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Molecular and Chemical Evaluation of Water Tank Contamination with *Escherichia coli* and Microplastics in Baghdad Schools Using 16S rRNA and FTIR

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Abstract

Water pollution remains a critical environmental and public health concern worldwide, particularly in urban areas with deteriorating infrastructure and limited water quality monitoring. In Iraq, school water tanks—often made of plastic—serve as a primary source of water in school but may be prone to contamination.

This study investigates the presence of Escherichia coli and microplastic particles in water tanks from eight schools across Baghdad's Karkh and Rusafa districts, using 16S rRNA gene sequencing and Fourier Transform Infrared Spectroscopy (FTIR). A total of 96 water samples were collected during both dry and wet seasons, from December 2023 to August 2024.

The results revealed a high prevalence of microplastics, primarily fibers and fragments composed of polyethylene (PE) and polypropylene (PP), along with significant detection of E. coli. A positive correlation was observed between microplastic concentration and bacterial density, indicating that microplastics may serve as a medium for bacterial growth. These findings underscore the health risks posed by aging plastic water tanks in schools and highlight the need for regular monitoring and improved infrastructure to ensure safe drinking water for students.

Keywords: Escherichia coli; Drinking water tanks; Molecular diagnosis; 16S rRNA technique; Polymer analysis; Drinking water quality; Fourier Transform Infrared Spectroscopy (FTIR)

1- Introduction

Water is an abundant and important natural resource for all living things, including humans, animals, plants, and other species. Increasing population, increased economic activity, and urbanization have increased the demand for water, contributing to the increased risk of declining levels and deteriorating water quality. (Al-Janabi, Z. Z., *et al.*2025)

Consequently, water pollution poses a significant risk to all life forms, especially humans. Therefore, drinking water must be free from chemical, physical, and biological pollutants, and it should be palatable, colorless, tasteless, and odorless (Al-Fatlawi, 2007; Hassan and Khalaf, 2022)

The focus on water pollution in particular comes because it is one of the most dangerous types of environmental pollution, Water quality refers to the concentrations of both organic and inorganic pollutant in water and changes in water properties (Hassan, F. M.,*et al.* 2016)

Pollution occurs in the environment either by non-living pollutants (biotic pollutants), which can be in the form of physical and chemical or pollution can be caused by living pollutants (biotic pollutants), which are attributed to a group of microscopic organisms that cause serious health effects and problems and

which are either naturally present or parasitic organisms on the component. Therefore, it is not possible to assert the possibility of a clean environment completely free of pollution.(Naji,H. f., et al., 2007)

In Iraq, the problem of the availability of drinking water is increasing day after day. the lack of enough attention to filtering and sterilizing water, as well as the old and breakdown of distribution networks, which led to a mixture between municipality water and sewage water, and thus water became the first source of various diseases such as typhoid, cholera, viral hepatitis, and cases of severe diarrhea, especially in children, and most of these pathological factors reach into water by humans and animals, especially coliform, Streptococcus, and anaerobic bacteria , these species coexist naturally in the large intestine of humans and animals, their presence is definitive proof of fecal contamination. from a human or animal source (Khaleel and Al-Nazzal, 2009) Water pollution is an issue that can be exacerbated by drought as increased concentrations of unwanted substances are a consequence of lower water levels (Fazaa,N. A., et al.,2021)

The water distribution system in the city of Baghdad consists of a network of pipes with different diameters. This network is old and has suffered a lot of erosion, calcification, and destruction, which reduced its efficiency by a high percentage and was a major cause of pollution by mixing with sewage or groundwater.

Many people use pumps to draw water directly from the network and then increasing the possibility of pollution as a result of low pressure and leakage of polluted sewage or groundwater into the distribution network(AL-Rawi and Razuki 2010)

The issue of quality for domestic water tanks and dependence on it to provide the various daily needs of the population is an important matter, as the water of the tanks is likely to be affected by many biological, organic, and other factors that lead to pollution of the water of domestic tanks and thus affecting the health of its users, especially a large number of the population Depends on the water of domestic tanks even for drinking (WHO., 2003; Slavik, *et al*., 2020)

In recent times, many people have resorted to the use of plastic storage tanks based on their belief that they are good for the safety of their health and environment. However, many recent studies indicate some of plastic materal organic compounds may leak into the water and provide a conducive environment for the growth of bacteria, and lead to several serious diseases (Mahmood, A. K., *et al.*,2019)

2- AIMS OF STUDY

- Investigate bacterial contamination in municipal water and tank water in some schools in Baghdad city
- Investigate the presence of microplastic particles in water storage tanks
- Provide recommendations to improve water quality and reduce health risks resulting from these pollutants

3-METHODOLOGY

3-1 Area of study

Samples of tap and tank water were collected from different schools in Baghdad city on both sides of Al-Karkh and Al-Rusafa. The areas of (Al-Ghazaliya, Al-Khadhraa neighborhood, Al-Jamiah neighborhood, and Al-Yarmouk) were selected on the Al-Karkh side, and the areas of (Al-Bunuk, Al-Shaab, Al-Sadr, and Al-Salikh) were selected on the Al-Rusafa side show Figure (1).

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Sample collection

- Drinking water samples have been collected from tap water and tank in the schools of the study area, taking into sterilization conditions in clean glass bottles
- 100 ml of each water sample was collected for microbiological analysis; 1 L of the sample was used for FTIR analysis of plastic contaminants.
- For microbial examination (0.5 ml) sodium thiosulphat solution ($Na_2S_20_3.5H_20$) at concentration (3%) was added to neutralize chlorine effect for microbial sample (APHA, 2017).
- Water taps were sterilized with ethanol and cotton with using candle flame and later the water taps were opened for half a minute to get rid of the thermal effects of cleaning
- Water samples were collected in sterile bottles and saved in cooler box to be transported to the laboratory for analyzing (Baird, Rice *et al.*, 2017)
- The microplastics were analyzed and examined in the laboratory to determine the type and size of plastic particles that were collected (Kalčíková *et al.*, 2017; Kovács *et al.*, 2022 ; Shukur, S. A*et al.,2023*)

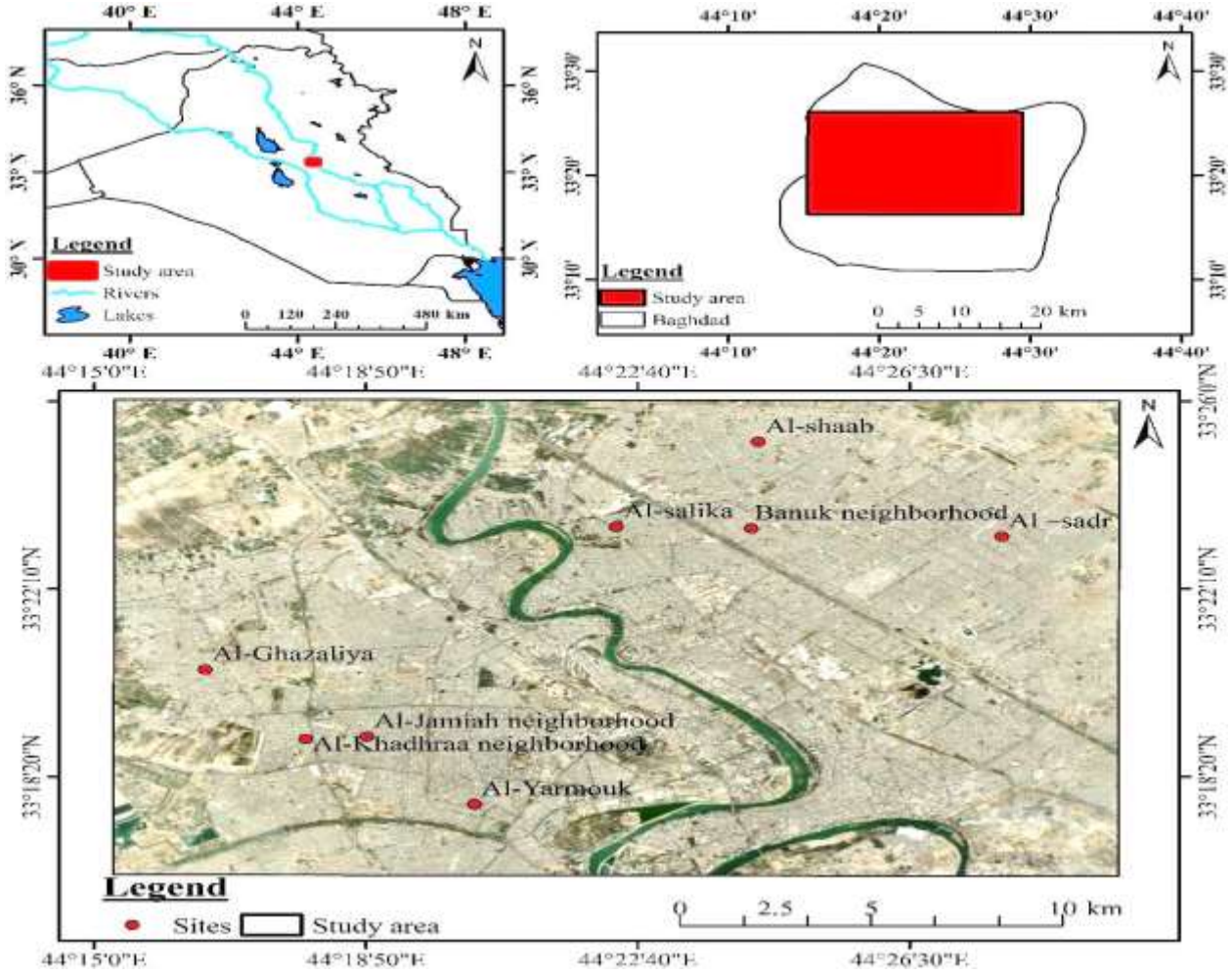


Figure (1) the area study in Baghdad city

3-2 Bacteria identification

Bacterial isolation and identification including:-

1. **Conventional tests** :- MacConkey agar and Eosin Methylene Blue Agar (EMB) media were used to detect Escherichia coli (*E. coli*), as the colonies showed a bright green appearance on EMB media, indicating their ability to ferment lactose

2. **Microscopic examination**:- by using Gram stain, and the results showed that the bacteria were Gram-negative in the form of short rods (Levinson, 2016).

3. **Biochemical tests:-**

- Catalase Test: The result was positive, indicating the ability of the bacteria to decompose hydrogen peroxide into water and oxygen

- Oxidase Test: The result was negative, meaning that the bacteria do not contain the cytochrome oxidase enzyme (Sharmin *et al., 2010*; Tille, 2017; Brown and Smith, 2017)

3-2-1 Polymerase Chain Reaction (PCR)

The bacterial isolates by molecular diagnosis using PCR technology, based on the diagnostic gene 16S rRNA, which is characterized by being a stable gene with little variation for a long time in the bacterial species it is a benchmark in bacterial identification and classification due to its high sequence conservation across bacterial species, with variable regions that can be used to distinguish species.

3-2-2 The methodologies used for DNA extraction and analysis in the project include

1. DNA Extraction: Genomic DNA was isolated from bacterial growth using the ABIO pure Extraction protocol, as the following steps:-

    - For pellet cells, 1ml from the overnight culture for 2min at 13000 rpm. The supernatant was then discarded.

    - The cell pellet was re-suspended completely in 200µl of Buffer.

    - For protein digestion and cell lysis, 20µl of Proteinase K solution (20 mg/ml) was added to 200µl of Buffer and cell pellet, and then the tube was mixed vigorously using vortex and incubated at 56°C for 30 min.

2. PCR Amplification: For bacteria, PCR was performed on 16S rRNA using 27F and 1492R primers, yielding sequencing data of 1,300 bp or more Table (1) showing Primers sequences used for PCR and Table (2) showing PCR-Thermal-Cycling – Protocol

3. Agar's Gel Electrophoresis: After PCR amplification, agar's gel electrophoresis used to confirm the presence of amplification as Figure (2) shows the electrophoresis results, indicating successful amplification of the target gene.

4. Quantitation of DNA: The concentration of extracted DNA was detected using a Quantus Fluorometer, which involved mixing diluted Quantus fluor dye with the DNA sample

5. Sanger Sequencing: PCR products were sent for Sanger sequencing

Table1: Primers sequences used for PCR

Primer Name	Sequence	Annealing Temp. (°C)	Product Size (bp)
27F	5`-AGAGTTTGATCCTGGCTCAG-3`	60	1500
1492R	5`-TACGGTTACCTTGTTACGACTT-3`	60	1500

Table2: PCR-Thermal-Cycling – Protocol

Step	Temperature (°C)	Time (m:s)	Cycles
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60	00:30	
Extension	72	00:30	
Final Extension	72	07:00	1
Hold	10	10:00	

3-3 Extraction and Identification of Microplastics (MPs)

Diagnosing the identity of MPs using the Fourier Transform Infrared Spectrophotometer (FTIR) to determine the type of polymer and compare it with the standard polymer (Masura *et al.*, 2015).

4- RESULTS AND DISCUSSION

Identification and Isolation of *E. coli*

Water samples were collected from school taps and water tanks to isolate and identify bacteria. Results showed the presence of Escherichia coli based on morphological characteristics (pink colonies on MacConkey agar and bright green on EMB agar), microscopic characteristics (Gram-negative rods), and biochemical tests (catalase positive and oxidase negative) (Hammad, & Hassan,2020) A novel *E. coli* gene was identified, which is considered a new record for this bacterium See Figure 3, which presents the BLAST analysis showing the alignment between the amplified gene sequence and the closest database entries See also Figure 4, which illustrates the phylogenetic tree and the affiliation of the isolated strains to their nearest neighbors based on 16S rRNA gene sequence similarity.

Molecular identification

The 16S rRNA gene was targeted to identify Escherichia coli (E. coli) using primers 27F and 1492R, and the results of DNA electrophoresis showed a clear band of 1500bp, confirming the success of the gene amplification process

The gene sequence was analyzed using the BLAST tool, and the result showed 100% matching with the E. coli genome, confirming the accuracy of the diagnosis (Jenkins *et al.*,2012; Alkhowaiter *et al.*, 2023)

The results showed that the sequence of the diagnostic gene for E. coli bacteria is present at a rate of (100%)

The results of the current study are also consistent with the study by Lai *et al.* ,(2016) in Malaysia and the study by Maleki *et al.*, (2017) in Iran, where all bacterial isolates were molecularly diagnosed using the 16S-RNA diagnostic gene. The results of the current study are also consistent with the results of the study by Jenkins et al., 2012, which showed that all Ecoli bacterial isolates had the 16SRNA diagnostic gene.

Table 3: Results of diagnostic tests for E.coli

Test	Result
cell shape and assembly	bacilli rods
MacConkey agar	lactose fermenting
EMB agar	green metallic sheen coline
gram stain	-
Oxidase	-
Catalase	+
16SrRNA	+

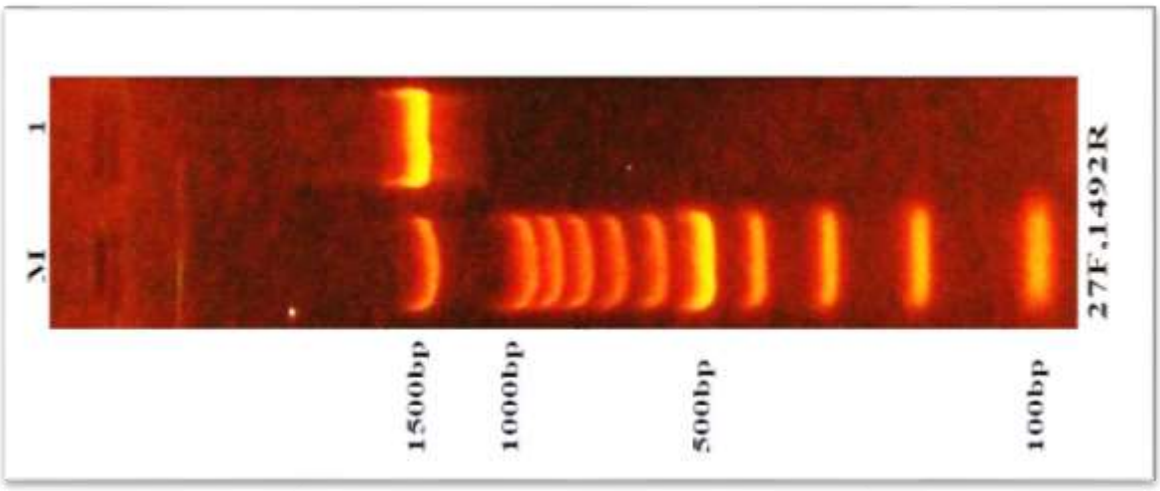


Figure 2: the results of agarose gel electrophoresis, showing DNA ladders (M) of different sizes (100-1500bp). In lane 1, the PCR product using primers 27F-1492R is shown, indicating successful amplification of the 16S rRNA gene with a size of approximately 1500bp.

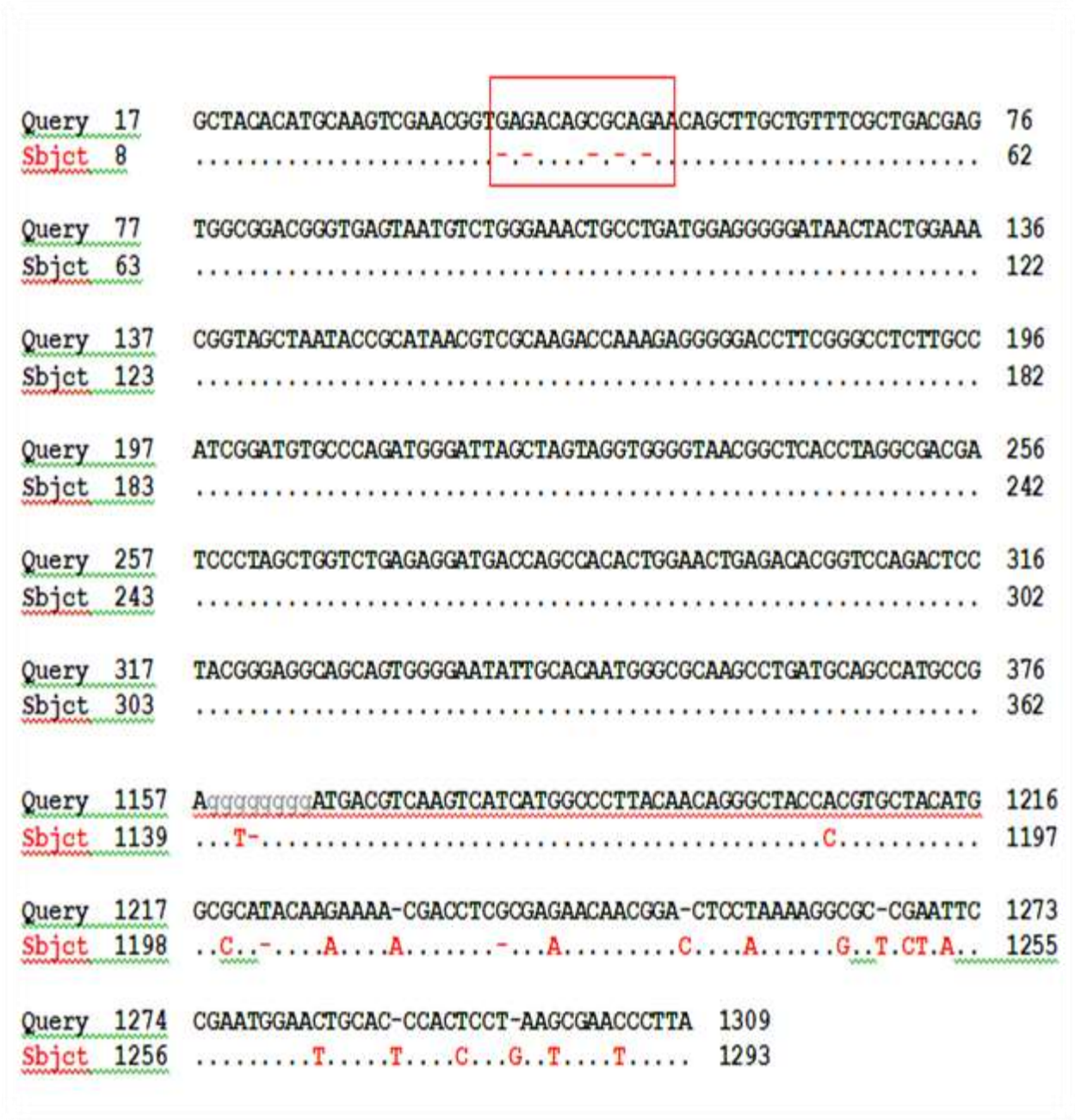


Figure3: BLAST (Basic Local Alignment Search Tool) analysis which shows the match between the amplified gene sequence and genes in a database

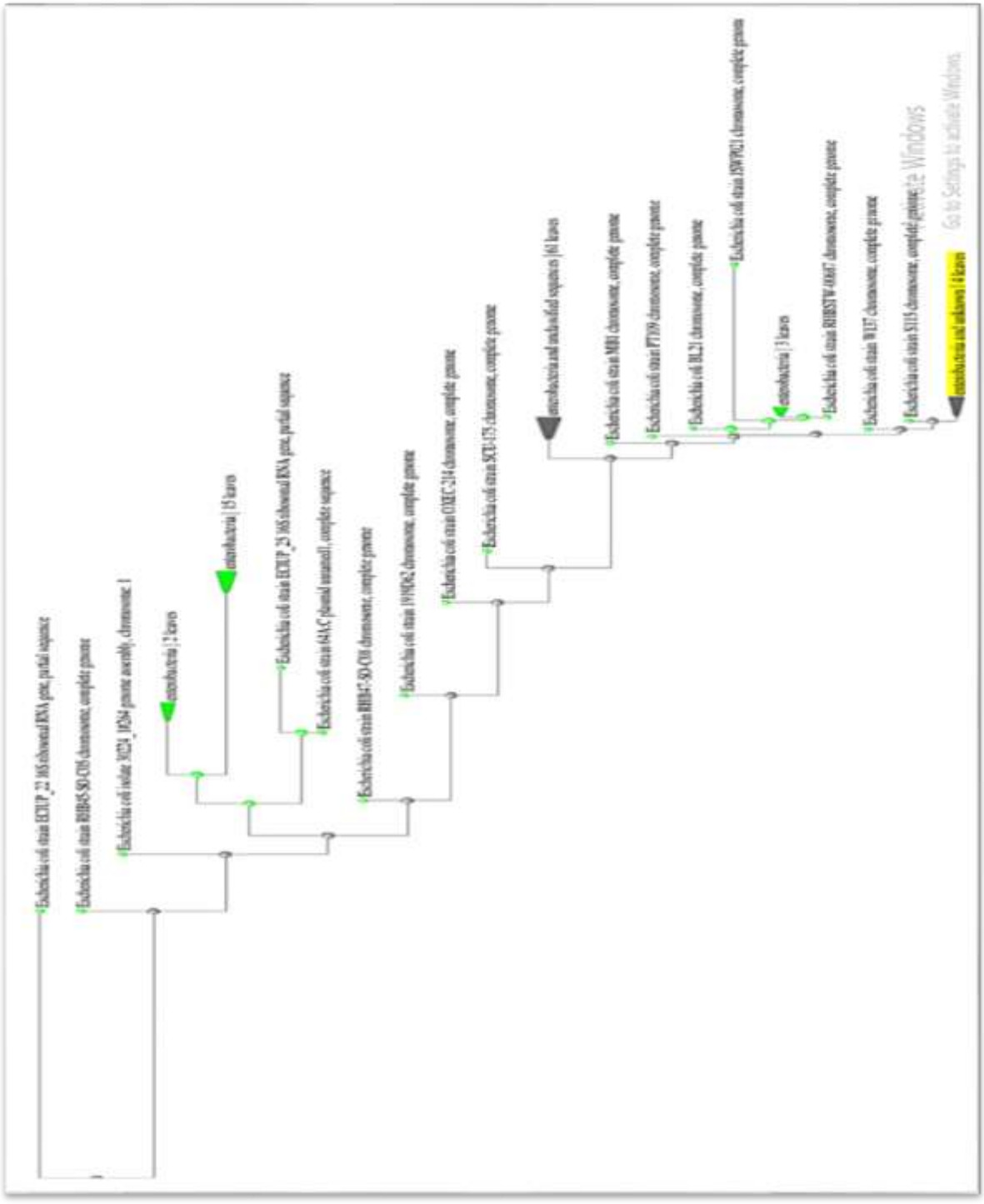


Figure 4: The affiliation of the strains to the nearest phylogenetic neighbor and the percentage of 16S rRNA gene sequence similarities

Microplastic Identification

MPs had no significance temporal variation in tank water in both seasons. Our findings indicate that the levels of MPs pollution remain consistently throughout the year, regardless of climatic factors**.**

Several researchers have noted that the impact of seasonal variation on the abundance of microplastics (MPs) remaine inconclusive. Some studies have reported no statistically significant differences across various ecosystems (Phuong *et al.,* 2018; Cha *et al*., 2023; Suteja *et al.,* 2021; Shekoohiyan *et al*., 2022) for instance, reported no temporal fluctuations in MP concentrations were observed in the Jajroud River (Iran). Instead, the widespread presence of MPs has been mainly linked to anthropogenic factors such as urbanization, industrial activities, and daily human behavior. These factors show a strong correlation with the consistent levels of microplastic pollution throughout the year, regardless of season or location.

Several types of microplastics were detected in the school's water tanks, including common polymers such as polyethylene (PE) and polypropylene (PP). (These types are common in the manufacture of water tanks, supporting the hypothesis of plastic degradation as one of the sources.) These particles were small in size (<0.5 mm), indicating advanced sources of degradation of the plastics used in the tanks.

The results also showed that fragmented plastic particles (Fragments) and fibers (Fibers) were the most common forms, reflecting long-term mechanical and thermal degradation of the plastic(Shekoohiyan *et al*.,2022)

Most of the particles were small, less than 0.5 mm in size, highlighting the potential risk of inhalation or ingestion.

Various colors were identified, such as white, transparent, and black. White and transparent indicate natural degradation of the plastic, while black indicates possible external contamination or degradation of the dark layers of the tank.

Identifying the MP polymers by FTIR

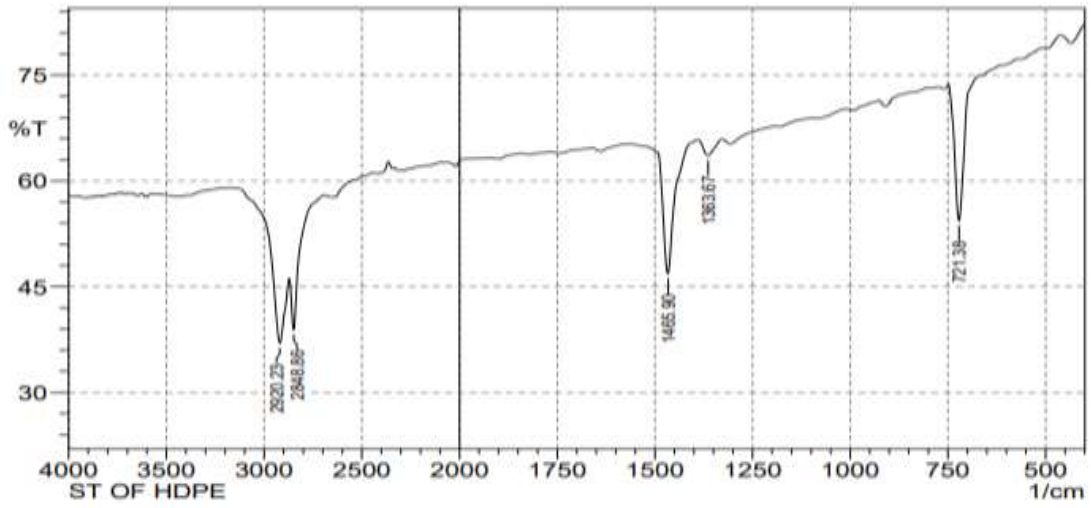
Fourier Transform Infrared Spectroscopy (FTIR) was employed to analyze suspected microplastic particles, This technique is widely recognized for its effectiveness in identifying microplastic polymers, owing to its high reliability in determining the chemical composition of unknown plastic fragments(Hidalgo-Ruz *et al.,* 2012; Shim *et al.,* 2017). This technique is based on measuring molecular vibrations and provides a distinct spectral signature for each type of polymer.

The main spectral peaks were shown at wavelengths such as 723 cm^{-1}, 1363 cm^{-1} and 2920 cm^{-1} as show in Figure(5).

The peaks at 723 cm^{-1} and 1363 cm^{-1} indicate the presence of polyethylene (PE), while the peaks at 2920 cm^{-1} indicate the presence of polypropylene (PP).

The matching of the spectral peaks with the PE and PP signatures confirms that the detected microplastics are mainly from the plastic materials used in the manufacture of tanks.

The low-intensity peaks may indicate partial degradation of the plastic due to prolonged exposure to heat and sunlight. The chart 1: showing the percentage of microplastics detected**.**



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	721.38	54.364	19.93	748.38	613.36	20.331	3.527
2	1363.67	63.604	2.269	1388.75	1328.95	11.293	0.456
3	1465.9	46.854	17.622	1490.97	1390.68	22.863	4.056
4	2848.86	38.902	8.612	2870.08	2690.7	50.846	1.996
5	2920.23	36.991	11.205	3174.83	2872.01	86.863	5.433

Figure5: The FTIR spectrum plot of the high density polyethylene (HDPE) sample

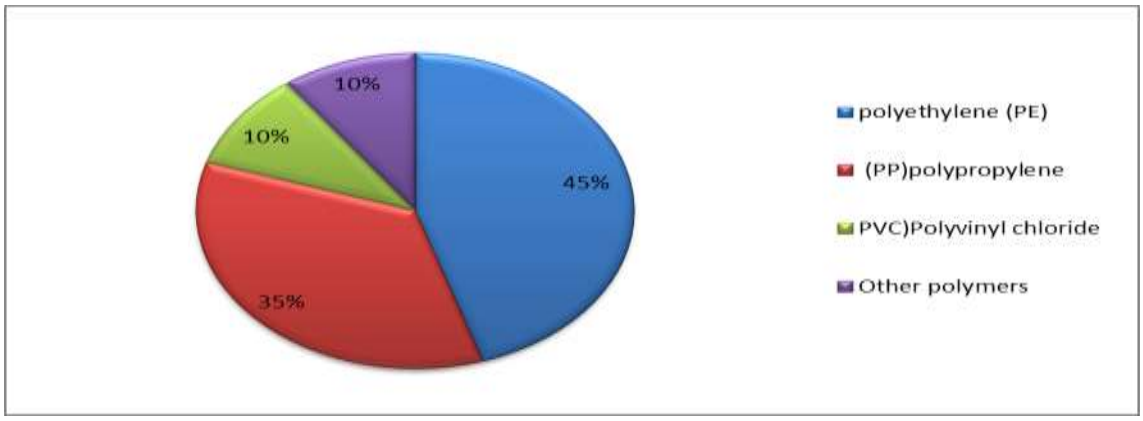


chart 1: showing the percentage of microplastics detected.

Microplastics and bacteria relationship

Linear regression analysis was used to assess the relationship between microplastic concentration and bacterial growth. The results showed a statistically significant positive relationship ($p < 0.05$) between microplastic concentration and bacterial colony count, suggesting that microplastics may provide a favorable surface for bacterial growth**.**

The study reports a strong positive correlation between the concentration of microplastics (MPs) and bacterial abundance as shown in Table 4 "which presents Pearson's correlations among MPs and other parameters", this suggesting that high concentrations of plastic particles may influence bacterial activity this

is attributed to the role of MPs as mediators of bacterial growth by providing a surface for microorganisms to adhere to or increasing the availability of organic matter degraded from plastic. Zhang,*et al.*(2021) and (Du *et al.*(2021), have described MPs as a novel habitat for microbial community; this habitat known as the "plastisphere" Furthermore, several recent studies; including Keswani ***et al.*** (2016), have demonstrated that bacteria and fecal indicator organisms (FIOs), such as ***Escherichia coli,*** can form biofilms on plastic pipes used in water distribution systems. (Keswani *et al.*, 2016) Turbidity showed the strongest correlation with MPs, suggesting that plastic particles directly contribute to increased water turbidity, either through their suspension in the water column or their interaction with other suspended particles. This result is consistent with previous studies linking plastic pollution to the deterioration of water optical properties (Moore *et al.*,2001, Cole *et al.* 2011).

Table 4: The Pearson's Correlations among all the parameters and MPs

Parameter1	Parameter2	Pearson Correlation	Sig. (2-tailed)	explanation
MPs	Bacteria	0.568*	0.043	Increased plastic particles may provide a surface for bacteria to grow
MPs	(TUR)	0.699*	0.011	Plastic particles increase water turbidity.
MPs	(W.T.)	0.423	0.170	High temperature may increase the decomposition of plastic.
MPs	(pH)	0.119	0.713	There is no significant effect of pH on the concentration of plastic particles.
MPs	(SAL)	0.391	0.208	Increased salinity may affect the stability of plastic particles.
MPs	(DO)	-0.428	0.165	Increased plastic particles may reduce the amount of dissolved oxygen in the water.
MPs	(BOD)	0.127	0.694	There is no significant effect on the concentration of plastic particles.

*. Correlation is significance at the 0.05 level (2-tailed)

The Results indicate that microplastics may pose a potential health risk, particularly to children, who constitute a large proportion of schoolchildren. These particles can cause health problems, such as gastrointestinal infections and immune disorders, if swallowed or inhaled. Additionally, plastic particles may act as vectors for pathogenic bacteria; increasing the risk of disease this is evident from Table (5), which compares tap water and tank water in terms of bacterial and plastic contamination.

Table5: Comparison between tap water and plastic tank water in terms of the number of bacterial colonies, plastic particles,

Factor	Tap water	Plastic tank water
Colony count (CFU/ml)	Low	High
Plastic particle concentration (MPs/L)	Low	High
Bacterial species detected	E. coli	E. coli.

CONCLUSION

During the current study, the following conclusions can be drawn:

1. Water samples collected from school tanks containing microplastic contamination had higher bacterial growth rates than the rest of the samples that did not contain microplastic.

2. The deterioration of water distribution network infrastructure is a major factor, as aging pipes and water leakage lead to drinking water mixing with polluted sources such as sewage or contaminated groundwater.

3. Climatic conditions play an important role in exacerbating the pollution problem, as high temperatures in the summer accelerate the decomposition of plastic materials used in water tanks, which increases the concentration of microplastic particles in stored water.

Recommendations

The following recommendations can be used in light of the results of this study:

1. The need to work on adopting environmental and health conditions related to drinking water tanks, while giving great importance to the supervisory and awareness role by the relevant supervisory authorities such as the Ministry of Health and the Ministry of Environment and School Health.

2. The manufacture of drinking water tanks from materials that are not susceptible to corrosion and rust, so as not to affect the natural properties of the water, and in a way that facilitates the exchange of water and cleaning it easily and a cover attached to it.

Renew and replace the networks for transporting water with modern networks, taking into account their distance from sewage drainage networks

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Competing Interests The authors declare that they have no competing interests.

Data Availability All data generated or analyzed during this study are included in this article or are available from the corresponding author upon reasonable request.

Author Contributions

Entesar Shehab Ahmed: Led the research design, methodology development, data collection, analysis, and initial manuscript preparation.
Jinan S. Al-Hassany: Provided continuous academic supervision, insightful guidance, and contributed to the critical revision of the manuscript.

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