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Assessing the Microbial Safety of Drinking Water in Basrah Province: A study of Three Water Treatment Plants

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Abstract. Investigation of the bacterial factors affecting the three main drinking water purification stations (Al-Qurna, Al-Abbas, and Al-Buradieiah) located in various regions of Basrah Governorate was done. Water samples were taken monthly from September 2019 to August 2020. Water samples from the three stations, before and after purification (raw and treated water) were all subjected to microbial investigations that included estimation of the total viable microbial counts, total coliform, total fecal *Escherichia coli*, *Salmonella* and *Shigella* bacteria, and other dangerous bacteria that might be present. The current findings show that all of the stations under study had ineffective purification processes, resulting in pathogen levels in drinkable water that was higher than the limit set by Iraqi criteria and WHO's desirable limits for drinking water. In the evaluation of all WTPs' efficacy in terms of microbial elimination, the results show an efficiency rate ranging from 92.09 to 99.72.

Keywords. Evaluation, Water Quality Treatment Plants, Basrah Province.

1. Introduction

Water is an essential factor to all life forms on the earth and is associated directly or indirectly with every aspect of human activities. The current issue facing many nations is their decreasing supply of water [1]. One of the most basic human needs is access to safe water in sufficient quantities for drinking, cooking, personal hygiene, and sanitation [2]. Since the industrial revolution, new pollution sources have risen daily, leading to contamination of the environment. Air and water are the most potential target for this pollution. The increase in water-borne diseases considers an obvious indicator of the environment's pollution level [3] [4].

Access to clean, safe drinking water has indeed been proclaimed a fundamental human right by the United Nations and many countries, as well as an important step toward improving living standards worldwide. According to the United Nations and WHO, 780 million people around the world still lack access to safe drinking water [5-7]

According to WHO, approximately 80% of human diseases are caused by contaminated water [8] [9]. Therefore, the regular testing of water quality is a fundamental issue to prevent water-borne diseases [10] and improving quality of life [11] [12]. Water-borne pathogens' impact is due to the morbidity and mortality they cause and the high cost of their prevention and treatment [10].

The main goal of the current study is to assess the performance of three of Basrah's main water treatment plants based on bacteriological properties as there have been insufficient previous studies on this important public health and environmental issue in the Basrah Province.



2. Material and Methods

This study was undertaken from September 2019 until August 2020 on three main water treatment plants (WTPs) in Basrah Province including Al-Buradieiah, Al-Abbas, and Al-Qurna WTPs. Those stations were chosen due to their location, importance, and source of raw water. Each of these is supplied with water from a different source of water, as Al-Qurna WTP is supplied with water from the Tigris River and Al-Abbas WTP from the Al-Bada'a Canal (sweet canal) which takes its water from the Al-Gharraf river, while the raw water of Al-Buradieiah WTP mainly comes from the Shatt Al-Arab River (SAAR). All these WTPs use the same conventional treatment technique.

Basrah is one of the South Iraq Provinces, located at the Shatt Al-Arab River (SAAR) where the Tigris and Euphrates rivers met. The area of the province is (19,070 km²) and the population of Basrah is around 4 million.

Table 1. The water treatment plant used in this study.

Water treatment plant	Longitude	Latitude	Established year	Water source
Al Qurna	47.1727	30.537	1977	Tigers River
Al-Abbas	47.7094	30.5302	1990	Freshwater Canal
Al-Buradieiah 1	47.855	30.503	1957	Shatt Al-Arab River

2.1. Sampling Strategy and Procedure

The raw and treated water samples were collected monthly from each station for investigating bacteriological properties. To achieve this, MPN methods were used for this purpose.

The samples were collected before and after treatment for all selected WTPs as a follow:

Stage 1: Raw water before treatment (R).

Stage 2: Water after treatment with chlorine and before pumping station to distribution network termed (T1)

Stage 3: Two home tap water samples are situated in different residential areas fed by each WTP and at different distances from the WTP termed T2 (a point near the water treatment plant) and T3 (a point away from the water treatment plant).

All samples were taken with three replicates of each site and each season as a follow:

Samples were collected in sterilized glass bottles of 250 ml for bacteriological tests. The bottles were then kept in an icebox until examination. Two procedures were used to collect the water samples using the bottles include:

- Raw water was collected directly from the river by holding the bottle in hand near its base and plunging it below the surface, neck down. The bottle has been turned slightly upward until the neck points, and the mouth is directed towards the current.
- Treated water samples were collected from house tapes supplied by the municipal water plants' distribution network. Water from home storage tanks was not included in this sampling technique. Fully opened the water tap and allowed the water to run to waste for at least 2 minutes to eliminate any waste associated with the tap water. After reducing the water flow, water samples were then collected to fill the sampling bottle thoroughly without splashing. Disinfect if the clean tap was questionable (inside and outside).

Sample air was left in the bottle (at least 2-5cm) in the two collected processes to facilitate mixing by shaking before the examination. The bottles were tightly closed and taken to the laboratory in a cooling box for a period not exceeding 24 hours to conduct bacteriological measurements [13]. The most Probable Number (MPN) method was used to assess the bacteriological water quality.

The drinking water testing procedure was performed aseptically. The MPN method has been carried out in different steps as fellows [13].

Lauryl broth media was prepared with Durham's tube in test tubes and autoclaved. Three sets of test tubes were prepared, each containing five tubes with 10 ml of broth. Ten ml of water sample was transferred to each of the first sets of test tubes using sterile pipettes. One ml and 0.1 ml were transferred to the second and third set of tubes, respectively. All tubes were then incubated at 37°C for 24 hours. After incubation, gas production in Durham's tube and the change of media color (positive

result) were checked. Positive results were compared with the WHO stander chart as acid and gas. Sugar fermentation is also produced by some microorganisms other than coliform. Confirmatory testing was performed to confirm the presence of coliforms. For this, a loopful of positive broth suspension was inoculated into EMB agar.

Moreover, *Salmonella-Shigella* (SS) agar was used to confirm *Salmonella's* presence and *Shigella* types in water samples. Produced colonies demonstrated positive results for the presence of coliform bacteria with a greenish metallic sheen. On the other hand, the presence of colonies with a black center on SS agar was referred to as *Salmonella's* presence, and light colonies represented *Shigella* in the water sample. Negative results were recorded by the absence of growth and gas formation in the broth and gram, non-spore-forming rods on Gram staining.

2.2. The Efficiency of the Water Treatment Plants

The efficiency (E%) of the water treatment plants was measured for the removal of bacteria according to the formula that was used to calculate the efficiency by Al-Fatlawy [14].

2.3. Statistical Analysis

Statistical package for social sciences (SPSS) IBM Statistics 22 was applied. The data were analyzed with a one-way analysis of variance (ANOVA), using complete randomized design (CRD) and Least Significant Difference (LSD) under the level of significance (0.05) among water treatment plants during the study period. The mean, standard deviations (SD), and correlation coefficient were calculated.

3. Results and Discussion

The current results show that the residual chlorine values of the treated waterfall are between (0.0) and (3.4) mg/L. The maximum mean value was recorded in site T1 of Al-Qurna WTP during Winter, while the minimum mean value was detected in the water samples of most treated water sites during Summer. Analysis of the variance of these data reveals significant differences ($P \leq 0.05$) among seasons only at Al-Qurna WTP.

3.1. MPN Method for Total Coliform (TC) Detection

In this study, the TC of raw water samples was 900 MPN/100ml using MPN methods as a maximum mean value was observed during Winter and Spring at Al-Qurna station and during Winter at Al-Buradieiah station. A minimum means the value of 170 MPN/100 ml was recorded at Al-Abbas station during the Summer.

Concerning treated water sampled from different sites, the maximum mean of TC value 170 MPN/100 ml was recorded in Autumn from site T3 of the Al-Abbas WTP distribution system. In contrast, the minimum TC value of 0.0 MPN/100 ml was detected at all seasons in water samples from sites T1 and T2 of the Al-Qurna WTPs.

Higher TC content was observed in the study area located at the Al-Buradieiah and Al-Qurna WTPs intake rivers. This is attributed to the discharge of effluent enriched with urban organic matter that plays an essential role in the severity of water source fecal contamination.

The statistical analysis has found significant differences ($P \leq 0.05$) in TC water content among seasons (Fig. 1), as well as between raw and treated of each station. This study has found significant differences ($P \leq 0.05$) in raw water TC content between Al-Qurna and Al-Buradieiah compared to Al-Abbas station. The same significant differences ($P \leq 0.05$) were also found in terms of treated water but between Al-Buradieiah and Al-Abbas WTPs compared to Al-Qurna WTP, which has less content TC (Tables 1-4).

For both raw and treated water, bacteriological water testing for the total coliform count was performed using the MPN method.

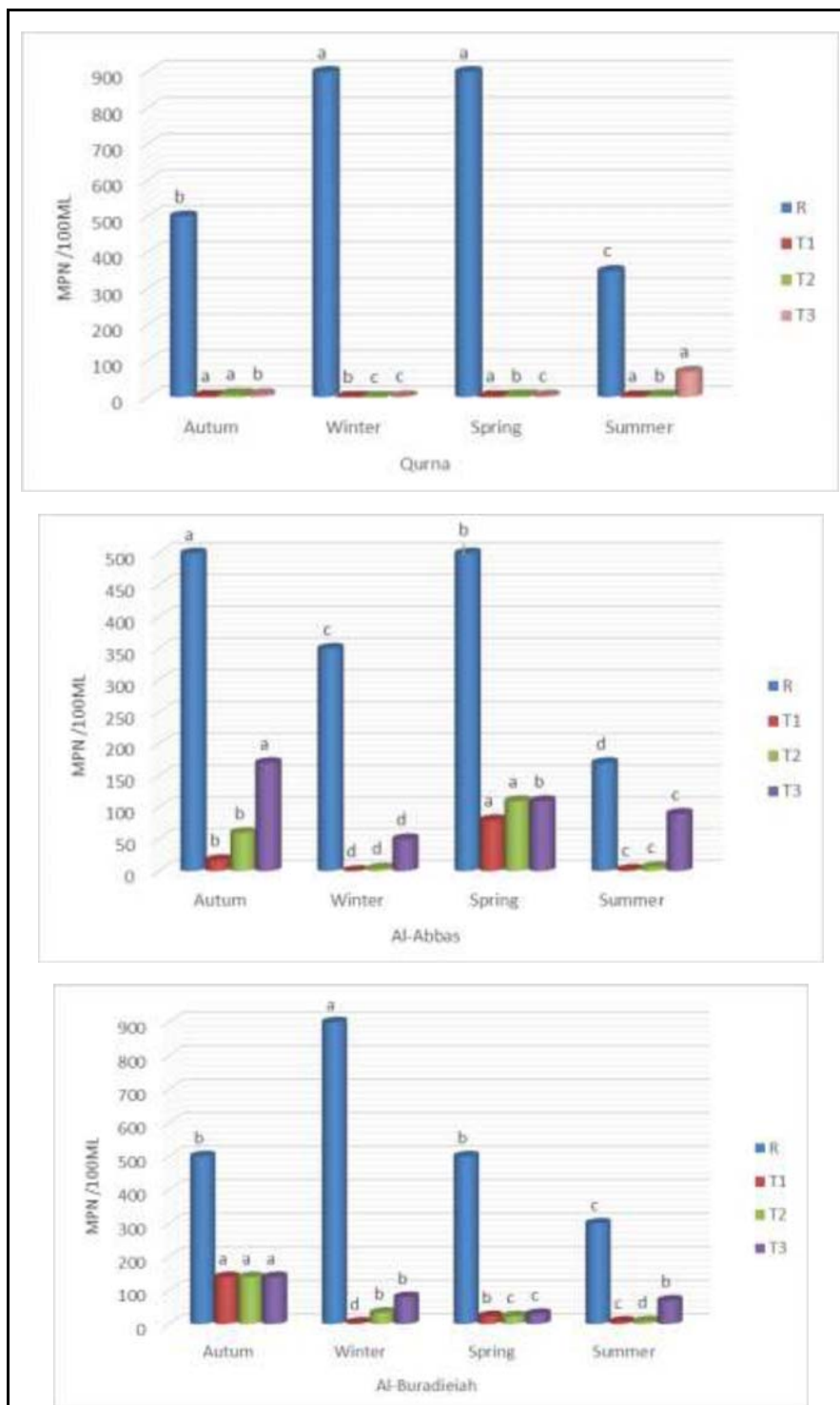


Figure 1. Total coliform value (MPN/100ml) in WTPs during the study period.

The presumptive MPN method coliform count was shown in Tables 1, 2, 3, and 4. Raw water had a total coliform count ranging from 170 MPN/100 ml to 900 MPN/100 ml, while the range of total treated water coliforms was between (<2-280 MPN / 100ml) (Fig. 1).

The presence of the fecal contamination indicator organism *Escherichia coli* isolates was confirmed by the production of a greenish metallic sheen on EMB media, as shown in Figure 2.

The confirmed MPN method test results for raw water have been presented in Table 1, while the confirmed MPN method for treated water sites, T1, T2, and T3, have been presented in tables 2, 3, and

4 respectively. MPN tests have been established for the possible presence of indicator species by fermentation of lactose with acid and gas production at 37°C.

However, the MPN method does not detect lactose-negative *Salmonella* and *Shigella* species that can cause waterborne outbreaks of diseases. Therefore, determination of the presence of other microbial contamination, and different types of contaminating bacteria, including *Salmonella* and *Shigella*, were also investigated. The presence of these bacteria (non-lactose fermentation bacteria) was confirmed using specific selective media named *Salmonella-Shigella* agar (SS agar). The colorless production with black centers colonies on SS agar media refers to *Salmonella*'s presence, while colorless colonies indicate *Shigella*, as shown in Figure 3. The black centers of colonies attributed to the production of hydrogen sulfide (H₂S) by *Salmonella* but not by *Shigella*. In addition, coliform bacteria such as *E. coli* can also grow in SS agar, resulting in bacterial growth with a pink color due to the fermentation of lactose in media but without producing hydrogen sulfide.

Table 2. Raw water (R) total coliform count by MPN method.

Station	Season	Combination of positive	MPN Index per 100 ml	Limit Upper-lower	Confirmed test		Results
					EMB Agar	SS Agar	
Al-Qurna	Autumn	05-05-02	500		+	+	Unacceptable
	Winter	05-05-03	900		+	+	Unacceptable
	Spring	05-05-03	900		+	+	Unacceptable
	Summer	05-04-04	350		+	+	Unacceptable
Al-Abbas	Autumn	05-05-02	500		+	+	Unacceptable
	Winter	05-04-04	350		+	+	Unacceptable
	Spring	05-05-02	500		+	+	Unacceptable
	Summer	05-03-03	170		+	+	Unacceptable
Al-Bradiciah	Autumn	05-05-02	500		+	+	Unacceptable
	Winter	05-05-03	900		+	+	Unacceptable
	Spring	05-0502	500		+	+	Unacceptable
	Summer	05-0501	300		+	+	Unacceptable
Percentage WHO Limit		0 MPN/100 ml			100%	100%	100%

Table 3. Treated water (T1) total coliform count by MPN method.

Station	Season	Combination of positive	MPN Index per 100 ml	Limit Upper-lower	Confirmed test		Result
					EMB Agar	SS Agar	
Al-Qurna	Autumn	01-0-0	2	1-11	-	-	Unacceptable
	winter	0-0-0	<2	-	-	-	Acceptable
	spring	01-0-0	2	1-11	+	-	Unacceptable
	summer	01-0-0	2	1-11	+	-	Unacceptable
Al-Abbas	Autumn	03-02-01	17	7-40	+	+	Unacceptable
	Winter	0-0-0	<2	-	-	-	Acceptable
	Spring	05-03-0	80	30-250	+	+	Unacceptable
	Summer	01-0-0	2	1-11	-	-	Unacceptable
Al-Buradiciah	Autumn	05-03-02	140	60-360	+	+	Unacceptable
	Winter	02-0-0	4	1-17	+	+	Unacceptable
	Spring	05-0-0	23	9-86	+	+	Unacceptable
	Summer	02-01-0	7	2-21	+	+	Unacceptable
Percentage WHO Limit		0 MPN/100 ml			66.6%	50%	83.3%

Additional complete identification of *Escherichia coli*, *Salmonella*, and *Shigella* isolates were performed by gram staining as gram-negative, rod cells bacteria visualized by light microscope

The current study of the MPN method bacteriological analysis of raw water found that (100% percent) of all stations' raw water samples were contaminated with coliform bacteria and *Salmonella* or

Shigella species (Table 1). Representatives from different treated water sites show different microbial contamination levels as 66.6% of site T1 samples were contaminated with *E. coli*, while 50% of samples were positive for *Salmonella-Shigella* media (Table 2). Regarding samples from site T2, this study recorded that 91.6% of samples were positive for *E. coli* and 83.3% for *Salmonella* or *Shigella* species (Table 3). Compared with T1 and T2 sites, site T3 revealed a high level of contamination as 91.6% contained *E. coli* as well as *Salmonella* or *Shigella* (Table 4). In general, 100% of raw water samples and 83-100% of treated water samples of all stations have not reached the standard of Most Probable Number (MPN) per 100 ml of water recommended by WHO drinking water guidelines (WHO, 2018). The presence of bacterial indicators indicated the possible presence of fecal contamination.

A similar problem like bacterial contamination in drinking water was also found in other local studies like [16] and [17].

High coliform content of treated water was found in the Autumn seasons may be due to several factors, such as adequate growth temperatures, microorganism activity, and increased concentrations of organic nutrients and salts in surface water sources, resulting in an increase in biomass in water sources [17,18].

Table 4. Treated water (T2) total coliform count by MPN method.

Station	Season	Combination of positive	MPN Index per 100 ml	Limit Upper-lower	Confirmed test		Results
					EMB Agar	SS Agar	
Al-Qurna	Autumn	02-01-01	9	3-24	+	+	Unacceptable
	Winter	0-0-0	<2	-	-	-	Acceptable
	Spring	02-0-0	4	1-17	+	+	Unacceptable
	Summer	02-0-0	4	1-17	+	+	Unacceptable
Al-Abbas	Autumn	05-01-02	60	30-180	+	+	Unacceptable
	Winter	01-0-0	2	1-11	+	-	Unacceptable
	Spring	05-03-01	110	40-300	+	+	Unacceptable
	Summer	01-02-0	6	2-18	+	+	Unacceptable
Al-Buradiah	Autumn	05-03-02	140	60-360	+	+	Unacceptable
	Winter	04-03-01	33	15-77	+	+	Unacceptable
	Spring	05-0-0	23	9-86	+	+	Unacceptable
	Summer	3-0-0	8	3-24	+	+	Unacceptable
Percentage WHO Limit	0 MPN/ 100 ml				91.6%	83.3%	91.6%

Table 5. Treated water (T3) total coliform count by MPN method.

Station	Season	Combination of positive	MPN Index per 100 ml	Limit Upper-lower	Confirmed test		Result
					EMB Agar	SS Agar	
Al-Qurna	Autumn	02-02-0	9	3-25	+	+	Unacceptable
	winter	01-0-0	2	1-11	-	-	Unacceptable
	Spring	02-0-0	4	1-17	+	+	Unacceptable
	Summer	05-02-01	70	30-210	+	+	Unacceptable
Al-Abbas	Autumn	05-04-01	170	70-480	+	+	Unacceptable
	Winter	05-04-03	280	20-150	+	+	Unacceptable
	Spring	05-03-01	110	40-300	+	+	Unacceptable
	Summer	05-02-02	90	40-250	+	+	Unacceptable
Al-Buradiah	Autumn	05-03-02	140	60-360	+	+	Unacceptable
	Winter	05-02-02	90	40-250	+	+	Unacceptable
	Spring	05-01-0	30	10-120	+	+	Unacceptable
	Summer	05-02-01	70	30-210	+	+	Unacceptable
Percentage WHO Limit	0 MPN/ 100 ml				91.6%	91.6%	100.0%

Treated water (T2 and T3) was contaminated with bacteria, and this may be due to fractures and defects affecting the pipeline of the distribution system. However, previous studies have reported similar findings, such as Eassa [16].

The presence of coliform bacteria in treated water samples may be due to the re-growth of those bacteria in the distribution system as a result of insufficient chlorination, insufficient contact time, and poor maintenance of service reservoirs [20].

The results of this study show that according to the Iraqi Criteria and WHO Standards, which is zero MPN/100ml, several drinking water samples have exceeded the allowable limit for the validity of treated water.



Figure 2. *E. coli* isolate by the production of greenish metallic sheen on Eosin Methylene Blue (EMB) agar.

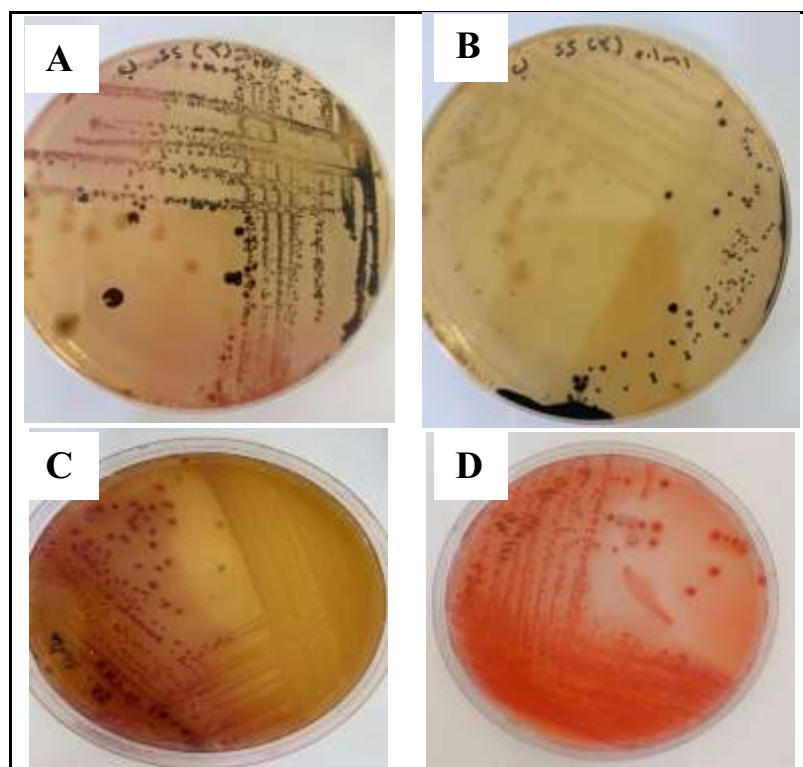


Figure 3. Colonies of *Salmonella* sp. (A,B), *Shigella* sp. (A,B and C) and *E. coli* (C and D) in SS agar.

The efficiency range of the bacteria removal by WTPs (Table 5) was between 92.1-99.7%, where the Al-Qurna showed the highest percentage at 99.7%. This may be because the Al-Qurna WTP used a high dose of chlorine that affected bacteria compared with the rest WTPs.

Table 6. The percentage of efficiency of WTPs MPN.

CFU				
Station	Season	Raw Water	Treated Water	Efficiency
Al-Qurna	Autumn	500.0	3.3	99.33
	Winter	900.0	0.0	100.00
	Spring	900.0	2.2	99.78
	Summer	350.0	2.0	99.43
Mean		662.5b	1.8c	99.72
T-Test (P<0.05)		0.001		
Al-Qurna	Autumn	500.0	17.0	96.60
	Winter	350.0	0.0	100.00
	Spring	500.0	80.0	84.00
	Summer	170.0	2.0	98.82
Mean		380.0c	24.8 b	93.48
T-Test (P<0.05)		0.001		
Al-Qurna	Autumn	500.0	140.0	72.00
	Winter	900.0	4.0	99.56
	Spring	500.0	23.0	95.40
	Summer	300.0	7.0	97.67
Mean		550.0 a	43.5 a	92.09
T-Test (P<0.05)		0.001		

Similar letters in the column mean no significant difference between them according to Duncan's multiple range test ($P \leq 0.05$).

To ensure that water is safe to drink in terms of bacteriological composition, chlorine must be used. The values of free chlorine in the current investigation ranged from zero at most WTPs to 3.4 mg/l at the Al-Qurna WTP, above the [15]. recommended residual chlorine range of 0.2-0.5 mg/l for water that is centrally treated at the point of delivery. The present study concludes that all WTPs did not use the recommended chlorine for disinfection because all sampling sites revealed free residual chlorine, with the majority of them falling below the set target of 0.5 mg/l. Hence, the maintenance of residual chlorine must be maintained at all points of the distribution pipelines to prevent microbial growth. As a result, pathogens survive in the drinking water distribution system and reach customers at the tap if no residual disinfectant is used as recommended [21].

In regards to bacteriological examination, the research area, which included the Al-Buradieiah and Al-Qurna WTP intake rivers, had a higher TC level. This is due to the discharge of effluent enriched with urban organic matter, which plays an important role in the severity of fecal pollution in water sources. The current study of the MPN method bacteriological analysis of raw water found that (100% percent) of all stations' raw water samples were contaminated with coliform bacteria and *Salmonella* or *Shigella* species (Table 1). Representatives from different treated water sites show different microbial contamination levels as 66.6% of site T1 samples were contaminated with *E. coli*, while 50% of samples were positive for *Salmonella-Shigella* media (Table 2). Regarding samples from site T2, this study recorded that 91.6% of samples were positive for *E. coli* and 83% for *Salmonella* or *Shigella* species (Table 3). Compared with T1 and T2 sites, site T3 revealed a high level of contamination as 91.6% contained *E. coli* as well as *Salmonella* or *Shigella* (Table 2). In general, 100% of raw water samples and 83-100% of treated water samples of all stations have not reached the standard of Most Probable Number (MPN) per 100 ml of water recommended by WHO drinking water guidelines [15]. The presence of bacterial indicators indicated the possible presence of fecal contamination.

The high coliform content of treated water found in the Autumn months could be due to several factors, including adequate growth temperatures, microorganism activity, and increased concentrations of organic nutrients and salts in surface water sources, all of which could lead to an increase in biomass in water sources [18] [19]. Besides, bacterial contamination was found in treated water (T2 and T3), which could be attributed to fractures and flaws in the distribution system's pipeline. Moreover, the presence of coliform bacteria in treated water samples could be attributable to bacteria re-growing in the distribution system as a result of insufficient chlorination, insufficient contact time, and poor service reservoir management [20].

Conclusion

The study concludes that the use of chlorine is essential to ensure the safety of drinking water. However, all water treatment plants did not use the recommended chlorine for disinfection, and most sampling sites fell below the set target of 0.5 mg/l. The discharge of effluent enriched with urban organic matter contributed to fecal pollution in water sources. The presence of bacterial indicators in treated water samples indicates possible fecal contamination. The high coliform content in treated water during Autumn may be due to several factors. Bacterial contamination in treated water may be due to pipeline flaws, insufficient chlorination, contact time, and reservoir management.

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