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Isolation and Diagnosis of Bacteria and Fungi from Some Areas of Tikrit and Some Villages

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Abstract: ***The purpose of the study was to evaluate the amount of air pollution caused by bacteria and fungi in specific areas of Tikrit, including the Al-Alam area, Al-Bu Ajil, Al-Buhyazaa, and the village of Al-Karaat. The study was conducted in November and January of 2021–2022, using petri dishes filled with nutrient agar (potato dextrose agar, or PDA) distributed throughout the study areas. The petri dish was left open for fifteen minutes before being closed and incubated with an incubator. After that, the dish was closed and the species was allowed to grow on the media, including isolates and differential media like Macconkey agar and Eosin Methylene Blue (EMB). For microscopical diagnosis, gram stain was applied to the bacterial samples, and fungal stain Lactophenol was used for fungus isolates. The findings of the current study showed that the proportion of Penicillium sp colonies colonized agricultural areas was twice as high, particularly in the Albu-Ajil area, where the dish G2.1 had more than 96 colonies, which was twice or three times more than the rest of the petri dishes in the study area for remote areas. This is because the area had ideal growth conditions in terms of temperature, humidity, and other factorsThe analysis revealed that the majority of bacteria were negative, particularly those belonging to the genus Klebsiella sp. which showed that it could grow on a Macconkey agar dish in Tikrit, the village of Al-Karaat, and Al-Alam. This suggests that it can grow in a variety of environments. However, in the Albu Hayaza area, no bacterial growth was found on the Macconkey agar dish, which was designated with the symbol 3.1 H. This could be because the environmental conditions at the time were insufficient for bacterial growth.***

Keyword: Isolation, Diagnosis, Bacteria and Villages.

1. INTRODUCTION

The common environmental pollutants found in the air are chemicals, particles, or biological materials that have an impact on human and other organism health (Obanya et al., 2018).

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Furthermore, indoor sources of air pollution include hairspray, room deodorizers, photocopiers, paints, solvents, printers, computers, and air purifiers (Rylander, 2004).
Due to people's excessive time spent at home and at work, household air pollution has a negative impact on health (Chao et al., 2003; Molhave, 2011).

Many microbes, the majority of which are pathogenic, proliferate in the human environment. While some bacteria were not infectious when they were small, they have the potential to cause infections when they grow to optimal levels. These bacteria are known as opportunistic bacteria (Hancpck et al., 2010).

2. RELATED WORKS

Many of the fungi that cause allergies in humans are classified as Ascomycota, Basidiomycota, or yeast (Khan et al., 2009). These fungi include Aspergillus, Rhizopus, Penicillium, Cladosporium, and Alternaria, and they can grow in the air and on surfaces made of natural and artificial materials. They are present throughout the year, but are most prevalent in the fall (Sailer et al., 2010; Shirakawa et al., 2011). Moreover, temperature, humidity, air movement, and air exchange rate are the primary environmental factors that support the development and reproduction of airborne microorganisms (Meadow et al., 2014).

The Aim of this Study:
The purpose of this study was to evaluate the amount of fungi and bacteria that are causing air pollution in some locations of Tikrit, including the Al-Alam region, Al-Bu Ajil, Al-Buhyazaa, and the town of Al-Karaat.

3. METHODOLOGY

Material and Methods
The biology labs at Tikrit University in Iraq's College of Science conducted the current study. The purpose of the study was to evaluate the amount of bacteria and fungi that cause air pollution in a few different areas of Tikrit, including the Al-Alam area, Al-Bu Ajil, Al-Buhyazaa, and the village of Al-Karaat. Samples for the study were first collected in November and January of 2021–2022.

1. Preparation of Culture
The media are prepared in accordance with the manufacturer's instructions, which are attached to the packages. They are then sterilized for 20 minutes at 121 degrees Celsius, 15 percent pressure, and poured into petri dishes. After that, they are incubated for 24 hours at 37 degrees Celsius to make sure they are not contaminated, and they are stored in the refrigerator at 4 degrees Celsius until they are needed (Ahmed, 2020).

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2. Macconkey Agar:
To prepare this medium, dissolve 51.5 g of MacConkey agar in 1 liter of distilled water. Transfer the mixture into a sterilizing container. Distribute the mixture into petri dishes and store in the refrigerator at 4 oC until needed (Ahmed, 2020).

3. Nutrinet Agar:
To prepare this media, dissolve 28 grams in one liter of distilled water, put it in a sterilizing container, pour it into sterile Petri dishes, and store it in the refrigerator until needed (Ahmed, 2020).

4. Eosin Methylene Blue:
As directed by the manufacturer, 35.96 g of this medium are dissolved in one liter of distilled water to prepare it. It is then transferred into a sterilizing container, distributed among plates, and kept in the refrigerator until needed.

5. Potato Dextrose Agar:
This medium is made by dissolving 39 grams of it in one liter of distilled water, then putting it in an antibiotic to sterilize it and an antibiotic—such as an anti-tetracycline—with the medium. After that, the dishes are distributed, the incubator is used to make sure there is no contamination, and the media is refrigerated until needed (Ahmed, 2020).

Sterilization:
All culture media is sterilized by autoclave at a temperature of 121°C \ 15 bar for 15 minutes.

Sample Collection
In order to determine the kind and quantity of pollutants present in the atmosphere of the study areas, Nutrient Agar, Potato Agar, and Potato Dextrose Agar media were distributed throughout the villages of Al-Karaat, Tikrit, Al-Alam, Al-Bu Ajil, and Al-Buhyazaa. The petri dish was then closed and placed in the incubator after being left open for 15 minutes. The number of colonies that grew on the aforementioned dishes was calculated by counting them and recording the growth.

Samples Culture:
After the isolates from the study areas were gathered, they were cultivated on eosin methylene blue agar, MacConkeys agar, and incubated at 37 ºC for a full day. Following this, the first growth observations were recorded and used for diagnosis.

Cultural Characteristics
On Nutrient Agar, MacConkeys Agar, Eosin Methylene Blue, and Potato Dextrose Agar, the features of the developing colonies were observed, including their size, shape, color, and capacity for differential growth as well as their ability to ferment. Shape, color, size, ability to grow on one medium without needing another, and fermentation were among the attributes noted.

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Microscopic Characteristics:
The bacteria underwent gram stain staining, followed by a microscope examination to document their features, including size, type (positive or negative), and how their cells were arranged in a chain or doubled up. Meanwhile, the fungi were dyed blue with lactophenol to observe their external structures, including thalus and clipboard spores, and how their cells were arranged in single or doubled up groups (Alhayfat).

4. RESULTS AND DISCUSSION

Total Number of Colonies:

Table (1): Represents the Total Bacteria Numbers in Different areas

Date	Bacterial Types	Tikrit	Alam	Krayat	Albu Ajil	Albu Hayaza
7/11	Klebsiella, Lactococcus, Enterococcus, Brucella	30	47	45	55	41
22/11	Klebsiella, Lactococcus, Enterococcus, Brucella	29	48	44	45	42
5/12	Klebsiella, Lactococcus, Enterococcus, Brucella	34	37	42	33	40
19/12	Klebsiella, Lactococcus, Enterococcus, Brucella	31	36	40	32	41
10/1	Klebsiella, Lactococcus, Enterococcus, Brucella	32	42	47	36	45
22/1	Klebsiella, Lactococcus, Enterococcus, Brucella	33	44	47	39	47

2. Total Number of Fungi:
Table 1 displays the findings of the current study. It shows that a variety of bacteria, including lactobacilli and klebsiella, grew most in November in Albu Ajil. This was attributed to the favorable environmental conditions, which included a temperature range of 12 to 43 C° and humidity fluctuations. These species also increased in the spring and autumn because of the presence of virulent factors, such as toxins, capsules, and antibiotic resistance, which aid in their survival, spread, and infection (Jump, 2006).

The results showed that the months with the lowest growth ratio were December and November, when the temperature dropped due to the cold weather. Nonetheless, certain anaerobic bacteria were able to withstand inappropriate environmental conditions (MCB Omald, et al., 2007). Because the formation of lactic acid lowers pH, which acts as an inhibiting substance, these bacteria are also important in the production of natural preservatives and foods (Noordianu, 2013). However, the presence of farm animals in these villages is what caused the bacteria to spread throughout the Al-Bu Ajil and Al-Buhayaz area. They were spread through contaminated soil, milk products, and direct human contact

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(Xovier, 2010). Some of them, like Enterococcus, can grow in both rich and poor environments with oxygen because they can withstand high temperatures between 10 and 45 degrees Celsius, a pH range of 4.6 to 9.9, and high sodium chloride concentrations (Fisher, 2009).

Table (2): represents the total numbers of fungi

Date	Fungus Strain	Tikrit	Alam	Krayat	Albu Ajil	Albu Hayaza
7/11	EXpansum Penicillium, Aspergillus Nijer, Rhizopus, Arthrographis	9	34	11	47	43
22/11	EXpansum Penicillium, Aspergillus Nijer, Rhizopus, Arthrographis	10	33	12	49	45
5/12	EXpansum Penicillium, Aspergillus Nijer, Rhizopus, Arthrographis	8	28	14	96	40
19/12	EXpansum Penicillium, Aspergillus Nijer, Rhizopus, Arthrographis	10	26	12	94	41
10/1	EXpansum Penicillium, Aspergillus Nijer, Rhizopus, Arthrographis	11	30	13	82	42
22/1	EXpansum Penicillium, Aspergillus Nijer, Rhizopus, Arthrographis	12	31	14	85	43

The results of the current study were displayed in Table (2), where it was found that the predominant fungus strain is related to the genera Penicillium and Rhizobus. Arthrographis is responsible for blue mold disease, which is the most common fruit disease that affects apples. It also infects a variety of hosts, including pears, tomatoes, corn, and rice, with Albu Ajil and Al-Buhayaz having the highest ratio of growth due to the area's agricultural nature and humidity availability (Morales, 2007). These fungi are abundant in agricultural areas because some of them can harm citrus fruits (Janisiewicz et al., 2012).

Because Rhizopus fungi are heterotrophic fungi, they feed on human and plant parasites and can be found in a variety of organic materials, including fruits and vegetables (Kirk, 2008Arthrographis is a type of fungus that grows in wood, soil, marine sediments, and agricultural environments. Due to the abundance of animals and air pollution from dust and high humidity, it was the cause of infectious animal disease (Sugiura yositug, 2010). The areas with the largest percentage of fungal growth were Albu Ajil and Al-Buhyazaa, and the areas with the lowest percentage were Tikrit, Alam, and Al-Karaat.

5. CONCLUSION

This could be because the environmental conditions at the time were insufficient for bacterial growth.

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