



## Spatial Variability Analysis of Enteric Bacteria Species in Water Contamination (Tap and Filtered) Using GIS: A Case Study of Maysan District, Najaf, Iraq

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تحليل التباين المكاني لأنواع البكتيريا المعوية في تلوث المياه (مياه الصنبور والمياه المفلترة) باستخدام نظم المعلومات الجغرافية: دراسة حالة في حي ميسان، النجف، العراق

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### الملخص:

تهدف هذه الدراسة إلى بيان تراكيز بكتريا الايكولاي و بكتريا Proteus mirabilis في المياه وبيان مدى تلوث المياه بها من عدمه، وكذلك معرفة مدى تأثيرها على الانسان، وأن أكثر الاعراض الذي تظهر على الانسان عند أصابته ببكتريا المعوية هي الاسهال والذي قد يتراوح من الخفيف والمائي الى الشديد والدموي كذلك تقلصات بالمعدة او شعور بألم أو وجع عند لمسها وغثيان وقيء لدى بعض الاشخاص وتأتي هذه الإصابة من المياه الملوثة حيث يؤدي براز الحيوان والانسان الى تلوث المياه السطحية بما في ذلك مياه الجداول والانهار فقد ارتبط تفشي هذه البكتريا بتلوث الموارد المائية المحلية. تم جمع (8) عينات من منطقة الدراسة لنوعين من المياه (الصنبور وفلتر المنازل) وأظهرت نوعين من البكتريا الممرضة جداً وهي بكتريا الايكولاي وبكتريا ميرابولس بتريوس بعد اختبارها في المختبر. بالاعتماد على التحليل المكاني والعددي للعينات المدروسة باستخدام نظام ال GIS pro في رسم خرائط منطقة الدراسة أظهرت النتائج للنمذجة المكانية لبكتريا الايكولاي وبكتريا ميرابولس بتريوس انها تتباين في التركيز من حيث التواجد المكاني. افضت الدراسة إلى أن المنطقة المدروسة وفق النتائج المخبرية قد تكون ملوثة بالبكتريا المعوية، ومن أهم الأسباب لتلوث مياه الواصلة الى المنازل هو تلوثها من مياه المجاري والصرف الصحي وضعف تصفية المحطات الرئيسية في المنطقة، ولهذه المياه تأثيرات وأن أكثرها هي الاسهال والذي قد يتراوح من الخفيف

والمائي الى الشديد والدموي كذلك تقلصات بالمعدة او شعور بألم أو وجع عند لمسها وغثيان وقيء لدى بعض الأشخاص.

**الكلمات الدالة:** التحليل المكاني، نظم المعلومات الجغرافية، الإشريكية القولونية، بروتوس ميرابيليس، جودة المياه، منطقة ميسان، الصحة العامة.

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### Abstract

This study aims to determine the concentrations of *E. coli* and *Proteus mirabilis* bacteria in water and assess the extent of water contamination. It also seeks to determine their impact on humans. The most common symptoms of enterobacteria infection are diarrhea, which can range from mild and watery to severe and bloody, as well as stomach cramps, pain or tenderness upon touch, nausea, and vomiting in some individuals. This infection is caused by contaminated water, as animal and human feces pollute surface water, including streams and rivers. Outbreaks of these bacteria have been linked to the pollution of local water resources. Eight water samples were collected from the study area, representing two types of water (tap and household filter). Laboratory testing revealed the presence of two highly pathogenic bacteria: *E. coli* and *Proteus mirabilis*. Based on spatial and numerical analysis of the studied samples using the ARCGIS Pro system to map the study area, the results of the spatial modeling of *E. coli* and *Proteus mirabilis* bacteria showed varying concentrations in terms of their spatial distribution. The study concluded that, according to laboratory results, the studied area may be contaminated with enteric bacteria. One of the most important causes of contamination of household water is its contamination with sewage and wastewater, and the inadequate filtration of the main treatment plants in the area. This contaminated water has several effects, the most common of which is diarrhea, which can range from mild and watery to severe and bloody, as well as stomach cramps, pain or tenderness upon touch, nausea, and vomiting in some individuals.

**Keywords:** Spatial Analysis, GIS, *E. coli*, *Proteus mirabilis*, Water Quality, Maysan District, Public Health.

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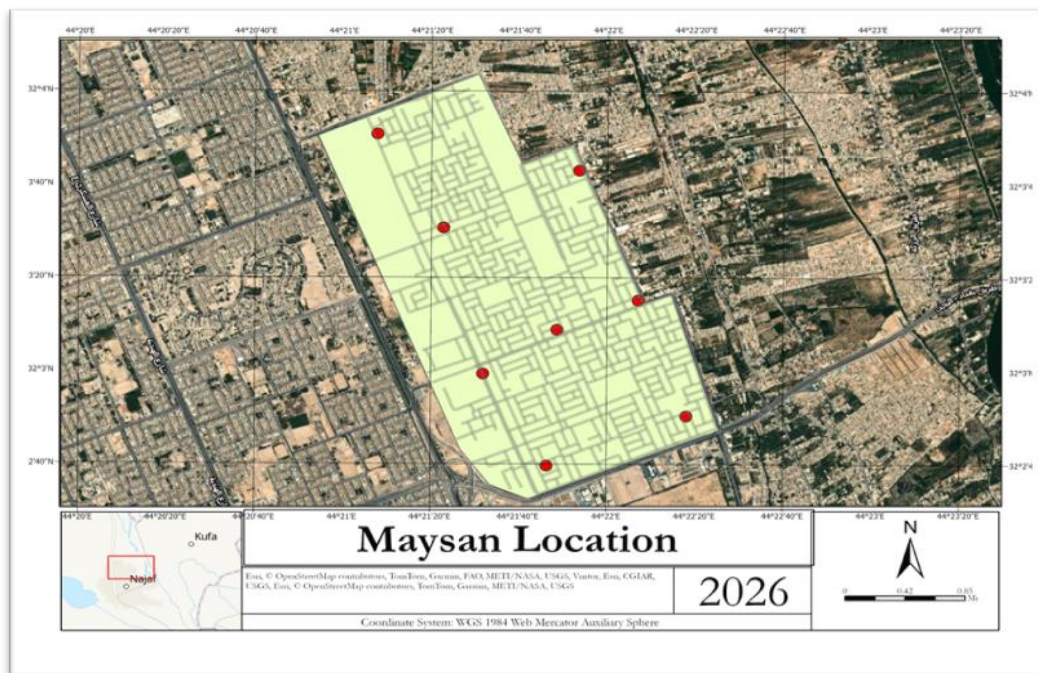
### Introduction

The bacterial characteristics of tap and filtered water directly impact human health (World Health Organization [WHO], 2022). Therefore, this study aimed to identify indicators of pollution in tap and filtered water in the Maysan neighborhood of the Najaf Governorate, and to determine the most important types of intestinal bacteria identified through laboratory analysis of water samples (Edberg et al., 2000).

This research included samples of tap water and filtered water from the neighborhood within the study area over a specific time period. It revealed the presence of several enteric bacteria, namely *Escherichia coli* (*E. coli*) and *Proteus mirabilis*, in very high numbers and concentrations in both types of water (Cabral, 2010). The researchers also investigated whether these bacteria were pathogenic or non-pathogenic at all locations. The results showed that they were highly pathogenic and directly impacted human health (Ashbolt, 2004). It is recommended to pay attention to the wastewater treatment process and to adopt treatment technology before discharging it into rivers, in order to maintain public health and reduce water pollution and the spread of diseases before this water reaches the drinking water directly (WHO, 2022). These actions are important to protect public health, reduce water pollution and reduce the spread of waterborne diseases before dirty water enters the drinking water supply (United Nations Environment Programme [UNEP], 2016).

The study aims to identify the main reasons for the spread of such bacteria in the studied waters. The study aims to reveal the sources of pollution in these waters with enteric bacteria and their temporal and spatial variation. The study aims to study the effect of the factors determining the growth and increase of enteric bacteria in the studied samples (Cabral, 2010). The relevance of this work is that it deals with one of the most important health and environmental problems, the pollution of drinking water with enteric bacteria such as *E. coli* and *Proteus mirabilis*,

which is directly related to human health. This was selected for this research since it is a heavily inhabited region. The Maysan neighbourhood is located within the Kufa District, administratively belonging to the Najaf Governorate. Its geographical coordinates are: 32° 2' 57" North and 44° 22' 1" East. The Maysan district has a population of 94740 and an area of 3.603 km<sup>2</sup> ("Kufa district", 2024).



**Figure 1:** Administrative boundaries of the Al-Nidaa neighborhood with sample locations (Created by using ArcGIS Pro).

#### Materials and Methods:

The microbiological quality of tap and filtered water samples was examined by traditional bacteriological procedures, which are often employed for the identification of symptoms of water contamination. The research was mainly focused on the determination of total aerobic bacterial count using the membrane filtration approach described by Dufour et al. (1981) using selective and differential culture medium such as eosin methylene blue (EMB) agar for identification of enteric bacteria. The bacterial contamination density in the samples was expressed as colony-forming units per millilitre (CFU/ml).

All laboratory work was conducted under sterile conditions to avoid contamination from outside sources. The glass containers used for sample handling were sterilised by direct flame exposure before filtration. The membrane filtering apparatus and the supporting base were carefully sterilised. Sterile forceps were flamed before putting the membrane filter paper on the filtration device. Each water sample was filtered via a membrane using a suction pump of around 100 cc.

The membrane filter was aseptically transferred to prepared growth medium in sterile Petri plates after completion of the filtering operation. The inoculation plates were then placed in the laboratory incubator shown in Figure 2a and incubated at 38°C for 24 hours. Incubation sample plates are shown in Figure 2b. After incubation, bacterial colonies that grew on the medium were examined for colony colour, texture and morphological features for bacterial isolates identification.

The auxiliary laboratory equipment and instruments employed in the inquiry are shown in Table 1, while Table 2 enumerates the culture medium used for bacterial isolation and identification.

**Table 1:** Equipment Used in the Laboratory

| No. | Equipment Name     | Manufacturer |
|-----|--------------------|--------------|
| 1   | Incubator          | Gallen Kamp  |
| 2   | Autoclave          | Gallen Kamp  |
| 3   | water distillatory | Gallen Kamp  |
| 4   | Analytical balance | Mettler      |



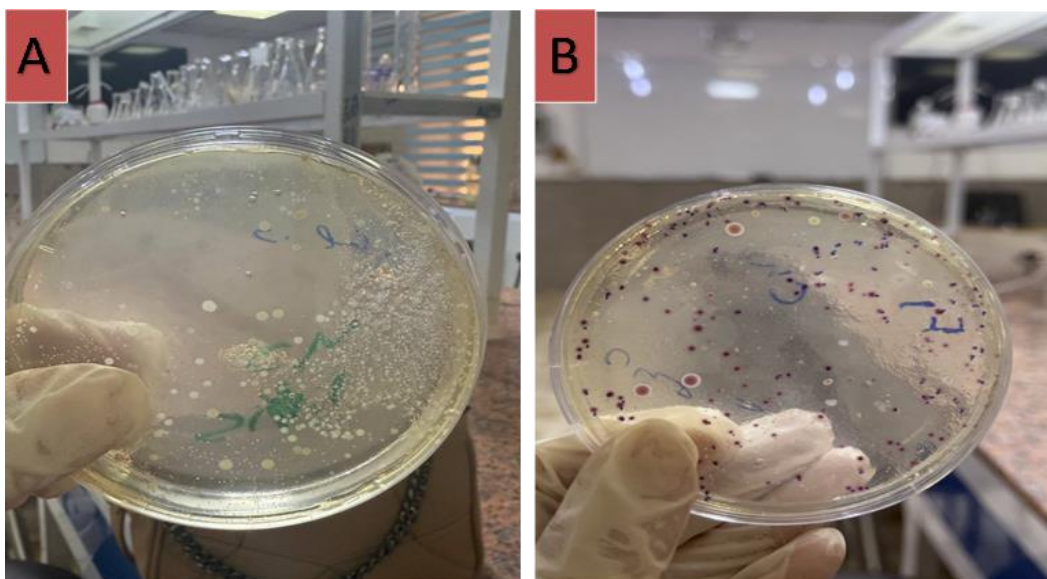
**Figure 2:** A. The incubator used for microbiological cultivation; B. The Petri plates were chosen for bacterial culture examination.

The EMB agar results suggested that the colonies of *Escherichia coli* had a metallic green sheen or dark blue colour while *Salmonella* species had light white colonies. *Klebsiella* species, on the other hand, produced light pink colonies (Figure 3). It was placed in the incubator for 24 hours at 38 degrees Celsius Figure 2, during which *Methylobacterium bifidum* formed yellow, mucoid colonies. The identification process relied on both morphological characteristics and conventional culture-based methods (Atlas, 2010).

The bacteria that appeared in the plates in Figure 3 are bacterial colonies, as these colonies contain millions or thousands of bacterial cells. The bacterial isolates were identified using morphological and culture methods. The results of the analysis were converted to spatial data in the form of points using the ArcGIS Pro program. A shapefile for the study site was created to conform to the study area, and spatial analysis and modeling were performed on it.

**Table 2:** Culture Media Used in the Study

| No. | Medium Name           | Manufacturer     |
|-----|-----------------------|------------------|
| 1   | MacConkey agar medium | HI media (India) |
| 2   | Nutrient agar         |                  |
| 3   | Eosin methylene blue  |                  |



**Figure 3:** A. *Escherichia coli* (*E. coli*) isolated from water samples, B. *Proteus mirabilis* isolated from water samples.

## Results

### The results of the tap water analysis

Figure 4 illustrates a noticeable spatial variation in the concentration of bacterial colonies among the investigated sampling sites, where the recorded levels ranged from low to high values. The highest abundance of *Escherichia coli* (*E. coli*) was observed at site (S5), reaching approximately 231 bacterial cells at the third dilution level ( $10^3$ ). In contrast, several sampling locations, including P1, P2, P4, P5, and P8, exhibited moderate bacterial concentrations ranging between 111 and 231 cells, as presented in Table 3. The lowest bacterial density was recorded at site (P7), where the concentration reached approximately 88 bacterial cells.

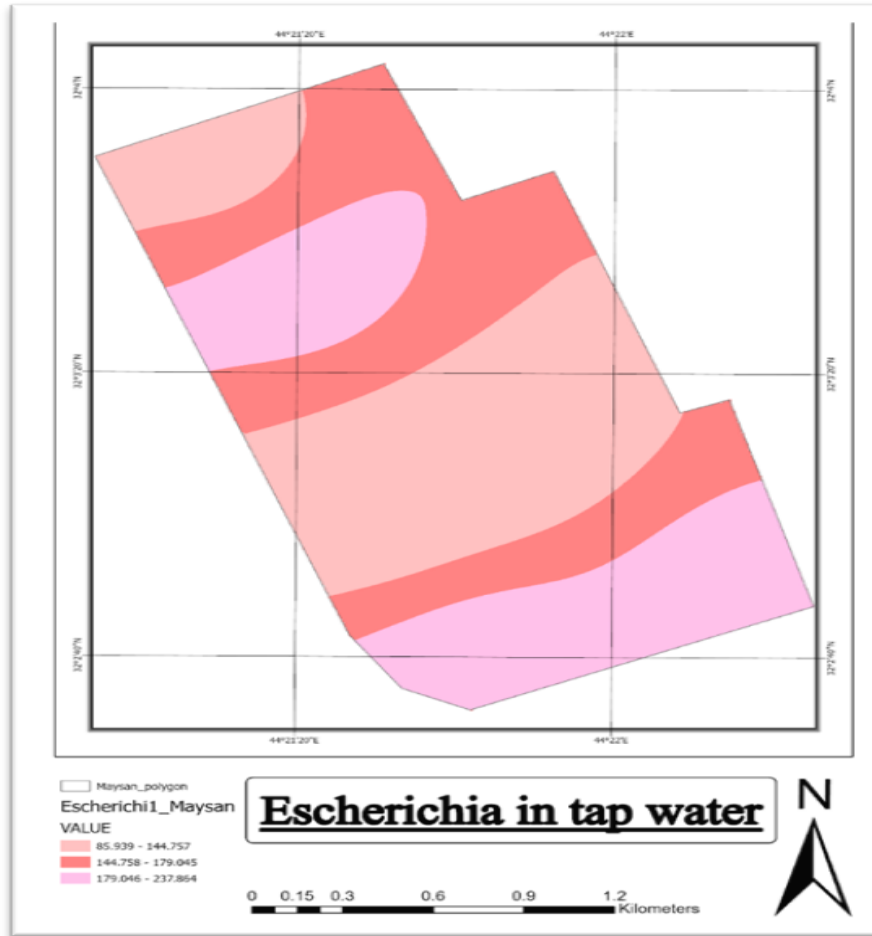
The spatial variation in bacterial concentrations recorded within the study area can be associated with differences in environmental and sanitary conditions among sampling locations. Elevated concentrations of enteric bacteria were mainly observed in areas affected by the discharge of untreated domestic wastewater into nearby rivers and water sources. The direct amalgamation of sewage with surface water substantially facilitates the transmission of microbiological pollutants to water treatment systems, especially when treatment efficacy is inconsistent or inadequate (Cabral, 2010). Thus, tainted water may harbour significant quantities of harmful bacteria prior to consumption.

The moderate to low concentration in some locations is due to their distance from food sources such as animal waste, human feces, or other human waste that alters the area's ecosystem (Ashbolt, 2004). These bacteria cause significant problems for pregnant women because they settle heavily in the female reproductive system due to the weakened immune systems of pregnant women, especially in areas with poor drainage systems, such as those where domestic sewage is discharged near rivers or where public sewage is discharged into the city without local government oversight to address the problem.

**Table 3:** Laboratory results of intestinal bacterial species in tap water in Maysan district.

| Sample No. | <i>E. coli</i> (CFU/100ml) | <i>Proteus mirabilis</i> (CFU/100ml) |
|------------|----------------------------|--------------------------------------|
| P1         | 111                        | 230                                  |
| P2         | 200                        | 94                                   |
| P3         | 150                        | 312                                  |
| P4         | 110                        | 55                                   |

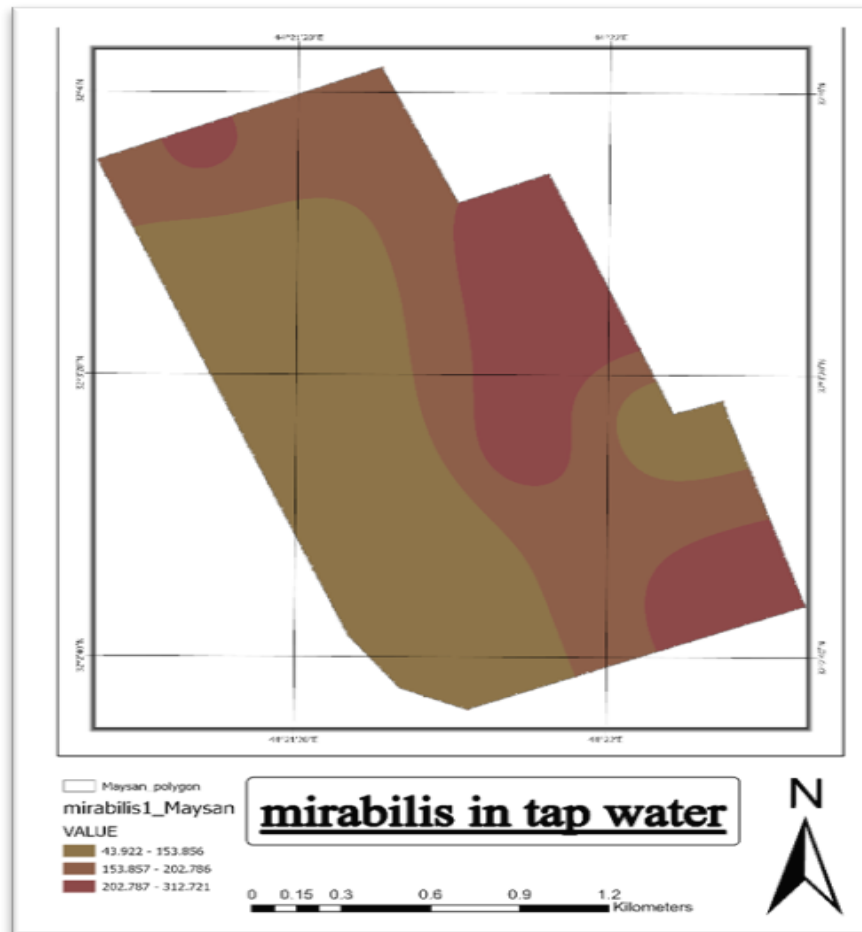
|    |     |     |
|----|-----|-----|
| P5 | 231 | 120 |
| P6 | 234 | 244 |
| P7 | 88  | 211 |
| P8 | 140 | 100 |



**Figure 4:** Spatial modeling map of E. coli bacteria in tap water at the Masan location using ArcGIS Pro.

One of the most important diseases caused by this bacterium in the population in the study area is profuse watery diarrhea that lasts for several days, which leads to dehydration and malnutrition in children. It also causes severe abdominal pain and bloody diarrhea, and in many cases, it causes acute kidney failure. This heavy wastewater changes the aquatic ecosystem and thus affects other components of the ecosystem (farm environment - livestock and poultry field environment) (World Health Organization [WHO], 2022). Pregnant women may be more susceptible to bacterial infections owing to the reduced immune response during pregnancy. This is especially true in areas without proper sanitation infrastructure and uncontrolled sewage disposal near rivers and residential areas (Leclerc et al., 2001).

As for Mirabus Proteus bacteria in the drinking water, the results showed through Table (3) and the spatial modeling Figure 5 that it has varying concentrations from one location to another, ranging between (high, medium, and low). It was shown that the highest concentration of Mirabus Proteus bacteria was in each of the locations (S1-S6 -S3-S2 S7), and the concentration ranged between 230 and 312. The locations with medium concentration were represented by (S5 - S8), and the concentrations ranged between (100-120) bacterial cells (103 dilution). The locations with low concentration were represented by location (w4), where the total concentration of this bacteria was shown to be (55) bacterial cells (103 dilution).



**Figure 5:** Spatial modelling of *Proteus mirabilis* in the tap water of the Maysan district

Laboratory data for *Proteus mirabilis* revealed a clear regional variance in the quantity of microorganisms throughout the tested sites (see Table 3 and spatial Modelling Figure 5). In P1, P6, P3, P2 and P7 elevated levels were observed. The bacterial count ranged from 230 to 312 cells at the third dilution stage. Moderate levels of bacterial counts (100-120 cells) were seen at sites P5 and P8, while the lowest concentration was observed at site P4 (~55 cells). This difference is due to the weakness of treatment systems when water purification plants are inefficient or malfunctioning, and bacteria are not completely eliminated (Edberg et al., 2000).

If the concentration of *Proteus mirabilis* bacteria is moderate in water, this is not considered normal or completely safe. Rather, it is a warning sign that requires attention according to international standards. The World Health Organization states that *Proteus mirabilis* bacteria should not be present in drinking water at all: the safe limit is 0 CFU/100 mL (i.e., its complete absence). However, in practice, analyses show: low: 1-10 CFU/100 mL, medium: 10-100 CFU/100 mL, high: more than 100 CFU/100 mL. CFU indicates the number of bacterial colonies, and this indicates that all sources are unfit for human consumption unless proper treatment is carried out (WHO, 2022).

#### **The results of the filtered water analysis**

In filtered water taken from homes in the study area (Maysan neighborhood), variations and differences in the types of bacteria (*E. coli* and *Proteus mirabilis*) were observed, as shown in Table (4) and the spatial modeling Figure 6 for *E. coli*. There were three concentrations detected: high, medium and low. High concentration was found at site (2S) with concentration (212) ( $10^3$  dilution) (Geldreich, 1996). The medium concentration was obtained at sites (S3, S4, S7 and S8) with bacterial concentration of (200-210) (for  $10^3$  dilution). (S1) The location had a concentration of (100). This variance in *E. coli* is due to an inefficient filter, a requirement for replacement or insufficient disinfection (e.g. UV radiation or chlorination) (WHO, 2022). *E. coli* is utilised as a signal of more harmful bacteria and its

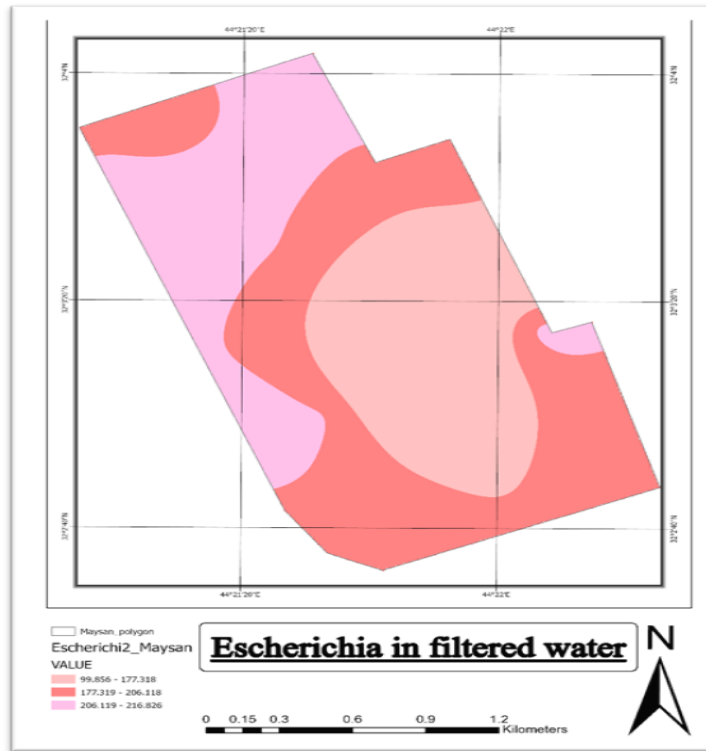
presence may suggest that other bacteria are also present, Other pathogens, viruses, or parasites (Ashbolt et al., 2001).

**Table 4:** Laboratory results for enteric bacteria species in filtered water from the Maysan neighborhood.

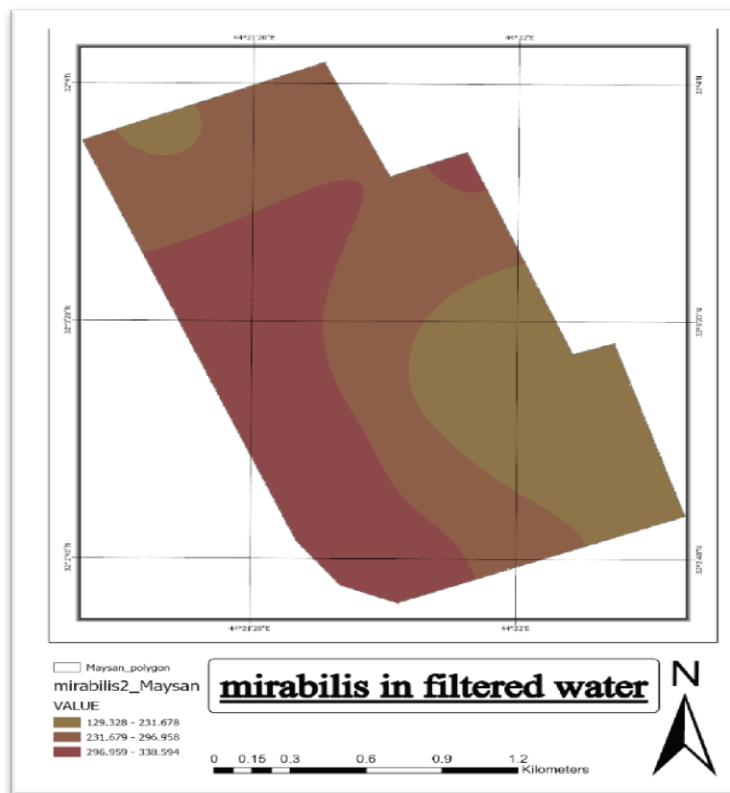
| Sample No. | E. coli (CFU/100ml) | Proteus mirabilis (CFU/100ml) |
|------------|---------------------|-------------------------------|
| S1         | 100                 | 200                           |
| S2         | 212                 | 312                           |
| S3         | 200                 | 300                           |
| S4         | 210                 | 330                           |
| S5         | 195                 | 321                           |
| S6         | 190                 | 216                           |
| S7         | 200                 | 219                           |
| S8         | 210                 | 130                           |

While the bacteria of *Proteus mirabilis* were represented in several locations, as observed from Table 4, and the spatial modeling map of *Proteus mirabilis* in Figure 7, the concentrations ranged between high, medium, and low. The following locations showed a high concentration, namely (S2- S3- S4- S5), and the concentrations of these locations ranged between (300-321) (103 dilution). The medium concentration was represented in the following locations (S1- S6- S7), and the bacterial concentration of these locations ranged between 200 and 219 (103 dilution). The low concentration was observed in location (S8) with a concentration of 130 (103 dilution).

The presence of *Proteus mirabilis* in filtered water samples is a significant signal of systemic deficiencies in the domestic water treatment process. The concentration of these bacteria in filtered water is due to several reasons, including contamination of the primary water source. If the water entering the filter is contaminated (sewage or organic matter), the bacteria may exceed the filter's capacity. Other factors include the expiration of filters (filters have a limited lifespan; if not replaced, they become a suitable environment for bacterial growth instead of being eliminated), the accumulation of dirt inside the filter, and the sediment and organic matter within the filter that provide food for bacteria, leading to their proliferation. The absence of sterilization (UV or chlorine) is another contributing factor. Some filtration systems rely solely on filtration (without sterilization), which does not completely eliminate bacteria. Contamination of the tank or pipes is also a factor. Similarly, unclean tanks and old or dirty pipes can re-contaminate the water after it leaves the filter. Stagnant water inside the system (when water remains in the filter for extended periods due to disuse, bacteria multiply) is another issue. In all cases, the water is considered unfit for human consumption due to the presence of these pathogenic bacteria, which pose a serious health risk.



**Figure 6:** Spatial modeling map of E. coli bacteria in filtered water at the Masan location using ArcGIS Pro



**Figure 7:** Spatial modeling map of Proteus mirabilis bacteria in filtered water for the Maysan neighborhood.

## Discussion

The geographical distribution of bacteria, including *Proteus*, in filtered water from household water networks was determined using a purposive random sampling method. This sample is influenced by both environmental and infrastructural factors. Spatial modeling data, obtained through maps, revealed a clear and significant concentration of bacterial spread and activity in the western part of the neighborhood, indicating very high levels of contamination. This concentration suggests several contributing factors, including the dilapidated and outdated water pipes supplying homes, neglect by the residents of the sampled house, and the fact that the agricultural areas in the Maysan neighborhood are newly developed residential areas lacking many essential services, such as regular and continuous maintenance of water and sewage networks. The mixing of sewage with the main water supply has led to clear and significant contamination in the study area.

These laboratory diagnoses, combined with spatial modeling, provide a comprehensive framework for the environment. These findings necessitate the restoration of infrastructure and regular maintenance of public systems in the city and private systems in homes to mitigate the risk of bacterial concentration and activity. This is especially important given that bacteria are active year-round, as temperature does not affect their growth and activity, provided they thrive in suitable conditions such as a dark environment and direct food sources.

The GIS based research also demonstrates a substantial relationship between high bacteria counts and the presence of decrepit sanitation facilities in close vicinity. The geographical link is consistent with global research that shows that urban water pollution typically results from the cross infection of drinking water pipelines with old sewage systems. *Proteus mirabilis* is an opportunistic bacterium that causes serious UTIs and urolithiasis, and its prevalence in residential water sources is a serious health problem. In conclusion, the combination of laboratory diagnoses and geographical modelling gives a full perspective of environmental auditing. The findings need an urgent examination of water treatment techniques in Maysan governorate, the restoration of infrastructure and the sensitisation of the public on the importance of periodic maintenance of home filtration systems to prevent water-borne diseases. This geographical link is in keeping with global research that have identified cross-contamination between ancient sewage systems and potable water pipes as a common cause of urban water pollution. *Proteus mirabilis* is a common opportunistic bacteria associated with complicated urinary tract infections (UTIs) and urolithiasis and is an important public health issue when present in home water supplies.

The combination of test diagnostics and spatial modelling ultimately gives a complete framework for environmental audits. These findings need an immediate re-evaluation of water treatment protocols in the Maysan region, emphasising the need for rehabilitation of infrastructure and public awareness about the meticulous maintenance of home filtration units to minimise the risk of waterborne infections.

## Conclusion

Based on spatial and numerical analysis of the studied samples using the ProGIS system to map the study area, the results of spatial modeling of *E. coli* bacteria in tap water showed that its highest concentration was in the southeastern part of the neighborhood, while *Proteus mirabilis* was concentrated in the northeastern and southeastern parts. In household filtered water, *E. coli* was concentrated in the northwestern and southwestern areas, while *Proteus mirabilis* was concentrated only in the northwestern and southwestern areas, with anomalies in concentration in the northeastern part. The study concluded that, according to the laboratory results, the studied area may be contaminated with enteric bacteria. One of the most important reasons for the contamination of the water supplied to homes is contamination from sewage and wastewater, and the inadequate filtration of the main treatment plants in the area. These waters have several effects, the most common being diarrhea, which can range from mild and watery to severe and bloody. Other symptoms include stomach cramps, pain or tenderness upon touch, nausea, and vomiting in some individuals. Two highly pathogenic bacteria have been identified: *E. coli* and *Mirabulus* bacterium.

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