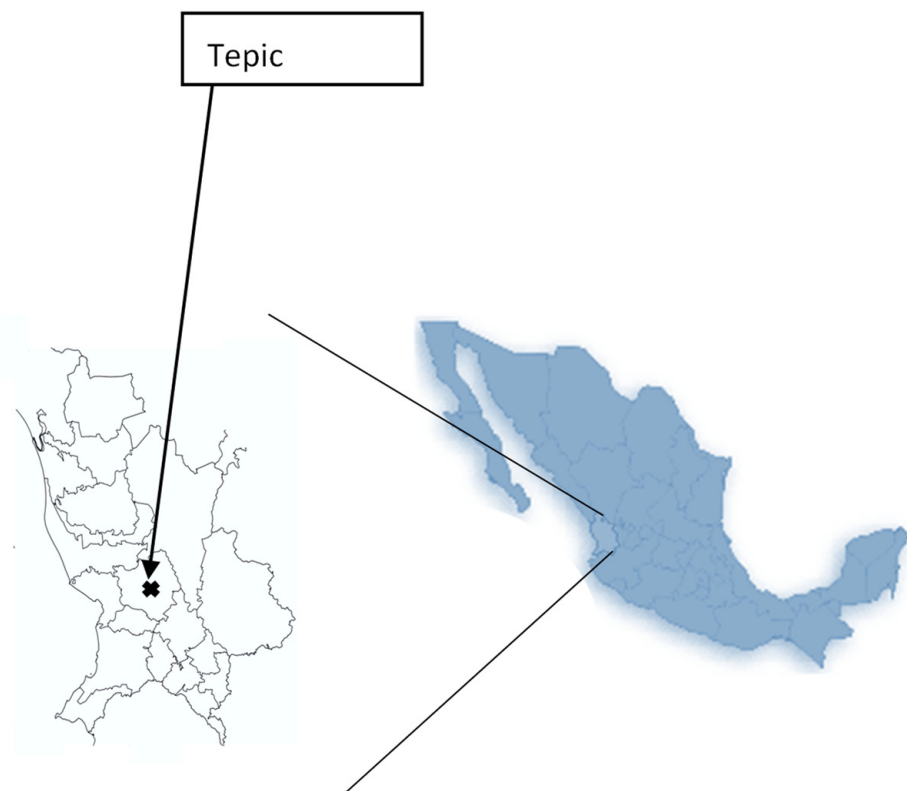
Because bioaerosols are of considerable importance in the food sector, their control is becoming standard practice as an approach to the monitoring of total viable microorganisms. Air monitoring can also be included as part of a hazard analysis and critical control point (HACCP) system in the food industry [5,18].

Microbiological analysis of the air is carried out primarily to determine the microbiological conditions of areas where processing activities are performed and must be under hygiene control to estimate the risk of contamination [19]. Evaluating and maintaining the microbiological control of the air is essential to ensure the quality of the environment and of manufactured products and is an indicator of the hygienic state around the facilities [19–21].

The objective of this study was to analyze the microbiological quality of the air in a popular area of the city of Tepic, Nayarit, where fish processing and marketing activities are carried out for the general population. Information regarding microbiological air conditions in fishery product processing and marketing areas around the world is limited. To date, there are no known microbiological studies of the air in this popular area of the city of Tepic, Nayarit. Thus, this study will allow us to assess the microbiological conditions of the air and whether these conditions may constitute a risk to the health of occupants and consumers and the food quality.

## 2. Materials and Methods

The study was conducted in the central area for the processing and marketing of fishery products, corresponding to the popular market known as the “market of the sea” in the city of Tepic, Nayarit, Mexico (Figure 1). The market’s geographical coordinates are  $21^{\circ}31'25''$  N and  $104^{\circ}53'27''$  W [22]. The study was performed between the months of February and March 2022; samples were collected one day a week for four weeks at 10 a.m. and 11 a.m., concurrent with the usual activities of the processing and marketing area.

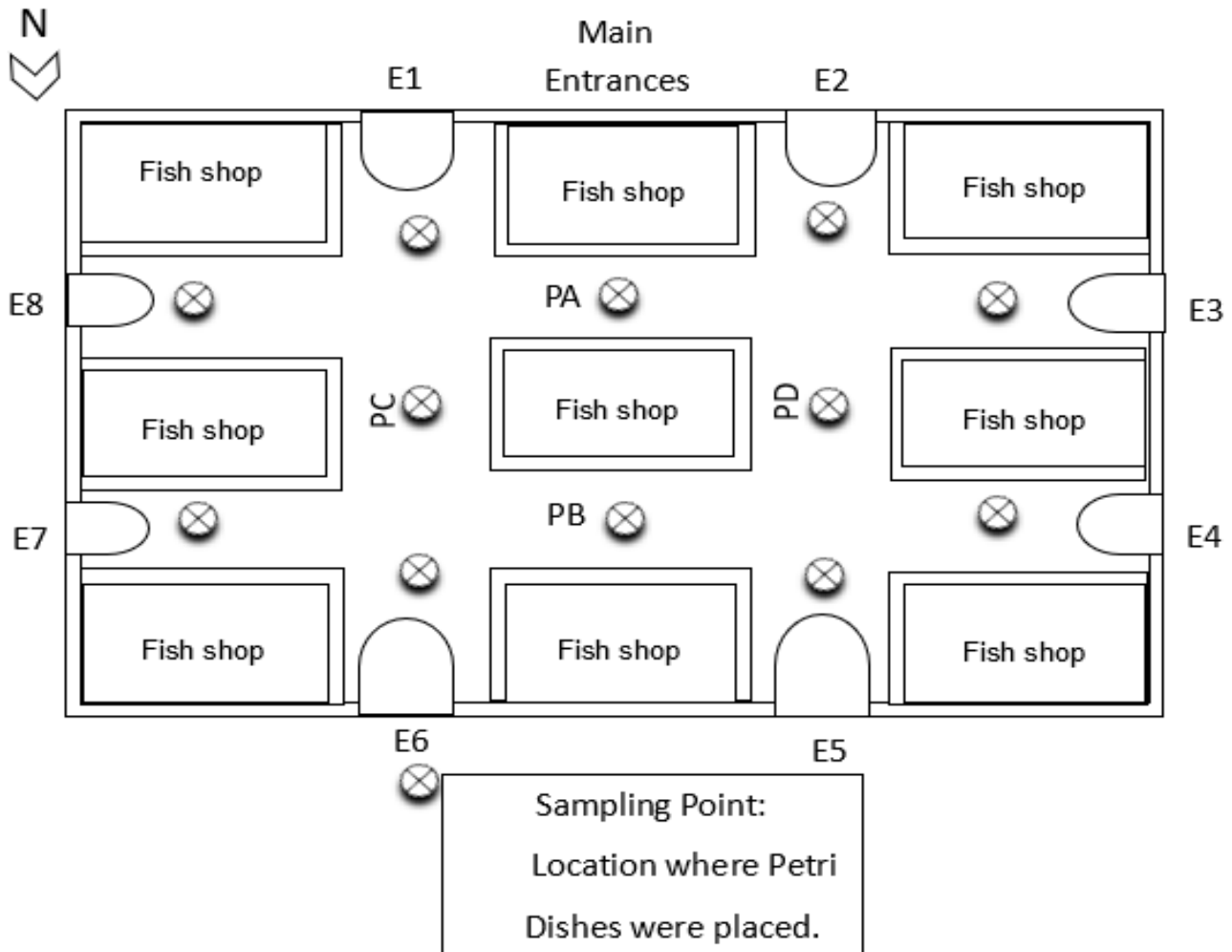


**Figure 1.** Location of the city of Tepic within the state of Nayarit in the Mexican Republic [23].

### 2.1. Air Sampling

Twelve sampling points were selected within the total area of the sea market facilities (Figure 2). At each sampling point, at a height of 1 m, Petri dishes with culture media

were placed in duplicate for the analysis of aerobic mesophiles, coliforms, fungi, and yeast. The Petri dishes were kept open for 1 h and then closed and incubated. During sampling, Petri dishes with closed culture medium were also placed and incubated under similar conditions to act as a control.



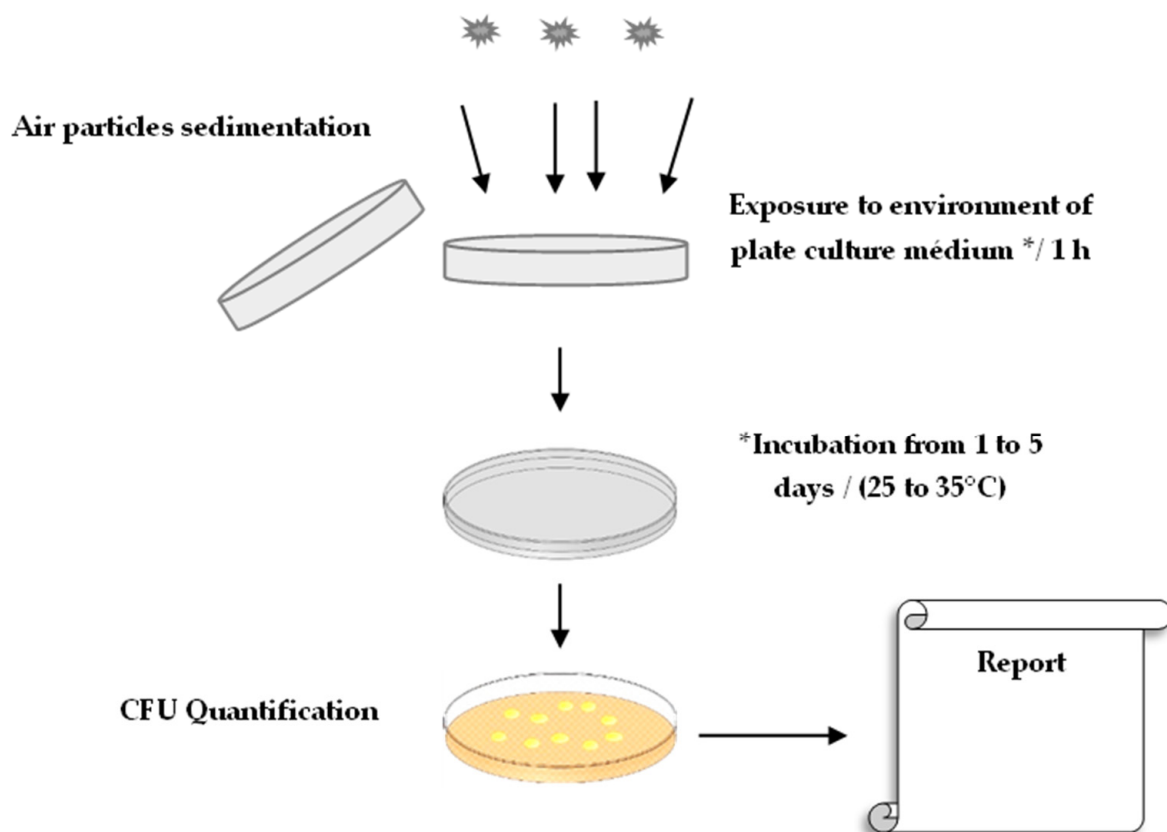
**Figure 2.** Layout of the area for the processing and marketing of fishery products and sampling sites. E1, entrance 1; E2, entrance 2; E3, entrance 3; E4, entrance 4; E5, entrance 5; E6, entrance 6; E7, entrance 7; E8, entrance 8; PA, corridor A; PB, corridor B; PC, corridor C; and PD, corridor D.

## 2.2. Temperature and Humidity Analysis of the Marketing and Processing Area

The measurement of ambient temperature and relative humidity was performed at all the sampling points of the processing and marketing area of the sea market using an HTC-2 thermohydrometer, China. Brand SG.

## 2.3. Microbiological Analysis

The microbiological analysis of the air was conducted using the passive gravity sedimentation method, which consists of viable microorganisms present in the air being transported and deposited onto the surface of a solid culture medium (in a 90 mm diameter Petri dish) by air currents present in the area (Figure 3) [15,19]; Petri dishes were open for 1 h, located at a height of 1 m from the ground and 1 m from walls, and contained agar tryptone–yeast extract for the isolation and quantification of aerobic mesophiles [24], violet red bile lactose agar (RVBA) for coliforms [25], and potato dextrose agar for molds and yeast [26]. The Petri dishes were subsequently incubated at  $35 \pm 2^\circ\text{C}/48\text{ h}$  for aerobic mesophiles,  $35 \pm 2^\circ\text{C}/24\text{ h}$  for coliforms, and  $25 \pm 1^\circ\text{C}/5\text{ d}$  for molds and yeast [19,24–26].



**Figure 3.** Passive plate sedimentation method for analysis of microbiological air quality [19].

\* AM, aerobic mesophiles; TC, total coliforms; MY, molds and yeasts.

At the end of incubation, the colonies were counted and reported as colony forming units (CFU)/m<sup>3</sup>/h of air according to the following equation:

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

where  $N$  is the microbial CFU/m<sup>3</sup> of indoor air,  $a$  is the number of colonies per Petri dish,  $b$  is the dish surface (cm<sup>2</sup>), and  $t$  is the exposure time (min) [15,18,27].

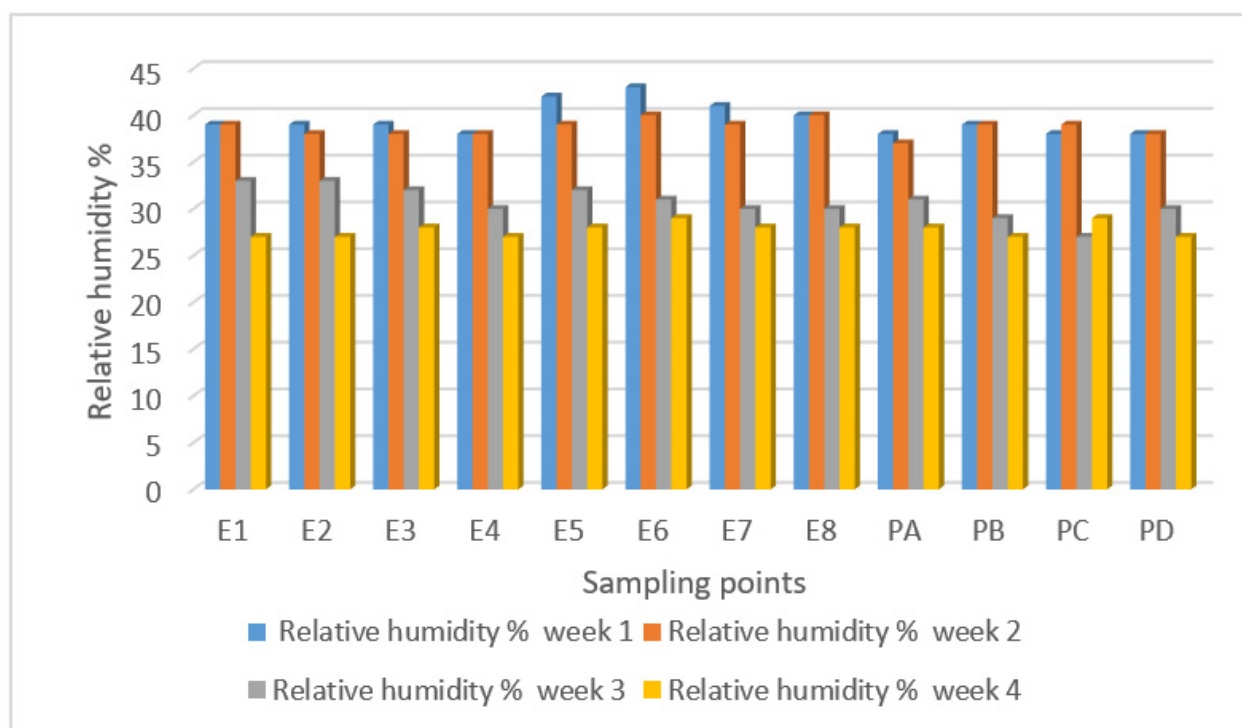
#### 2.4. Data Analysis

Data analysis was performed using Microsoft Excel for Windows version 15 (2013).

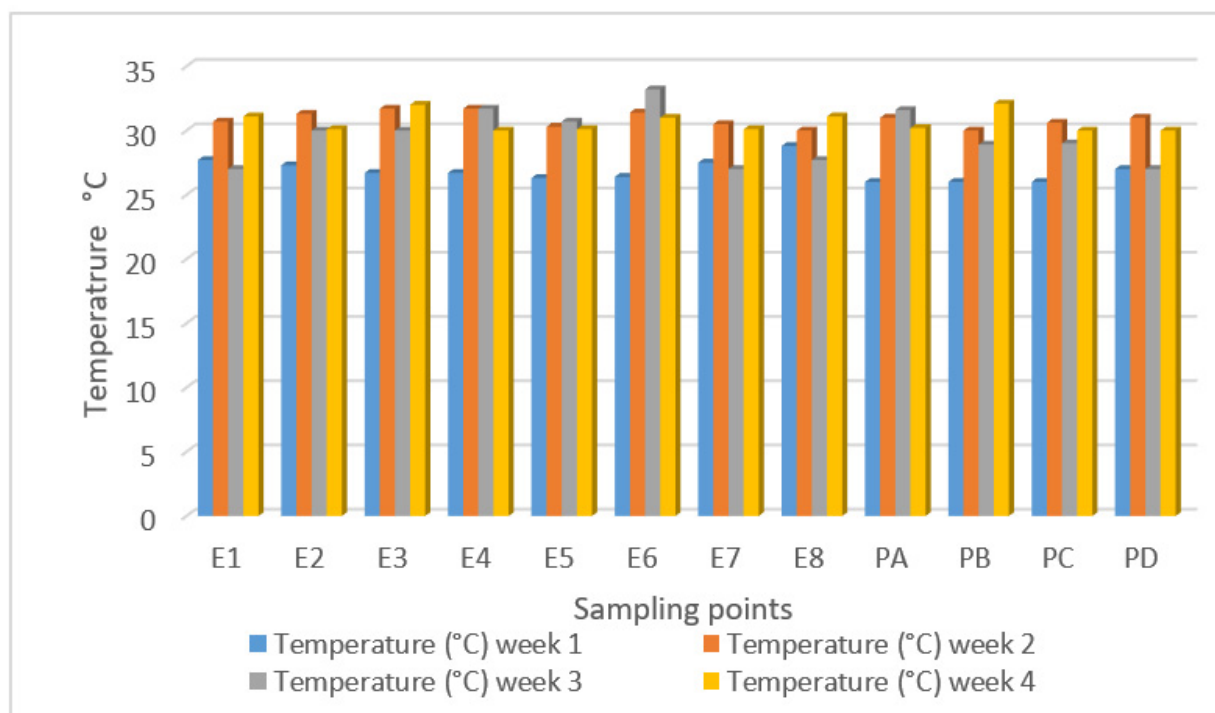
### 3. Results and Discussion

#### 3.1. Relative Humidity and Temperature in the Fish Processing and Marketing Areas

During the four weeks of the study, the temperature and relative humidity of the fishery product processing and marketing area were assessed at each sampling point, with the following results: in week 1, the average ambient temperature was  $26.8 \pm 0.8$  °C and the relative humidity was  $39.5 \pm 1.6\%$ ; in week 2, the temperature was  $30.8 \pm 0.5$  °C and the relative humidity was  $38.6 \pm 0.8\%$ ; in week 3, the temperature was  $29.4 \pm 2$  °C and the relative humidity was  $30.6 \pm 1.7\%$ ; and in week 4, the temperature was  $0.6 \pm 0.7$  °C and the relative humidity was  $27.7 \pm 0.7\%$ . The highest average relative humidity and the lowest average environmental temperature were observed in the first week of the study. Point E6 in week 1 had the highest relative humidity (43%); the lowest values were recorded at PA and PB in week 3, with 29 and 27%, respectively (Figure 4). The highest environmental temperature was recorded at point E6 in week 3 (33.3 °C), and the lowest temperature (23 °C) was recorded at sampling points PA, PB, and PC in week 1 (Figure 5).



**Figure 4.** Analysis of relative air humidity at each sampling point in processing and marketing areas. E1, entrance 1; E2, entrance 2; E3, entrance 3; E4, entrance 4; E5, entrance 5; E6, entrance 6; E7, entrance 7; E8, entrance 8; PA, corridor A; PB, corridor B; PC, corridor C; and PD, corridor D.



**Figure 5.** Environmental temperature analysis at each sampling point in the fish processing and marketing area. E1, entrance 1; E2, entrance 2; E3, entrance 3; E4, entrance 4; E5, entrance 5; E6, entrance 6; E7, entrance 7; E8, entrance 8; PA, corridor A; PB, corridor B; PC, corridor C; and PD, corridor D.



The state of Nayarit generally has a warm, subhumid climate with an average annual temperature of 25 °C, a minimum average temperature of 12 °C in January, and an average maximum temperature slightly above 35 °C during the months of May and June [28]. Environmental factors such as seasonality, temperature, humidity, oxygen, organic matter, radiation (exposure to sunlight), ions, pressure, etc., influence the viability, distribution, and number of microorganisms present; the number of microorganisms in the air in inhabited areas is greater than that in less inhabited areas and is a function of the local activities (industrial, agricultural, or livestock) and the presence of living organisms and dust [11,13,29,30].

The physical–chemical conditions in the air do not favor the growth and survival of microorganisms, so they are viable for only a short period of time; relative humidity and temperature have an impact on microbial survival because, as they decrease, the water available for microorganisms also decreases, leading to their inactivation [11]. Relative humidity is also considered an important factor for health because it affects the respiratory tracts of humans and other animals, and the infectiousness of pathogens in the air depends on this parameter [13].

In this study, the proportions of analyzed microorganisms (aerobic mesophiles, coliforms, molds and yeast) during the four weeks were generally high at all sampling points for weeks 1 and 2; the respective numbers in weeks 1 and 2 ranged from 2.95 to 2.66 log CFU/m<sup>3</sup>/h and 2.82 to 2.61 log CFU/m<sup>3</sup>/h for aerobic mesophiles; from 1.68 to 1.06 log CFU/m<sup>3</sup>/h and 1.80 to 0.7 log CFU/m<sup>3</sup>/h for coliforms; from 2.56 to 1.42 log CFU/m<sup>3</sup>/h and 2.75 to 1.90 log CFU/m<sup>3</sup>/h for molds, and from 2.11 to 1.63 log CFU/m<sup>3</sup>/h and 1.82 to 1.33 log CFU/m<sup>3</sup>/h for yeast. The high values obtained for the different microbial groups (aerobic mesophiles, coliforms, molds and yeast) in weeks 1 and 2 were related to the higher relative humidity of the air, which ranged between 37 and 43%. Therefore, this factor may influence the presence and proportion of microorganisms in the fish processing and marketing area.

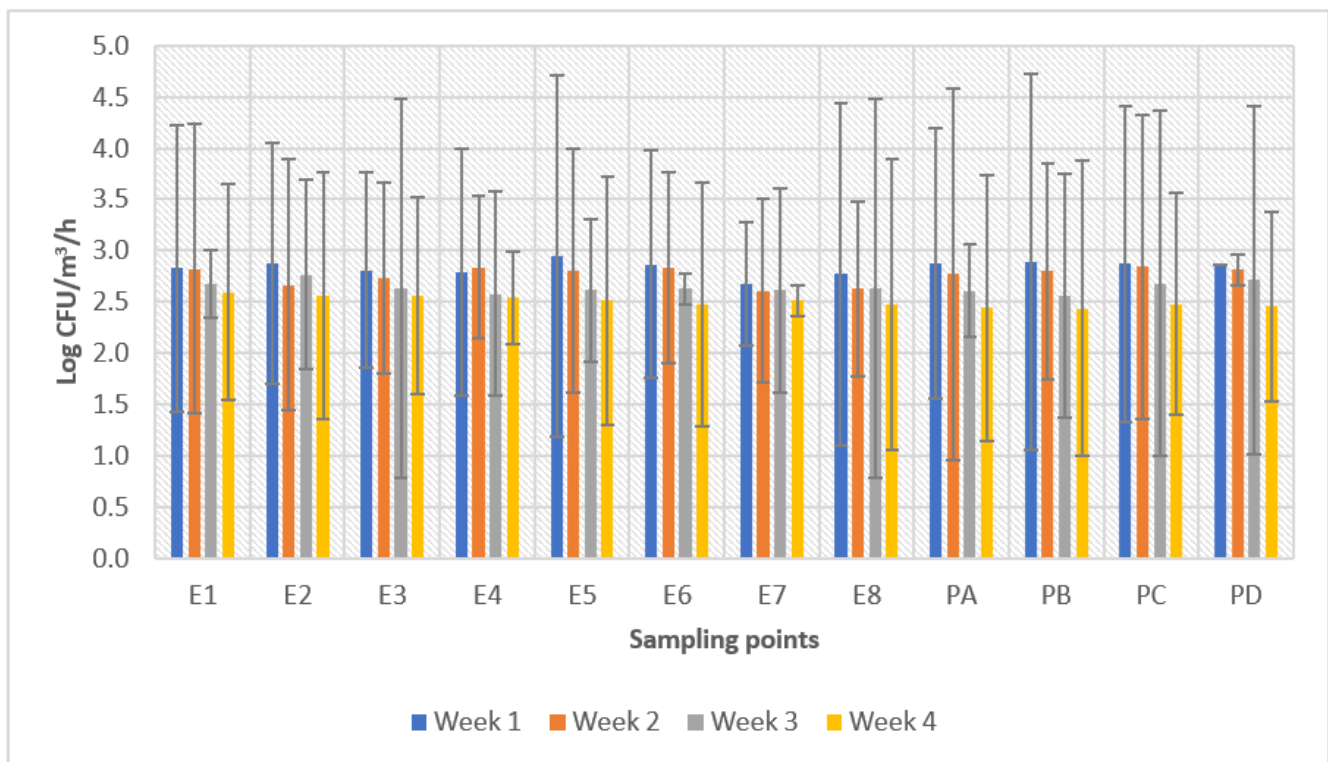
The preparation of some food products requires control over the quality of the ambient air to reduce the possibility of contamination. Thus, it is necessary to monitor the air that is in contact with food products. Studies related to the control of air properties, primarily parameters such as temperature and humidity, can potentially reduce the presence and growth of some microorganisms in manufacturing and storage areas, although studies have recognized that the geographical area and time of the year have a qualitative and quantitative influence on the microbial results [18].

### 3.2. Aerobic Mesophiles

In general, the highest values of mesophilic aerobic bacteria were found in the first week at all sampling points, particularly at point E5 (2.95 log CFU/m<sup>3</sup>/h), followed by point PB (2.89 log CFU/m<sup>3</sup>/h), in week 1; the PC point had the highest values (2.84 log CFU/m<sup>3</sup>/h) in week 2, the E2 point had the highest values in week 3 (2.77 log CFU/m<sup>3</sup>/h), and the E1 point had the highest values in week 4 (2.59 log CFU/m<sup>3</sup>/h). In week 4, the lowest values were observed at all the sampling points, and the lowest value was at the PB point (2.44 log CFU/m<sup>3</sup>/h) (Figure 6).

In the food industry, the presence of aerobic mesophiles is an indicator of the hygienic quality, handling conditions, and shelf life of foods, although a low proportion of aerobic mesophiles does not imply or ensure the absence of pathogens or toxins, and a high count does not demonstrate the presence of pathogens [31].

Microorganisms such as fungi and bacteria can be airborne as vegetative cells or spores [32]. For bacteria, survival is variable and is related to their structural and metabolic diversity, with Gram-positive bacteria often more resistant than Gram-negative bacteria (enterobacteria or coliforms) due to the composition of their cell wall; however, unlike the vegetative forms, spores exhibit higher survival in adverse conditions due to their proportion, low metabolism, and composition, and they do not require external nutrients or water for their long-term survival [11,32].



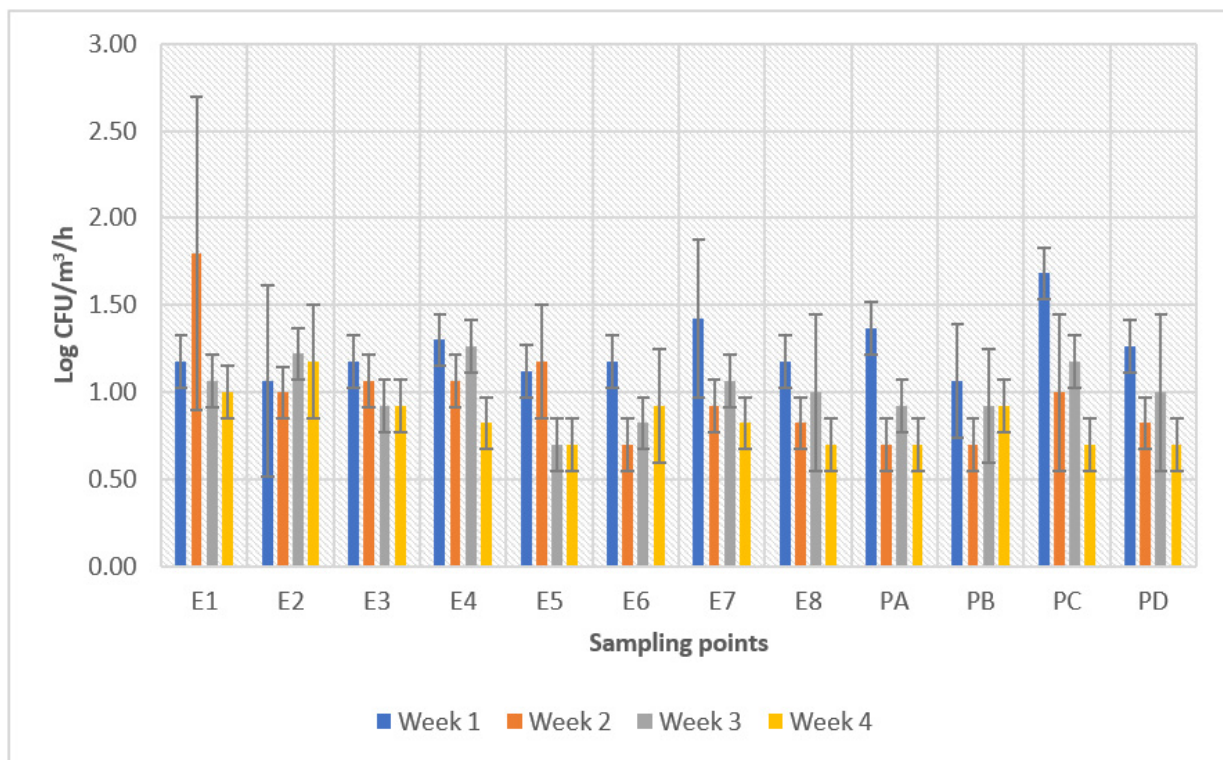
**Figure 6.** Analysis of aerobic mesophiles in the air by sampling point for 4 weeks in a popular fish processing and marketing area. E1, entrance 1; E2, entrance 2; E3, entrance 3; E4, entrance 4; E5, entrance 5; E6, entrance 6; E7, entrance 7; E8, entrance 8; PA, corridor A; PB, corridor B; PC, corridor C; and PD, corridor D.

For this study of aerobic mesophiles, the colonies that grew in the culture media frequently presented a response to Gram staining, mainly Gram-positive bacilli and cocci, as well as a positive response to the biochemical catalase test, which is consistent with reports on various microbiological analyses of the air [5,14,31]. It has been reported that the bacteria common in indoor air are saprophytic bacteria from the human skin, mouth, and nose that are emitted into the air; among the bacteria reported are micrococci, staphylococci, streptococci, and corynebacteria. Further reported are bacteria that grow in pipes, water tanks, refrigeration systems, or wet areas of a building, such as bathroom sinks (*Legionella* and mycobacteria), as well as Actinobacteria and species of *Clostridium*, *Bacillus*, and fungi that grow on moist building materials [8,32].

### 3.3. Coliforms

The coliform analysis at the different sampling points in the fish processing and marketing area showed that different numbers were obtained during the 4 weeks of analysis, with a maximum value of 1.80 log CFU/m<sup>3</sup>/h and a minimum value of 0.7 log CFU/m<sup>3</sup>/h. The highest coliform value of the 12 sampling points during the four weeks was at E1 in week 2 (1.80 CFU/m<sup>3</sup>/h), followed by the PC point (1.68 log CFU/m<sup>3</sup>/h) and the E7 point (1.42 log CFU/m<sup>3</sup>/h) in week 1. The lowest values of the 12 sampling points over the 4 weeks of the study were in week 4 at points E5, E8, PA, PC, and PD (0.7 log CFU/m<sup>3</sup>/h) (Figure 7).





**Figure 7.** Analysis of coliforms in the air by sampling point during 4 weeks in a popular fish processing and marketing area. E1, entrance 1; E2, entrance 2; E3, entrance 3; E4, entrance 4; E5, entrance 5; E6, entrance 6; E7, entrance 7; E8, entrance 8; PA, corridor A; PB, corridor B; PC, corridor C; and PD, corridor D.

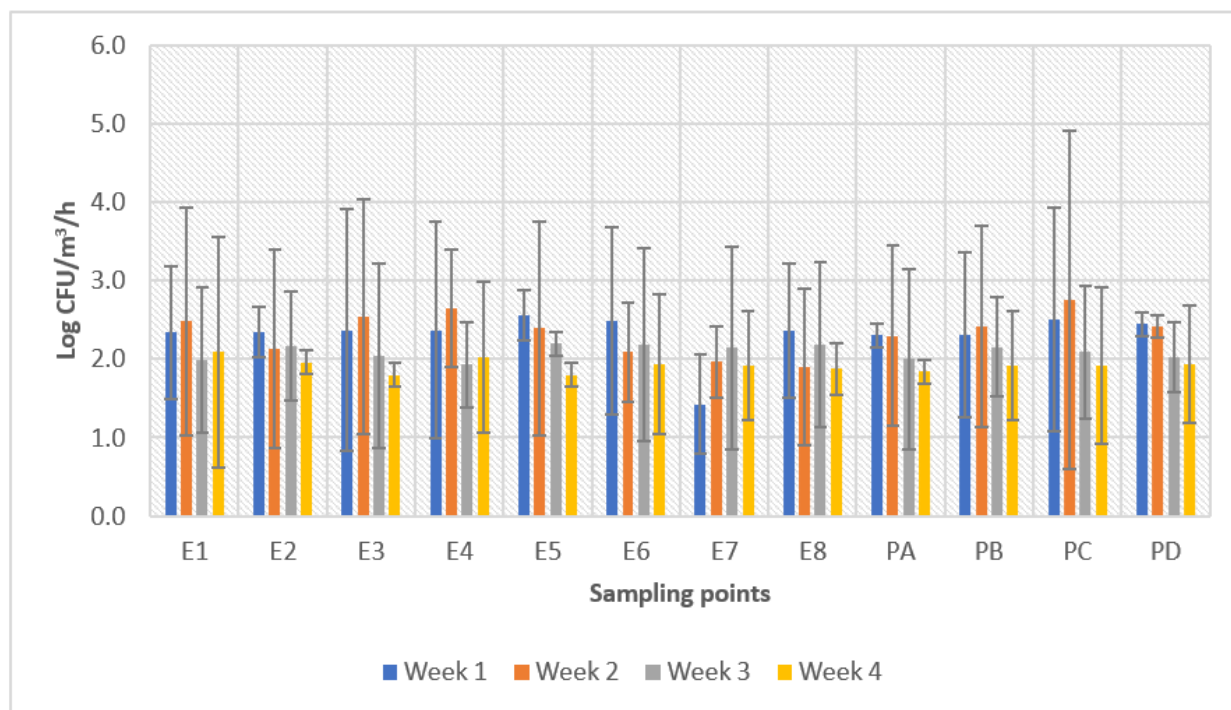
The coliforms are frequently used in the food production environment as an indicator of microbiological quality, as well as the hygienic conditions and practices to which food is subjected during processing and handling [26]. Coliforms are widespread in the environment; however, they are also found in greater proportions in the intestinal tract and animal feces, so their presence can be related to contamination of fecal origin. Moldoveanu [8] points out that the presence of Gram-negative bacteria or enterobacteria in indoor air is due to the presence of water inside, possibly from sewage leakages.

### 3.4. Molds and Yeast

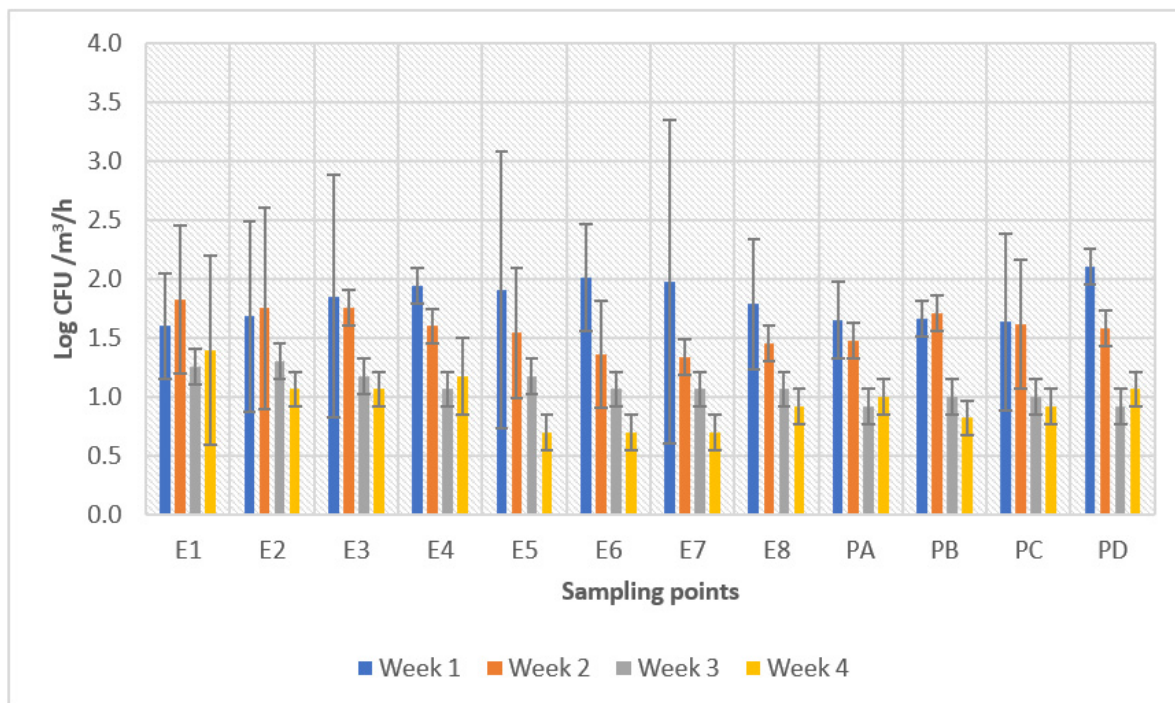
The analysis of molds in the air at different sampling points during the 4 weeks of the study showed a maximum value of 2.75 log CFU/m<sup>3</sup>/h and a minimum value of 1.42 log CFU/m<sup>3</sup>/h. The highest count among the 12 sampling points was at sampling point PC (2.75 log CFU/m<sup>3</sup>/h), followed by point E4 (2.65 log CFU/m<sup>3</sup>/h) in week 2. The lowest counts among all sampling points were obtained at point E7 in week 1 (1.42 log CFU/m<sup>3</sup>/h). Week 4 had the lowest counts at all sampling points (E2, E3, E5, E6, E8, PA, PB, PC, and PD) (Figure 8).

In the analysis of yeast during the 4 weeks of the study at the 12 different sampling points, values ranged from a maximum of 2.11 log CFU/m<sup>3</sup>/h to a minimum of 0.7 log CFU/m<sup>3</sup>/h.

The highest values were at the PD sampling point (2.11 log CFU/m<sup>3</sup>/h), followed by points E6 (2.01 CFU/m<sup>3</sup>/h), E7 (1.98 CFU/m<sup>3</sup>/h), E4 (1.94 CFU/m<sup>3</sup>/h), E5 (1.91 CFU/m<sup>3</sup>/h), and E3 (1.85 CFU/m<sup>3</sup>/h) in week 1 and sampling point E1 (log 1.82 CFU/m<sup>3</sup>/h) in week 2. During week 4, sampling points E5, E6, and E7 had the lowest counts among all the sampling points (0.7 log CFU/m<sup>3</sup>/h) (Figure 9).

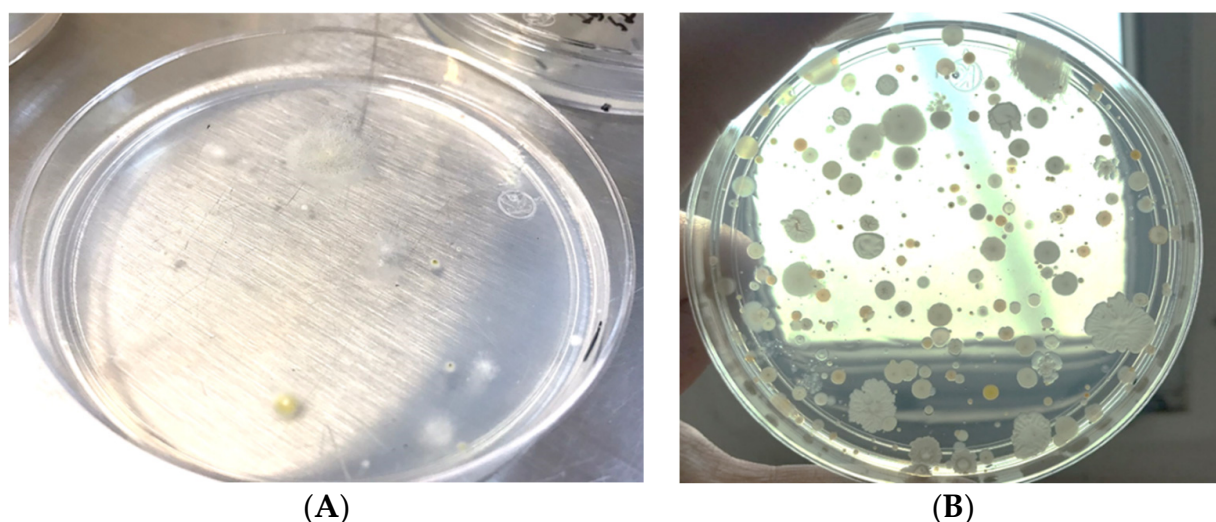


**Figure 8.** Analysis of molds in the air by sampling point over 4 weeks in a popular fish processing and marketing area. E1, entrance 1; E2, entrance 2; E3, entrance 3; E4, entrance 4; E5, entrance 5; E6, entrance 6; E7, entrance 7; E8, entrance 8; PA, corridor A; PB, corridor B; PC, corridor C; and PD, corridor D.



**Figure 9.** Analysis of yeasts in the air by sampling point over 4 weeks in a popular fish processing and marketing area. E1, entrance 1; E2, entrance 2; E3, entrance 3; E4, entrance 4; E5, entrance 5; E6, entrance; E7, entrance 7; E8, entrance 8; PA, corridor A; PB, corridor B; PC, corridor C; and PD, corridor D.

Concern regarding the presence of molds and yeasts in food or production environments is due to the ability of these microorganisms to contaminate these environments and produce different degrees of deterioration and decomposition (Figure 10A). Fungi can produce mycotoxins during their growth in food, which are not destroyed during food processing and are responsible for poisoning, with serious consequences for the health of consumers; the inhalation of mycotoxins has been considered to be even more dangerous than their consumption. In addition, these microorganisms are associated with allergic reactions and infections, mainly in immunocompromised populations, elderly individuals, and children [16,32–34].



**Figure 10.** Petri dishes with growth of molds and yeasts (A) and Petri dishes with growth of aerobic mesophilic bacteria (B).

In the food industry, bioaerosols are generally a mixture of many species of microorganisms, including bacterial endospores and exospores, such as members of the genera *Bacillus* and *Clostridium*; vegetative cells, mainly of Gram-positive bacteria, including *Micrococcus* and *Staphylococcus*; mold species of the genera *Aspergillus*, *Trichoderma*, *Penicillium*, *Cladosporium*, *Alternaria*, *Mucor*, and *Fusarium*; and yeasts, including *Saccharomyces*, *Torulaspora*, *Hanseniaspora*, and *Pichia* [5,18,30].

The microbiological analysis of the air can be conducted by two methods: the traditional culture-dependent method using culture media (liquid or solid) and culture-independent molecular methods based on the polymerase chain reaction (PCR). The choice of the analytical method is based on factors such as cost, analysis time, sensitivity, specificity, and sampling method [5,7].

Sampling for the microbiological evaluation of the air can be conducted using active or passive methods. The active or volumetric methods use devices that sample a defined volume of air, and the colony forming units (CFU) present are counted. The passive or sedimentation method in Petri dishes uses deposition on the surface of a solid medium by the air currents present in the study area [19].

Traditionally, culture-based methods for determining airborne microbiological concentrations prevail in the food industry. Frequently, the selection of general media is preferred for the characterization of the aerial microbiota because it favors the growth of a wide variety of species. However, the simultaneous sampling of various types of microorganisms (bacteria and fungi) using a single culture medium may not be satisfactory [5]. Among the advantages of plate culture methods is that they are cheap, simple, and relatively rapid, and they count the viable microorganisms [5,19]. However, the disadvantage of culture and plate count methods is that they only consider a part of the microbial population, because some microorganisms may be in a viable but nonculturable state (VBNC) due to



stress conditions. Despite this disadvantage, the plate count method is considered the gold standard in food microbiology [5].

Mansilla [31] conducted an air microbiology study of the indoor areas of school canteens (entrance, yard, and kitchen) used in food processing, handling, and service during the months of June, August, and October 2019 during regular activities, and they used the active or volumetric sampling method through repeated aspirations of air with a sterile syringe and subsequent incorporation into liquid culture medium (BHI). Their study reported an average of  $272 \times 10^3$  CFU/cm<sup>3</sup> at the point of entry, followed by the patio ( $141 \times 10^3$  CFU/cm<sup>3</sup>) and finally the kitchen ( $14 \times 10^3$  CFU/cm<sup>3</sup>). In all scenarios, the main taxon identified was enterobacteria, which are common inhabitants of the gastrointestinal tracts of animals and humans and thus a potential risk to the health of occupants and for food contamination.

Solano [35] conducted a mycological and relative humidity analysis of the air at eight points in the historic center of the city of Lambayeque, Peru, during its greatest influx of people. Using the passive sedimentation method for 10 min and Petri dishes 10 cm in diameter, concentrations were reported that ranged between 349 and 5026 CFU/m<sup>3</sup>. The study noted that the highest proportion of microorganisms was related to the high relative humidity (85%) and the influx of people, and these factors influenced the distribution, viability, and proportion of microorganisms in the air, creating a risk to public health and the need to implement actions to contain the impacts of their presence, as they could contaminate the food presented in nearby markets.

Finally, Anaya et al. [18] analyzed the relative humidity and microbiological quality of the air in various areas (processing, packaging, and storage) in two food production plants, one for artisanal chocolate (ACP) and another for diet products (SRP), in Cuba, during regular hours of industrial activity every 25 d between March and November. Using the sedimentation method in 90-cm-diameter Petri dishes exposed for 1 h, the microbial concentration ranged from 0 to 1507 CFU/m<sup>3</sup> and the relative humidity ranged from 60 to 95% for ACP, whereas the microbial concentration ranged from 39 to 1638 CFU/m<sup>3</sup> and the relative humidity ranged from 57 to 92% for SRP. The study concluded that the sampled areas presented high humidity levels that favored microbial development and contamination, and although the average microbial concentration detected was classified as low contamination in some areas, an adequate design for the construction of production factories, as well as reviewing and strengthening sanitary activities and the use and correct location of dehumidifiers, are essential to avoid contamination, spread, and health risks caused by microorganisms through food.

The results obtained in this study are consistent with those reported by other researchers. In all cases, the importance of microbiological monitoring of the air of the immediate environment where food is produced and shipped is highlighted. Across the different analysis methods, there is an emphasis on the relationship between the microbiological quality of the air, the environmental conditions (humidity), and the cleanliness of the study area and its effect on food safety and public health.

Microorganisms in the environment are an invisible hazard, and they spread contamination and increase the risk of contracting diseases. Because the levels of microorganisms depend on many variables, there are currently no universally accepted specific levels of CFU/m<sup>3</sup> [21]. Various researchers, along with international organizations, have proposed and recommended microbiological limits for the air. Borrego et al. [14] and Alonso and Amistad [36] indicated that a concentration of microorganisms exceeding 500 CFU/m<sup>3</sup> is considered a contaminated environment and a risk to the health of occupants. Mansilla [31] stated that the concentration of fungi must be less than 500 CFU/m<sup>3</sup> for the environment to be satisfactory, whereas, for bacteria, a concentration greater than 1000 CFU/m<sup>3</sup> in the air is considered contaminated, and, above 1500 CFU/m<sup>3</sup>, the environment is considered highly polluted.

Moldoveanu [8] points out that levels of bacterial concentrations less than 1000 CFU/m<sup>3</sup> in indoor air can be considered low and concentrations of more than 5000 CFU/m<sup>3</sup> can be

considered high; these numbers reflect the density or occupation of the interior of a facility and ventilation efficiency and are a measure of hygienic air quality.

The Environmental Protection Administration in Taiwan states that the concentration of bacteria in the air of sampled rooms should not be greater than 1500 CFU/m<sup>3</sup>, and the Department of Environmental Protection in Hong Kong states that the concentration of microorganisms in the air of offices and public places should not exceed 500 CFU/m<sup>3</sup> [37]. The World Health Organization (WHO) states that, for indoor environments, a concentration of fungal microorganisms greater than 1000 CFU/m<sup>3</sup> is considered to indicate contamination. In Brazil, the limit is 700 CFU/m<sup>3</sup>, and in the United States, the American Conference of Industrial Hygienists and the US Public Health Service indicate that levels above 200 CFU/m<sup>3</sup> represent a health risk [36].

To determine the environmental quality in a work area, microbiological limits must be established to determine the optimal cleaning conditions. Currently, in the food industry, there is no agreement or universal regulation that determines these limits. Therefore, regarding fungal organisms, it has been established that values of 101 to 140 CFU/m<sup>3</sup> are acceptable for healthy environments, and higher values are considered to indicate contaminated environments [38]. However, studies such as Salustiano et al. [30] report that, for the internal environments of food processing areas, such as dairies, they recommend 180 to 360 CFU/m<sup>3</sup> for aerobic mesophiles and 70 to 430 CFU/m<sup>3</sup> for fungi and yeast.

To prevent infections in high-risk internal environments such as hospitals, the WHO has suggested that the concentration of microorganisms in common interior areas of hospitals should be less than 300 CFU/m<sup>3</sup>. In areas occupied by individuals with compromised immune systems, the concentration should be less than 100 CFU/m<sup>3</sup>; the colony forming unit counting method is the most used and practical method to quantify microorganisms in the air [21].

In the present study, the counts for aerobic mesophiles ranged from 273 to 892 CFU/m<sup>3</sup>/h, coliforms ranged from 5 to 63 CFU/m<sup>3</sup>/h, fungi ranged from 542 to 568 CFU/m<sup>3</sup>/h, and yeast ranged from 122 to 127 CFU/m<sup>3</sup>/h during the four weeks of the study. The quantification of the different microbial groups in the fish processing and marketing area implies poor air quality and risks to the health of occupants and food contamination (Figure 10 B). Salustiano et al. [30] noted that the different proportions of microbial groups in the air at the various points analyzed may be due to the aerodynamic behavior of their bioaerosols, which are influenced by physical and biological characteristics such as particle diameter, humidity, temperature, ventilation, personnel activities in the area, and gravitational and electrostatic forces.

The air in food processing areas can be contaminated through sources of aerosols such as drains, personnel or handlers, ventilation systems, and water when applied under pressure in cleaning processes [30]. The safety and quality of fish are important throughout the production chain, because contamination by microorganisms, in combination with the extrinsic and intrinsic properties of the food, may greatly impact the degree of deterioration, health risk, and depreciation or rejection of products [1,29].

The analysis of the microbiological quality of the air has economic, social, and health impacts [18]. The monitoring of the microbiological quality of the air in food processing facilities is considered a basic point for safety management in the food industry; this contributes to the implementation of a proactive action to reduce sources of contamination (air, water, surfaces, handlers, raw materials, and supplies) of products by agents responsible for deterioration (disposal due to poor condition), reduce foodborne illnesses, improve compliance with legal requirements or guidelines that establish that the air in the food sector must be controlled, validate cleaning and disinfection processes and conditions in certain areas, generate a history of air quality parameters and trends, identify potential sources of new contamination and take corrective and control measures, and collect epidemiological data to establish exposure limits [5,18,20,39].

The prevention or control of indoor air pollution, mainly in food handling areas and areas requiring a low microbial concentration, should consider the adequate design or re-

modeling of facilities involving manufacturing practices, adequate ventilation, recirculation and internal renewal of air, occupant density, surveillance of the surrounding environments, control of contamination sources (presence of animals, pests, and organic waste), hygiene and disinfection processes, and the implementation of regular environmental microbiological monitoring of areas using general bioindicators such as aerobic mesophiles, fungi, and yeast [8,13,29,30,40,41].

#### 4. Conclusions

The microbiological indicators of mesophilic aerobes, coliforms, fungi, and yeast were determined, and their presence was quantified in the air in a popular fish processing and marketing area; throughout the study period and across sampling points, the highest counts were found for aerobic mesophiles (2.95–2.44 log CFU/m<sup>3</sup>/h), followed by molds (2.75–1.42 CFU/m<sup>3</sup>/h), yeasts (2.11–0.7 log CFU/m<sup>3</sup>/h), and coliforms (1.80–0.7 log CFU/m<sup>3</sup>/h). The presence of aerobic mesophiles and molds indicates air contamination in the fish processing and marketing area, exceeding the recommended microbiological limits at some sample points. The values obtained demonstrate the exposure to the microbiological load of the air in the fish processing and marketing area, and this may be considered a risk to the health of occupants and for food contamination.

In the area of fish processing and marketing, it is recommended to implement regular pest and animal control actions, cleaning, disinfection, maintenance of premises and facilities, and adequate ventilation systems (avoiding high relative humidity and condensation) to control and reduce microbial populations in the air, which lead to health risks for occupants and food contamination. Thus, the analysis and regular monitoring of the microbiological quality of the air in the food industry, within a total management system focused on the quality and safety control of the products, must be focused on detecting, controlling, and reducing contamination and health hazards for users and consumers through inhalation and food consumption.

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