ORIGINAL ARTICLE

Detection of Biological Contamination in Drinking Water Tanks and Sediments in some Areas of Mosul

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ABSTRACT

This study was conducted in 10 selected areas, three on the east side of Mousl city, three on the west side of the City and four areas in Badoush which affiliated to the center of Mousl. Some physical and chemical tests were performed on water samples, such as pH and electrical conductivity, because of their importance and their impact on the properties of water. The results showed that the values of pH ranged between (7.5 - 8.4), the electrical conductivity values between (263.3 - 392.4) µs/cm, The results of biological tests also showed the presence of small number of unsatisfactory TPC bacteria in all samples while no numbers of fecal coliform bacteria or any other pathogenic bacteria appeared, some types of fungi also appeared in 7 samples, one sample had a type of yeast and tow samples were free of fungi and yeast. The results were within the permissible limits according to Iraqi or international standard, as for the fungi and yeast that appeared in the samples there are no determinations about them in the Iraqi and international specifications.

Keywords: drinking water tanks, fungi, fecal coliform bacteria, biological contamination.

INTRODUCTION

Water is precious for life, since the dawn of human civilization, water supply is an integral part of society for various purposes like, drinking, agriculture, industry, household, etc, much of the health problems in the developing countries are largely due to the unavailability of safe drinking water⁽¹⁻³⁾.

The provision of safe drinking water has been one of humanity's most successful public health interventions and is a defining aspect of a developed country, nonetheless, ignorance of the potential risks and inappropriate training of staff and mangers working on drinking water systems still results in unnecessary waterborne disease outbreaks in affluent communities⁽⁴⁾.

Bacterial contamination of drinking water is a major contributor to waterborne diseases in rural areas of most developing countries where water sources are communally shared^(5,6), and exposed to multiple faecal – oral transmission path ways in their neighborhood boundaries^(7,8),waterborne illnesses caused by bacteria in contaminated household water tanks increases the risk of spreading waterborne diseases and may lead to many infectious outbreaks, World Health Organization (WHO) data on the burden of disease suggest that approximately 3.2% of deaths (1.8 million) and 4.2% of disability – adjusted – life years (61.9 million) worldwide are attributable to unsafe water, sanitation and hygiene⁽⁹⁾.

The source of microbiological pollution is often inadequately treated human sewage or runoff from animal husbandry facilities into streams or lakes, in addition, some microbial populations can increase in drinking water distribution systems, other factors may also influence microbial levels including, wild animals are reservoir for bacteria or protozoa that can infect humans, variations in turbidity or water chemistry can effect bacterial densities and algal blooms may increase bacterial abundance⁽¹⁰⁾.

Many studies have been conducted in different countries of the world about the biological pollution of water, such as UAE, Nepal and Nigeria⁽¹¹⁻¹³⁾. There are many studies about biological pollution of Iraqi rivers such as Tigris, Euphrates and Shatt AI – Arab⁽¹⁴⁻²⁰⁾.

The aim of the current study is to investigation of the biological pollution of drinking water in some areas in Mousl city and the identification of some physical and chemical properties of the water under study.

MATERIALS AND METHODS

Area of study: The samples in this study were collected from some areas of the western, eastern sides of Mosul city and Badoush region, which belongs to Mosul district center. 10 samples of sediment were taken from drinking water tanks from the targeted areas in the study, which are: (17 Tammuz, Musharrifah, Al-Yarmwk, Yarimjah Al Sharqiah, Al-Nwr, Al-Bakr and the other four samples were collected from Badoush area).After less than twenty-four hours, these samples were transferred to the laboratory for the purpose of conducting tests and measurements on them.



Figure 1: Map shows the area of study

Collection of samples: Ten samples were collected from the selected study areas in November of 2021 by using sterile 250 ml polyethylene bottles pre-treated with sodium thiosulfate for the purpose of conducting biological tests, as well as other samples were collected in clean 250 ml polyethylene bottles for the purpose of conducting physical and chemical tests on them and from the same locations mentioned above, and the samples were kept at a suitable temperature until they were delivered to the laboratory as mentioned in⁽²¹⁾, then 10 samples of sediment were collected by using Grab Sampler in glass containers of 500 ml capacity previously sterilized by the autoclave. The samples were kept in a cooled and light-blocking container and were transferred in less than 24 hours to the laboratory for the purpose of conducting laboratory measurements and tests.

Physical and chemical analysis: pH 10 g of air-dried precipitate was taken and sieved using a 2-ml sieve and placed inside a 50-ml beaker. 10 ml of distilled water was added at a ratio of (1:1). The solution was mixed well using a glass rod and left to the next day, the mixture was filtered and the filtrate was taken. After the filtrate was ready for reading, the measurement was made using a portable pH meter (Senz pH Dou, Trans instruments) after

calibrating it with standard solutions (buffers) of different values, usually (4, 7, 9)(22).

Conductivity: In the same way that samples were prepared for measuring pH, the samples were prepared for the purpose of measuring electrical conductivity, and then a solution of potassium chloride (Kcl) was prepared at a concentration of 0.01 where 186.4 mg of potassium chloride salt was dissolved in a small amount of deionized water to dissolve the salt, and then the volume was completed using distilled water to 250 ml using a graduated cylinder of 500 ml. The reading was taken for the calibration solution that was It was prepared in advance as mentioned above by the electrical conductivity measuring device (Company loviband). The reading of the device was 954 µs at a temperature of 13.8 °C. Knowing that the concentration of 0.01 of potassium chloride solution at a temperature of 25 °C (standard temperature) is 1413 µs, the conductivity of the prepared and prepared soil samples was measured. We took measurements by electrical conductivity device on the next day with three readings for each sample, and then we took the average readings for each sample in order to reduce the error rate and to obtain more accurate results. After that we obtain the final result for each sample accourding to the law below^(23,24).

Ec for sample

standard Kcl titration solution from device reading

Temperature: The temperature was measured using a mercury thermometer (Company Worner Lab).

Turbidity: The samples were shaken by turning them upside down three to five times and then left to settle. After that, readings were taken using a Turbidity meter TB300IR (company loviband). The device was calibrated with the special solutions attached to the device after shaking it and leaving it to settle, making sure that it was free of bubbles, and then we added the sample in the measurement tube. After cleaning it well with distilled water, calibration is performed after each reading with the previously mentioned solutions (800 NTU, 200 NTU, 20 NTU, > 0.1)(25)

Salinity: Salinity was calculated in terms of electrical conductivity by applying the equation mentioned below based on the method of (26 28)

Salinity =
$$\frac{\text{Elcetrical condactivity value} - 14.78}{1589.08}$$

Detection of biological contamination: Detection of biological contamination in water For the purpose of isolating fungi and bacteria from water samples, 10 ml of water was taken and placed in a sterile 100 ml glass beaker containing 90 ml of saline solution (N.S). The sample was shaken gently to homogenize the mixture and then 1 ml of the diluted solution10-1 was taken and added to sterile tubes containing 9 ml of saline solution to obtain a dilution of 10-2. The process was repeated by transferring 1 ml of dilution 10-2 to a new tube, to which 9 ml of saline was added, and thus we obtained a dilution of 10-3 by transferring 1 ml of the prepared dilutions to Petri dishes on which laboratory examinations were carried out in the presence of a comparison sample(Control) containing saline solution only. At least two replicates were made. For bacteria, petri dishes were incubated one to two days at 32 °C, and fungi, they were incubated for 7-9 days at 28 °C. (17).

Detection of biological contamination in sediments: Dilution plate method Fungi and bacteria were isolated by the same method used to isolate fungi and bacteria from water, but distilled water was used instead of saline solution.

Direct method of detection through soil samples: In this method, fungi and bacteria were isolated by transferring an appropriate amount of soil approximately 1 gm and spreading it on Petri dishes containing the nutrient medium, pre-equipped for this purpose.

purification and diagnosis: The purification process is carried out by transferring microorganism into fresh nutritive medium from its

stock culture(Sub-culturing). Using PDA medium to purify the fungi, bacterial isolates were purified on N.A medium. The pure isolates of both bacteria and fungi were preserved by making slant medium by adding N.A. medium and PDA medium into sterilizable plan tubes. It was placed in the sterilizer after adding the medium to it, at a temperature of 121 ° C and a pressure of 1 atmosphere, for 15 minutes, and after the sterilization was completed, it was placed diagonally on a special rack at an angle of approximately 5 ° inclination and left for the second day. The pure isolates were added to the prepared tilted media as mentioned above by means of a ring A sterile inoculation loop was kept in the refrigerator, with information and the date of adding the isolate to the slant, and it is renewed every month and used when needed. Through the use of a compound light microscope, methylene blue stain was used to identify the shape of the bacterial cell. A Gram stain was used to differentiate between gram-positive bacteria and gram-negative bacteria, Lactophenol cotton blue solution was used to identify the shape of the fungal hyphae and some types of the fungal hyphae were identified without adding stain^(29,30).

RESULT AND DISCUSSION

The results of physical and chemical analysis in this study showed that the water temperature ranged between (17-18.5)°C, As shown $= \frac{\text{mesured sample by device } \times \text{ standard Kcl concentration 0.1 at 25°C } \times \text{Varjation and the inference of the lowest temperature was recorded in the}$ Mushairfa area and the highest temperature was recorded in Yarimjah Al Sharqiah area, the water temperature was close between all areas of the study. Whereas the average temperatures were (18) and (19) degrees Celsius, respectively, and the average water temperature was (21.32, 23) °C. Turbidity ranged between (0.9-4.81) NTU, The lowest value of turbidity was recorded in the central Badoush region and the highest value in the region of Yarimjah Al Sharqiah, and the average turbidity values were (2.76) NTU. The turbidity values varied between the different study areas, and all values were within the WHO determinants, which determined the highest value for turbidity 5 NTU⁽³¹⁾ . This result agreed with the results of $^{(32)}$ and the results of $^{(33)}$, where the average turbidity values were (2.5) and (3.5) NTU, respectively, while this result did not agree with the results of (34) and the results of^(35_38), where the average turbidity values were (15) and (18.3) NTU, respectively, and Table (1) shows the turbidity values in the study areas. The electrical conductivity ranged between 263.3 and 392.4 µs/cm, The lowest value was recorded in Al-Nwr and the highest value was in the region of Yarimjah Al Sharqiah, and the average electrical conductivity values were (316.12) µs/cm, and the results were close in all study areas with some slight differences, and these results were within the Iraqi and international determinants, which determined the highest conductivity of 2000 µs. The water quality in the experiment within these results is considered to have good taste and high acceptability because electrical conductivity values are one of the most important factors for determining water quality. These results converge with the results of $^{(39)}$ (451.3) µs, while they do not agree with the results of ⁽³⁴⁾ and the results of ⁽⁴⁰⁾ where the results were (1166.10) and (7900) µs / cm respectively pH values ranged between (7.5 - 8.4) The lowest value was recorded in the central Badoush region, and the highest value was recorded in 17 Tammuz region The results are similar in all regions with slight relative differences, and the water in this study tends to be light basal, but it is within the parameters of the World Health Organization, as its determinants are (8.5-6.5). The average pH in this study was 7.99. The results of this study agreed with the results of [31] and the results of [28], where the pH values were (7.91) and (7.8), respectively, while they did not agree with the results of the study of [32] and the results of the study of [27], where the results were 6.95) and (7.6), respectively, and salinity values ranged between (0.156-0.237). The salinity values depended on electrical conductivity. If the value of electrical conductivity increases, the value of salinity also increasesand vice versa^(41,42). Table (1) indicates that the salinity values ranged between (0.156) and (0.237) parts per thousand, and the lowest

value was recorded in Al-Nwr neighborhood and the highest value was in the region of Yarimjah Al Sharqiah, and the average values were (0.198) parts per thousand, and the highest permissible concentration in water was 500 parts per million, i.e. (0.5 g salt/kg of water) ⁽⁴³⁾. The results of this study are acceptable and within the internationally permissible parameters, and these results are consistent with the results of the study of ⁽⁴⁴⁾, where the average salinity value was 0.274 parts per thousand, while these results differ from the results of the studies of [28] and [25], the results were (0.72) and (1.02), respectively. According to Iraqi and international standards, all the results were within acceptable limits. Chemical and physical properties were measured because they have a direct impact on water properties.

Table 1: shows the values of the following measurements (pH, conductivity, temperature, turbidity and salinity).

Sampling areas	рΗ	EC	Temp	TUR	Salinity
		µs/cm	C°	NTU	Ppt
17 Tammuz	8.4	307.1	18	2.61	0.184
Musharrifah	7.9	315	17	1.83	0.188
Al-Yarmwk	7.8	388.2	17.6	2.97	0.235
Al-Nwr	8.1	263.3	17.8	3.44	0.156
Al-Bakr	8.2	287.7	18.2	4.81	0.171
Yarimjah Al- Sharqiah	7.9	392.4	18.5	4.35	0.237
Upper Badoush	8.0	268.4	17.2	1.70	0.159
Middle Badoush	7.5	288.3	17.9	0.9	0.172
Lower Badoush	8.1	288.7	17.2	1.26	0.172
Agriculture Housing	8.0	362.1	18.2	3.79	0.218

Biological examination is one of an important examinations which give a clear view about extent of water contamination with microorganisms in water tanks in houses and residential neighborhoods, it also gives accurate information about the resulting health problems. The results of bacterial examination shown in table (2) the total number of bacteria (TPC) ranged between (3 - 8) cells/ml, all studies samples contained bacteria and these numbers were within the limits allowed by (WHO) and the central organization for standardization and quality control in Iraq (45_48). Who confirmed that the total number of bacteria must don't exceed 10 cells/ml (49). As shown in table (2) all samples are free of fecal coliform and E.coli, this gives a kind of reassurance that there is no fecal contamination and therefore the possibility of the presence of pathogens in the water studied. These results are in agreement with the results of⁽⁵⁰⁾ while they differ with the results of (51) in his study of bacterial contamination of drinking water in Najaf governorate, the results showed that the studied samples were contaminated by 53% coli bacteria and they attributed this to the dumping of sewage waste into the river directly without treatment.

Table 2: Results of bacteriological contamination of water samples in the study areas.

Location	Sampling area	TPC cell/ml	E.coli cell/10m	F. coliform Cell/100ml
west side of	17 Tammuz	4	0	0
the city	Musharrifah	4	0	0
	Al-Yarmwk	5	0	0
East side of the city	Al-Nwr	6	0	0
	Al-Bakr	8	0	0
	Yarimjah Al- Sharqiah	3	0	0
Badoush	Upper Badoush	4	0	0
region	Middle Badoush	8	0	0
	Lower Badoush	6	0	0
	Agriculture Housing	3	0	0



Figure 2: The colonies shape of bacteria under study



Figure 3: Bacillus subtilis and Bacillus mycoides under 1000x magnification power

The results of sediment samples also showed several types of fungi in (7) samples, while a type of yeast appeared in one sample and tow samples were free of fungi or yeast, where the type Aspergillus fumigatus appeared in (1,2,4,9,10), while the type Aspergillus terreus appeared in samples (2,3), the type Aspergillus niger appeared in sample (1), the type Aspergillus flavus appeared in sample(4), These types cause diseases to humans, especially people who have an altered immune system ⁽⁵²⁾, and the type Alternaria spp. appeared in sample (7), Most Alternaria species are common saprophytes that derive energy as a result of cellulytic activity and are found in a variety of habitats as ubiquitous agents of decay. Some species are plant pathogens that cause a range of economically important diseases like stem cancer, leaf blight or leaf spot on a large variety of crops (53), and a type of yeast Rhodotorula mucilagirosa appeared in sample (5), it is considered to be ubiquitous because of its worldwide distribution in terrestrial, freshwater, and marine habitats and its ability to colonize a large variety of substrates. Rhodotorula is naturally found in soil, water, and plants and is a constituent of the normal human respiratory, gastrointestinal, and genitourinary flora]. The results of ⁽⁵⁴⁾ converged with the results of this study through the emergence of several genera of fungi similar to what appeared in this study and differed with results of the studies (11, 54) due to the emergence of fungi of different types and the appearance of some types of pathogenic bacteria. The reason for the appearance of numbers of bacteria and fungi may be attributed to the presence of a break in the pipes of the water transmission network, which causes the entry of these organisms into drinking water and this is confirmed by many studies, or through negliecting the tanks cap and not closing it properly or breaking some of them, allowing entry of dust and microorganisms to water tanks (11). The researchers recommended the need to reconside the Iraqi standard specification for drinking water to include reference to the limits of the numbers of fungi in drinking water (55).

Rhodotorula mucilagirosa (Yeast)	Alternaria spp.	Aspergillus flavus	Aspergillu s niger	Aspergillus terreus	Aspergillus fumigatus	Types of fungi and yeast	
-	-	-	+	-	+	17 Tammuz	west side of
-	-	-	-	+	+	Musharrifah	the city

-	-	-	-	+	-	Al-Yarmwk	
-	-	+	-	-	+	Al-nwr	East side of
+	-	-	-	-	-	Al-Bakr	the city
-	-	-	-	-	-	Yarimjah Al- Sharqiah	
-	+	-	-	-	-	Upper Badoush	Badoush
-	-	-	-	-	-	Middle Badoush	region
-	-	-	-	-	+	Lower Badoush	
-	-	-	-	-	+	Agriculture Housing	



Figure 4: Shape of colonies of fungi and yeast on Petri dishes



Figure 5: The shape of the filaments of fungi, and yeast under the lens of a compound light microscope

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