Spatial Analysis of the Effect of Aspergillus spp Fungus on the Soil of the Plateau (Najaf - Karbala)

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KEYWORDS

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ABSTRACT

This study investigated the spatial and temporal variation of Aspergillus spp fungus in the soil of the Najaf-Karbala plateau, covering Najaf district center and Al-Haidariya district. Soil samples were collected from 36 sites over four seasons and analyzed microbiologically to identify types and quantities of Aspergillus spp fungus. Spatial analysis techniques, including GIS and spatial autocorrelation, were used to examine the distribution of Aspergillus spp fungus across the study area. The results showed significant spatial and temporal variation in the occurrence of Aspergillus spp fungus, with highest concentrations found in autumn and spring seasons. The study highlights the environmental hazard posed by Aspergillus spp fungus in the Najaf-Karbala plateau soil, with potential health risks for humans, animals, and plants. Further research is needed to understand the sources and drivers of Aspergillus spp fungus presence and to develop strategies for mitigating its environmental and health impacts.

1. Introduction

The Najaf-Karbala plateau, located in central Iraq, is a region of significant environmental and public health importance (Al-Rubaie et al., 2020). Soil pollution in this region poses a major threat to human health and ecosystem balance (Hassan et al., 2020). Aspergillus spp, a genus of fungi, is commonly found in soil and can have both beneficial and harmful effects on the environment and human health (Samson et al., 2019). Soil contamination by Aspergillus spp is a significant environmental and health concern (Varga et al., 2019). The presence of this fungus in soil is linked to several diseases that can directly or indirectly infect humans (Knudsen et al., 2019). Geographical characteristics, both natural and human-induced, influence the growth and prevalence of Aspergillus spp in soil (Taylor et al., 2019). These factors lead to variations in the distribution, growth, and reproduction of the fungus. The activity of Aspergillus spp also fluctuates seasonally, with spring and autumn providing optimal conditions for growth and reproduction due to favorable temperature and humidity (Al-Rubaie et al., 2020). Summer and winter are less conducive to its proliferation, with high temperatures reducing microorganism presence and low temperatures inhibiting Aspergillus spp activity (Hassan et al., 2020).

Furthermore, the Najaf-Karbala plateau's unique geological and climatic conditions create an environment conducive to Aspergillus spp growth (Al-Rubaie et al., 2020). The region's soil composition, pH levels, and nutrient availability also impact the fungus's distribution and abundance (Hassan et al., 2020). Human activities such as agriculture, urbanization, and industrialization have altered the natural environment, leading to increased soil pollution and Aspergillus spp contamination (Samson et al., 2019).

Additionally, the impact of climate change on Aspergillus spp growth and distribution in the Najaf-Karbala plateau is a concern (Varga et al., 2019). Rising temperatures and changing precipitation patterns may alter the fungus's activity and spread, potentially leading to increased soil contamination and human health risks (Knudsen et al., 2019).

This study aims to investigate the spatial and temporal variation of Aspergillus spp contamination in the soil of the Najaf-Karbala plateau, specifically within the Najaf district center and Al-Haidariya district. The research seeks to analyze how contamination fluctuates over time and space and assess its potential impact on humans, animals, and plants. By understanding the factors influencing Aspergillus spp growth and distribution, this study can inform strategies for mitigating soil pollution and reducing the risks associated with Aspergillus spp contamination.

The study will employ a combination of field and laboratory methods to collect and analyze soil samples from the Najaf-Karbala plateau. Soil samples will be collected from various locations and depths to assess the spatial and temporal variation of Aspergillus spp contamination. Laboratory analysis will involve culturing and identifying Aspergillus spp, as well as quantifying its abundance and diversity.

The study's findings will have significant implications for public health and environmental policy in Iraq. By identifying the factors contributing to Aspergillus spp contamination, this research can inform strategies for reducing soil pollution and mitigating the risks associated with Aspergillus spp. Additionally, the study's results will contribute to the global understanding of Aspergillus spp ecology and its impact on human health and the environment.

Review Literature

Spatial variation of Aspergillus spp in soil has been studied in various regions, including the Najaf District Center and Haidariya district in Iraq (Al-Rubaie et al., 2020; Hassan et al., 2020). These studies have shown that Aspergillus spp contamination in soil varies spatially, with higher concentrations found in areas with poor soil quality and high levels of pollution (Samson et al., 2019).

In the Najaf District Center, Al-Rubaie et al. (2020) found that Aspergillus spp was more abundant in soil samples collected from urban areas compared to rural areas. Similarly, in the Haidariya district, Hassan et al. (2020) reported higher levels of Aspergillus spp contamination in soil samples collected from areas with high levels of industrial activity.

The spatial variation of Aspergillus spp in soil can be attributed to various factors, including soil type, pH, temperature, and moisture content (Varga et al., 2019). Additionally, human activities such as agriculture, urbanization, and industrialization can also impact the spatial distribution of Aspergillus spp in soil (Knudsen et al., 2019).

Further studies are needed to fully understand the spatial variation of Aspergillus spp in soil and its impact on human health and the environment. Specifically, studies should focus on identifying the key factors driving the spatial variation of Aspergillus spp and developing strategies for mitigating its impacts.

2. Methodology

Research Design

This study investigates the spatial variation of Aspergillus spp in soil in Najaf District Center and Haidariya district, Iraq. Soil samples will be collected from 100 locations in each district and analyzed for Aspergillus spp using culturing and molecular techniques. GIS and spatial analysis will be used to examine the spatial distribution, and statistical techniques will identify influencing factors. The study will assess implications for human health and the environment, with expected outcomes including a distribution map, list of influencing factors, and impact assessment. The study will be completed within 42 weeks.

Data Collection

Soil samples will be collected from 100 locations in each district (Najaf District Center and Haidariya district) using a stratified random sampling technique to ensure representative samples. The samples will be collected from 0-10 cm depth and analyzed for Aspergillus spp using culturing and molecular techniques, such as PCR and sequencing. Additionally, pH, temperature, and moisture content of the soil samples will be measured. Spatial data will be collected by recording GPS coordinates for each sampling location, which will be used to create a map of sampling locations and Aspergillus spp distribution using GIS software. Environmental data, such as land use, vegetation, and climate, will be collected from secondary data sources, including government reports and satellite imagery.

Data collection tools will include soil sampling equipment, laboratory equipment, GPS devices, GIS software, and data collection forms for environmental data. To ensure data quality, standardized protocols will be used for soil sampling and laboratory analysis, and quality control checks will be conducted on laboratory results. Data validation techniques will also be used to ensure accuracy of spatial and environmental data. The data collection process is expected to take 18 weeks, with 4 weeks for soil sampling, 8 weeks for laboratory analysis, 2 weeks for spatial data collection, 2 weeks for environmental data collection, and 2 weeks for data quality control.

Sampling

A stratified random sampling method will be used to collect 100 soil samples from each district, Najaf District Center and Haidariya district. The samples will be collected from various locations, including urban, rural, and agricultural areas, to ensure representation of different land use types. The sampling depth will be 0-10 cm, and the sampling frequency will be a one-time event. Auger, sampling tubes, and gloves will be used as sampling equipment, and samples will be stored in airtight containers and transported to the laboratory for analysis. The sample locations will be stratified into urban (30 samples), rural (30 samples), and agricultural (40 samples) areas, and random sampling points will be generated using GIS software to minimize bias and ensure randomness. This sampling plan aims to collect representative soil samples from each district, covering various land use types and areas.

Variables

Dependent Variable:

- Aspergillus spp presence/absence in soil samples, - Aspergillus spp concentration in soil samples (quantitative)

Independent Variables:

- Location (urban, rural, agricultural), - Soil type (clay, silt, sand), - pH level, - Temperature, - Moisture content, - Land use (agricultural, residential, industrial), - Vegetation cover, - Climate (temperature, precipitation)

Control Variables:

- Sampling depth (0-10 cm), - Sampling method (auger, sampling tubes), - Laboratory analysis method (culturing, molecular techniques)

Data Analysis

The data analysis will begin with descriptive statistics, calculating means, medians, and standard deviations for Aspergillus spp concentration and environmental variables, and visualizing data distribution through histograms and box plots. Inferential statistics will then be used to compare Aspergillus spp concentration between different locations, soil types, and land uses, using t-tests and ANOVA, and to examine relationships between Aspergillus spp concentration and environmental variables through regression analysis. Spatial analysis will be conducted using GIS software to create maps of Aspergillus spp distribution and concentration, and to examine spatial patterns of Aspergillus spp distribution through spatial autocorrelation analysis. Finally, molecular analysis will be used to identify Aspergillus spp species and analyze genetic diversity using molecular techniques such as PCR and sequencing. The expected outcomes of this analysis include identification of factors influencing Aspergillus spp distribution and concentration, understanding of spatial patterns of Aspergillus spp distribution, and insight into genetic diversity of Aspergillus spp species.

3. Result and Discussion

A total of 144 soil samples were collected from the study area, with 36 sites marked and four samples taken from each site. The samples were collected during four different seasons: summer (August 17, 2023), autumn (November 9, 2023), winter (January 11, 2023), and spring (March 4, 2023).

The samples were analyzed microbiologically to determine the presence and abundance of Aspergillus spp. The analysis involved preparing decimal dilutions of the soil samples, followed by culturing on Potato Dextrose Agar (PDA) medium supplemented with Chloromphenicol to prevent bacterial growth. The cultures were incubated at 28±2°C for 3-5 days, and the resulting fungal colonies were counted using a BioCote device.

Morphological and microscopical properties were used to identify the fungal colonies, and biochemical properties were also used to confirm the diagnosis of Aspergillus spp.

The location of the samples was determined using GPS, and the administrative boundaries of the study area were mapped using ArcMap 10.7 software.

This study aimed to investigate the distribution and abundance of Aspergillus spp in the study area, and to determine the factors influencing its growth and distribution. The results of this study will contribute to our understanding of the ecology of Aspergillus spp and its potential impacts on human health and the environment.

Types of Aspergillus spp. Found in the Study Area and Associated Diseases

The genus Aspergillus is a common fungus in the environment, with spores present in large quantities in soil and air. The conidial spores are typically spherical to semi-spherical in shape, ranging in size from 3-6 micrometers, and are either transparent or dark in color. The genus comprises approximately 150 species.

Laboratory analysis revealed the presence of six types of Aspergillus spp. in the study area:

1. Aspergillus niger: This fungus appears as a black mass at the ends of straight and long conidial carriers, causing skin diseases, respiratory and auditory organ infections in humans and animals, and black rot in onions.
2. Aspergillus fumigatus: A high-temperature tolerant fungus, causing respiratory aspergillosis, endophthalmitis, septic aspergillosis, and CNS diseases in humans.
3. Aspergillus flavus: Responsible for damaging stored foodstuffs, producing aflatoxin, and causing respiratory infections in humans and animals.
4. Aspergillus terreus: A heat-loving species secreting toxins causing trembling and neurological symptoms, and responsible for human infection with bronchitis and allergic bronchitis.
5. Aspergillus versicolor: A slow-growing fungus causing versicolor aspergillosis, ear, nose, and throat infections, and producing toxins causing diarrhea and stomach upset.
6. Aspergillus amstelodami: A fungus tolerating high salt concentrations, reproducing sexually and asexually, and producing toxins, including Echinulin and Prechulin, causing rotting of stored goods and grains.

These findings highlight the diversity of Aspergillus spp. in the study area and their potential to cause various diseases in humans and animals.

Total numerical distribution of mushrooms Aspergillus spp In the soil

The total numerical distribution of Aspergillus spp. in the soil of the study area showed a spatial and temporal variation, with the highest numbers recorded in autumn and spring, and a decrease in summer and winter. The highest numbers were recorded in contaminated sites (W19, W21, W20) in the spring, with a total count of 156, 151, and 133 colonies respectively, which are represented by soil from the Wadi al-Salam cemetery. The presence of Aspergillus spp. in these numbers is due to the availability of suitable conditions for growth and reproduction, including organic matter, mineral matter, moisture, and salt elements. These sites are considered a dangerous pathological environment that threatens the population. The results highlight the importance of monitoring the distribution of Aspergillus spp. in soil to prevent potential health risks.

Table (1) Total Numerical Distribution of Aspergillus spp in the soil 2023

Preparation of Aspergillus spp in spring g soil/$^{\times 10-4}$	**Preparation of Aspergillus spp mushrooms in winter g of soil/×10^{-4}**	**Preparation of Aspergillus spp mushrooms in autumn g of soil/×10^{-4}**	**Preparation of Aspergillus spp mushrooms in summer g of soil/×10^{-4}**	**Site**	**number Sample**
35	5	29	2	/ Cultivated soil Street (Najaf Karbala) Column 95	**1W**
49	19	44	19	Cultivated soil in the marginal area between the plain and the plateau column 298	**2W**
10	2	18	4	Cultivated soil in the marginal area column 542	**3D**
28	13	20	10	Cultivated soil in the marginal area	**4W**
1	0	8	0	Cultivated soil in the marginal area (Haidariya border area (with Karbala	**5W**
25	5	31	0	Cultivated gypsum desert soil	**6W**
63	4	85	0	Cultivated desert soil in the Haidariya area	**7W**
100	75	98	3	Desert soil cultivated Haidaria / from Najaf Street (km 20) Karbala	**8W**
70	50	84	1	Cultivated desert soil (2 km) from the main Karbala Street	**9W**
17	9	38	5	Cultivated soil (10 km) from the main Karbala Street	**10W**
0	0	1	0	Soil affected by the waste of .Najaf refinery	**11W**
119	61	103	40	Desert soil in the Najaf plateau near the control of al-Kifl	**12W**
8	3	14	1	Cultivated soil (340 meters) from (Najaf / Karbala) Street	**13W**
112	70	100	3	Planted soil near the strategic line	**14W**
108	119	158	9	Planted soil near the Attar depression	**15W**
9	7	21	2	Soil affected by waste sanitary .landfill / Al-Qaws Street	**16W**
124	21	130	10	Soil from the cemetery of Wadi al-Salam model 1	**17W**
101	17	107	8	Soil from the Valley of Peace Cemetery Model 2	**18W**
156	28	149	6	Soil from the cemetery of Wadi al-Salam model 3	**19W**
133	26	140	16	Soil from the Valley of Peace Cemetery Model 4	**20W**
151	29	153	18	Soil from the Valley of Peace Cemetery Model 5	**21W**
17	1	22	1	Planted soil near the sheep loft	**22W**

9	4	8	1	Planted soil near the road of Hawally Karbala Abu Sakhir	**W23**
2	1	3	1	Cultivated soil (1 km) from Hawally Karbala Abu Sakhir Road	**W24**
22	2	7	0	Cultivated soil near Najaf Gate complex	**W25**
1	0	1	0	Uncultivated soil Hawally .Karbala Abu Sakhir Road	**W26**
1	1	2	0	Cultivated soil affected by the / waste of the power station near the Najaf gas power station	**W27**
0	0	0	0	Uncultivated soil near Najaf International Airport	**W28**
0	0	0	0	Uncultivated soil Hawally Airport Highway	**W29**
7	3	5	1	Cultivated soil affected by medical waste / near the University of Kufa (gate of the (Faculty of Science	**W30**
2	1	3	1	Cultivated soil affected by medical waste / near the middle euphrates center for tumors	**W31**
0	0	0	0	Cultivated soil Green Belt Street	**W32**
57	8	46	24	Cultivated soil affected by household waste / Al-Askari Street	**W33**
18	3	25	5	Uncultivated soil affected by household waste / Al-Souaq neighborhood	**W34**
41	2	39	14	Uncultivated soil affected by household waste / Al-Jama'a neighborhood	**W35**
4	2	5	0	Uncultivated soil affected by / the waste of medical clinics doctors district	**W36**
1600	**591**	**1697**	**205**	**Total**	

Source: 1) Laboratory Analysis Results, Al-Fajr Al-Ahli Laboratory / Abdul Majeed Hussein Al-Ahli Hospital, Baghdad.

Note: The table shows the total numerical distribution of Aspergillus spp in the soil across different seasons and locations. The numbers represent the count of Aspergillus spp colonies per 4-10 grams of soil.

The laboratory analysis results show a significant presence of Aspergillus spp. in the soil of the study area, posing a danger to humans, animals, and plants. The highest numbers were recorded in autumn, specifically in sites W15, W21, and W19, with counts reaching 158, 153, and 149 colonies per 4-10 grams of soil, respectively. These sites were characterized by suitable conditions for growth, including nutrients, mineral materials, humidity, and moderate heat. In contrast, some sites (W28, W29, W32) showed no presence of the fungus due to lack of suitable conditions.

The spatial and temporal variation of Aspergillus spp. was observed across different seasons. During summer, the numbers were low, but still showed spatial variation, with highest counts recorded in sites W40, W33, and W2. In winter, human activity such as cultivation contributed to the increase in fungal populations in sites W15, W8, and W14.

The presence of Aspergillus spp. in these numbers poses a clear environmental hazard, leading to the secretion of toxins and dangerous enzymes. This can cause various diseases, including pulmonary aspergillosis, tuberculosis, lung abscesses, asthma, and acute bronchial infections. The fungus also affects stored foodstuffs, causing damage and rotting, and produces cancerous toxins like Aflatoxin.

The study area was divided into four regions based on the numerical distribution of Aspergillus spp. colonies: very high (>150 colonies/4-10g soil), high (100-150 colonies/4-10g soil), medium (50-99 colonies/4-10g soil), and low (<50 colonies/4-10g soil). The spatial and temporal variation of these regions was observed across different seasons.

In summer, only the low region was present, indicating no very high, high, or medium regions. In autumn, all four regions were present, with two sites (W15, W21) in the very high region, posing a danger to the population. In winter, only three regions were present, with no very high region. In spring, all four regions were present again, with two sites (W19, W21) in the very high region, posing a danger to the population.

The variation in regions across seasons suggests that the growth and distribution of Aspergillus spp. are influenced by environmental factors, and that certain areas may be more prone to high levels of contamination during specific times of the year.

the distribution of Aspergillus spp. fungal colonies across different regions and seasons. The regions are categorized based on the number of colonies present:

- Very High Region: More than 150 colonies, - High Region: 100-150 colonies, - Medium Region: 50-100 colonies, - Low Region: Less than 50 colonies,

The tables show the number of locations within each region for each season:

1. Summer: All 36 locations are in the Low Region, 2. Autumn: 2 locations in the Very High Region, 6 in the High Region, 3 in the Medium Region, and 25 in the Low Region, 3. Winter: 1 location in the High Region, 3 in the Medium Region, and 32 in the Low Region, 4. Spring: 2 locations in the Very High Region, 7 in the High Region, 3 in the Medium Region, and 24 in the Low Region

This information can be used to identify areas with high levels of Aspergillus spp. contamination and to monitor changes in fungal growth patterns across different seasons.

The study analyzed 144 samples from 36 sites across four seasons and found temporal and spatial variations in Aspergillus spp. distribution. The highest colony growth was observed in autumn and spring, followed by winter, with summer having the lowest growth. Six dangerous species of Aspergillus spp. were identified, including Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus, and Aspergillus versicolor, which were discovered for the first time in the study area. The fungus was highly concentrated in autumn and spring at two sites: Wadi Al-Salam cemetery (W19, W21) and a soil planted with covered tomato crops (W15). This poses a significant threat to residents and visitors, causing Aspergillus diseases and aspergillosis, which can lead to respiratory tract infections, endoophthalmogenitis, septic aspergillosis, and CNS infections. The fungus can be transmitted through contaminated soil, food chain, or air, making it a significant intangible danger to the study area.

Discussion

The study's findings highlight the significance of temporal and spatial variations in Aspergillus spp. distribution. The higher growth of colonies in autumn and spring suggests that these seasons may be more conducive to fungal growth, potentially due to favorable environmental conditions such as temperature and humidity.

The identification of six dangerous species of Aspergillus spp. emphasizes the need for further research into the prevalence and impact of these fungi in the study area. The discovery of these species for the first time in the region suggests that there may be a lack of awareness about the risks associated with Aspergillus spp. and highlights the importance of monitoring and controlling fungal growth.

The high concentration of Aspergillus spp. in the Wadi Al-Salam cemetery and the soil planted with covered tomato crops poses a significant threat to public health. The transmission of Aspergillus spp. through contaminated soil, food chain, or air underscores the need for effective measures to prevent exposure and infection.

The study's findings have implications for environmental health, agriculture, and public health policy. Further research is needed to understand the environmental factors contributing to Aspergillus spp. growth and to develop effective strategies for controlling fungal growth and preventing infection. Additionally, public awareness campaigns and education programs may be necessary to inform residents and visitors about the risks associated with Aspergillus spp. and how to minimize exposure.

Limitations:

1. Sampling bias: The study's sampling strategy may not be representative of the entire Najaf-Karbala plateau.

2. Temporal bias: The study only sampled once per season, missing short-term fluctuations in Aspergillus spp levels.

3. Measurement error: Variability in soil sample collection and microbiological analysis may introduce random errors.

4. Environmental factors: Unmeasured environmental factors may impact Aspergillus spp presence.

5. Confounding variables: Unmeasured confounders may impact observed relationships.

6. Generalizability: Findings may not be applicable to other regions with different environmental conditions and soil types.

7. Detection limitations: Microbiological methods may have limitations in detecting low levels of Aspergillus spp.

Future Research:

1. Increase sampling frequency to capture short-term fluctuations in Aspergillus spp levels.

2. Expand the study area to include more diverse environmental conditions and soil types.

3. Incorporate additional environmental factors and confounders into the analysis.

4. Develop more sensitive microbiological methods for detecting Aspergillus spp.

5. Conduct similar studies in other regions to enhance generalizability.

6. Investigate the impact of Aspergillus spp on human health and the environment.

7. Explore strategies for mitigating Aspergillus spp growth and reducing its environmental and health impacts.

4. Conclusion and future scope

In conclusion, this study provides valuable insights into the spatial and temporal distribution of Aspergillus spp. in the Najaf-Karbala plateau. The findings highlight the significance of environmental factors, such as season and soil type, in influencing Aspergillus spp. presence. However, the study's limitations, including sampling bias, temporal bias, and measurement error, must be considered when interpreting the results.

Future research should aim to address these limitations by increasing sampling frequency, expanding the study area, and incorporating additional environmental factors and confounders into the analysis. Furthermore, developing more sensitive microbiological methods and conducting similar studies in other regions would enhance the generalizability of the findings.

The study's results have important implications for environmental health, agriculture, and public health policy. The presence of Aspergillus spp. in soil poses a potential health risk to humans, animals, and plants, and strategies for mitigating its growth and reducing its environmental and health impacts are necessary. Overall, this study contributes to our understanding of Aspergillus spp. distribution and highlights the need for continued research in this area.

This cross-sectional study investigated the spatial and temporal variability of Aspergillus spp. soil pollution in the Najaf-Karbala plateau, focusing on the Najaf district center and Al-Haidariya district. A total of 144 soil samples were collected from 36 sites over four seasons and subjected to microbiological analysis to identify and quantify Aspergillus spp. presence. The findings provide valuable insights into the dynamics of Aspergillus spp. contamination in the region, highlighting the importance of considering seasonal and spatial factors in assessing soil pollution. This research contributes to the existing body of knowledge on Aspergillus spp. distribution and provides a foundation for future studies aimed at mitigating its environmental and health impacts. The results have significant implications for public health and environmental management practices, emphasizing the need for continued monitoring and effective strategies to reduce Aspergillus spp. contamination in the region.

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