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Estimating the Concentrations of Toxic Elements and Contaminated Bacteria of Groundwater in the City of Al-Muthanna/Iraq

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ABSTRACT

Fifty samples of water were taken from 10 wells in the southern Iraqi city of Muthanna to investigate microbiological contamination in the wells, specifically *Pseudomonas aeruginosa*, coliform, and fecal coliform bacteria. Toxic ions (Cd^{+2}, B^{+3}, pb^{+2} and No^{-3}) were examined as part of the chemical contamination in the wells. Some phenotypic diagnostic tests, namely Gram stain, were performed to identify bacteria that were Stain-negative or positive. The presumptive, confirmatory tests demonstrated the presence of coliform bacteria and fecal coliform contamination in the well water. The bacteria *P. aeruginosa* were also found and separated using Cetrimed Agar media via biochemical and cultural analyses. The average most probable number (MPN) of bacteria in the well water was found to be varied. The findings demonstrated that all the hazardous ions under investigation were present at low amounts in comparison to the standard limits established by the World Health Organisation (WHO).

INTRODUCTION

Groundwater (including that of wells) is one of the most crucial water reserves that can be used in rural areas. It is the only source of the already scarce water for irrigation, industrial, and local purposes **(Chowdhury *et al.*, 2020)**. Wells have long been considered as a source of pure water that cannot be contaminated due to their filtration effect. Studies have indicated that since wells are typically situated close to the surface of the earth, they have a high chance of being exposed to biological pollution from many sources including human activities **(Sasakova *et al.*, 2018; Ateshan *et al.*, 2019)**. Pollutants from wastewater purification plants and animal husbandry plants would leak into the wells, resulting in fecal pollution. Pollution also occurs because of the interaction between groundwater and wastewater, especially in villages and cities that do not have

modern sewage networks **(Wear *et al.*, 2021)**. Groundwater could be polluted by wastewater from the irrigation of agricultural lands which contains a high percentage of coliform bacteria and fecal coliforms. Pathogens transmitted to humans through drinking water, such as Cholera, Vibrio, *Salmonella typhi*, *Escherichia coli*, and other Shigella bacteria, may therefore be present in well water due to improper human activities **(Murphy *et al.*, 2017; Ha *et al.*, 2019; Panizzolo *et al.*, 2023)**. The presence of coliforms in well water is also an indicator of the possible presence of pathogenic bacteria. The presence of fecal coliforms in water is another indication of wastewater contamination in the well. In both cases, the water must first be sterilized before being given to animals or used by humans **(Alexander, 2002; Turner *et al.*, 2011)**. The use of chemical fertilizers and pesticides in agricultural areas where water wells are present may cause toxic ions contained in these compounds to enter the wells. Incorrect handling of wastes containing toxic elements such as lead and cadmium may cause the transfer of these elements into the soil, and subsequently reaching the groundwater **(Mench *et al.*, 1994; Ateshan *et al.*, 2020; Ying *et al.*, 2024)**.

This study aimed to examine groundwater taken from wells for the presence of chemical and microbial elements and to evaluate the findings based on international specifications and standards.

MATERIALS AND METHODOLOGY

Well water samples

Water samples taken from 10 wells in the region of Al-Muthanna were examined for the presence of chemical and microbial elements. A total of 50 samples of water were gathered, i.e., 5 samples from each well. The water samples were collected in tightly sealed and sterile glass containers, which were then placed inside plastic boxes filled with ice for preservation purposes and taken to the laboratory for analysis.

Investigating the presence of coliform

The laboratory work included a series of tests to investigate the presence of coliforms in the well water. Three tests were conducted according to **A.P.H.A (2005)**.

1. Presumptive test

This test involved the use of tryptose lauryl broth with the following ingredients (g/L): tryptose – 20.0g, lactose – 5.0g, K_2HPO_4 – 2.75g, KH_2PO_4 – 2.75g, NaCl – 5.0g, sodium lauryl sulfate – 0.1g, and distilled water – 1 liter. This culture medium was prepared and distributed into 15 test tubes, each containing 15ml of the above culture medium. For every 5 tubes, one of the dilutions of 10, 1.0, 0.1, 5ml of the well water

sample was added. Next, the tubes were shaken for five minutes and were then incubated at a temperature of 50°C to detect the presence of gas.

2. Confirmatory test

All tubes that were positive in the presumptive test, regardless of the amount of gas produced or the occurrence of acid reaction, were taken within 22 hours of incubation. The tubes with negative test results in the first examination were incubated again for 11 hours to confirm the non-formation of gas and the non-occurrence of acid reaction. Tubes with positive results in the virtual test were taken, shaken well, and then transferred once or multiple times using a sterile loop from the culture to the fermentation tube filled with Brilliant green lactose bile broth of which components are grams/litter, Peptone = 10.0g, Lactose = 10.0g, Oxgall = 20.0g, Brilliant green = 0.0133g, and distilled water = 1 litter. The inoculated tubes were then incubated at 35°C, and the most likely number of positive tubes was calculated.

3. Fecal coliform bacteria detection (Completed test)

A supplementary examination was performed on the positive tubes, the result of which was in the confirmatory examination, as these tubes were inoculated again with the above liquid medium for the purpose of detecting total coliform bacteria and fecal coliform bacteria. This test used liquid culture medium (EC) of which ingredients are grams/litter: tryptose = 20.0g, Lactose = 5.0g, Bile salts mixture or bile salt No.3 = 1.5g, ، K_2HPO_4 = 4.0g, KH_2SO_4 = 1.5g, Nacl = 5.0g, and distilled water = 1 litter. Also, a liquid medium EC-MUG was used to detect *Escherichia coli* bacteria. Its ingredients are grams/litre: tryptose = 20.0g, Lactose = 5.0g, 4-methylumbelliferyl-β-D-glucuronide (MUG) = 0.05g, Nacl = 5.0g, ،KH_2PO_4 = 1.5g, and distilled water = 1 litter.

4. Most probable numbers (MPN)

The probability counts of total coliforms and *Pseudomonas aeruginosa* bacteria was calculated according to the following equation **(Australian Standards, 1995)**:

$$\frac{MPN}{100\,ml} = \left(\frac{MPN}{ml}\,(from\ table) \times dilution\ facter\ of\ P2\right) \times 100$$

Isolation and identification of *Pseudomonas aeruginosa* bacteria in the well water

The selective culture medium for isolating *P. aeruginosa* bacteria (Cetrimide Agar 0.03%) was prepared by dissolving 2.0 grams of K_2PO_4 and 0.3 grams of $MgSO_4.7H_2O$

and 0.3 grams streamide (N, Acetyl-N-N-N trimethyl ammonium bromide) and 15 grams of agar in a liter of distilled water, and then adjusting the pH to 4.2 using NaoH (1mM) and adding 15ml of glycerol. The mixture was then boiled and sterilized in an incubator. This medium was used to isolate and diagnose *P. aeruginosa* bacteria **(Hawkey *et al.*, 1989; Azemi *et al.*, 2021; Jasim *et al.*, 2021; Capatina *et al.*, 2022)**. A series of decimal dilutions of the well water samples were conducted, and 0.5ml of the water sample was transferred and planted on the surface of the culture medium. It was placed in Petri dishes in three replicates for each dilution. These dishes were then incubated at a temperature of 55°C for 22 hours. A study was conducted of the colonies of *P. aeruginosa* bacteria on the surface of the culture medium from a phenotypic and morphological standpoint, and biochemical tests were conducted for the purpose of confirming the presence of these bacteria in the well water **(Cabral, 2010)**.

Phenotypic and biochemical diagnostic tests

Some phenotypic diagnostic tests, namely Gram stain, were performed to identify bacteria that were Stain-negative or positive. The morphology of the bacterial colony growing on the culture medium was also examined. A set of biochemical tests were also conducted for the purpose of diagnosing the bacterial isolate. The first is the Oxidase test whereby a portion of the colony was transferred to the center of a filter paper saturated with a blue oxidase reagent solution. If the colony turns purple, it indicates a positive test **(Lennet *et al.*, 1985)**. Next is the bacterial growth test which was performed at 4 and 42 degrees Celsius. The hemolysin test was conducted to assess the ability of the bacteria to decompose blood; the citrate consumption test examined urease enzyme production and gelatin hydrolysis, whilst the methyl red test assessed lactose, glucose, and maltose fermentation.

Chemical analysis of well water samples

Some of the well water's chemical characteristics were examined using the methods suggested by **Napacho *et al.* (2010)**, as follows:

1. Degree of water reaction (pH) using pH meter.
2. Electrical conductivity (EC) using a meter-EC device.
3. Calcium and magnesium by plating with (2Na-EDTA).
4. Sodium and potassium using a photometer flame.
5. Carbonates and bicarbonates by decantation method with 0.01 sulphuric acid.
6. Chlorides by electrophoresis with 0.01 grams of silver nitrate.

Estimating the concentrations of toxic elements in the well water

1. Nitrate (No^{-3}) using the Phenoldisulfonic acid method.
2. Boron (B^{+3}) using the colorimetric method (involving carmine dye) via a spectrophotometer as described by **Page *et al.* (1982)** and **Al Sailawi *et al.* (2020)**.
3. Lead and cadmium using an atomic absorption device (AAS).

Table 1. Chemical characteristics of the well water under study

Well No.	Ec	pH	Dissolved ions (mg/L^{-1})						
			Ca^{+2}	Mg^{+2}	Na^{+1}	K^{+1}	Cl^{-1}	$CO3^{-2}$	$HCO3^{-1}$
1	3.50	7.20	12.53	10.90	22.38	0.59	9.48	Nil	3.52
2	3.8	7.28	10.88	8.13	18.29	0.10	10.23	Nil	2.93
3	2.83	7.13	15.68	11.28	17.48	0.48	14.8	Nil	4.58
4	3.88	7.12	5.78	7.18	11.18	0.29	7.38	Nil	3.20
5	4.03	7.8	6.23	5.48	9.43	0.07	6.48	Nil	5.68
6	2.53	7.53	8.83	7.59	10.09	0.05	13.9	Nil	3.9
7	3.73	7.18	14.10	12.39	13.33	0.13	8.18	Nil	4.23
8	2.87	7.23	9.79	8.00	12.19	0.11	9.11	Nil	3.88
9	4.8	7.09	11.28	10.19	13.29	0.05	5.42	Nil	3.13
10	3.38	7.20	7.25	5.78	10.60	0.05	10.10	Nil	4.8

RESULTS AND DISCUSSION

Microbial contamination of well water

Microbial contamination, specifically coliform bacteria and fecal coliform bacteria was shown to be present in the samples of well water. This is thought to be caused by the locations of the wells, i.e., in agricultural areas where an abundance of animal waste has seeped into the soil, through which the microbes reach the groundwater, thus increasing their number. These bacteria are present in the well water, consistent with the findings of **Entry *et al.* (2000)** and **Ateshan *et al.* (2020)**. Coliform bacteria are most likely to increase in large numbers due to contamination. The presence of coliform bacteria and fecal coliform bacteria in the samples of well water suggests that the water contains animal waste and wastewater from sewage, thus confirming contamination.

Calculating the most probable number (MPN) of well water

The results showed that there was a variation in the MPN of total coliform bacteria in the well water. Based on Table (2), the MPN of bacteria in Well 3 is the highest at 433/100ml, whilst the lowest is in Well 9 at 289/100ml. This is thought to be caused by the proximity of the wells to the pollution source. It may also be caused by the variations in pH or temperatures of the well water, which could influence the growth of Pathogenic

bacteria **(Borelia *et al.,* 2004; Kttafah *et al.,* 2020)**. The World Health Organisation and the US Environmental Protection Agency assert that coliform bacteria can be used as an appropriate microbial indicator for identifying the quality of drinking water. Coliform bacteria are determined by certain metabolic properties, as they are Gram-negative bacilli that can grow in the presence of bile salts. Lactose is fermented and converted into lactic acid and gas, which are oxidase-negative and non-spore-forming **(WHO, 2000)**.

Table 2. Most likely count rates for total coliform bacteria

Well Number	The highest number of colon bacteria / Total 100/ml of well water	Well Number	The highest number of colon bacteria / Total 100/ml of well water
1	335	6	382
2	420	7	295
3	433	8	420
4	394	9	289
5	406	10	410

Contamination of well water with bacteria: *Pseudomonas aeruginosa*

Isolation and diagnosis of *Pseudomonas aeruginosa* bacteria

The isolation results showed that the microbial colonies, after being grown on developmental and selective culture media and undergoing planning and purification, belong to bacteria of the genus Pseudomonas (Figs. 1 and 2).

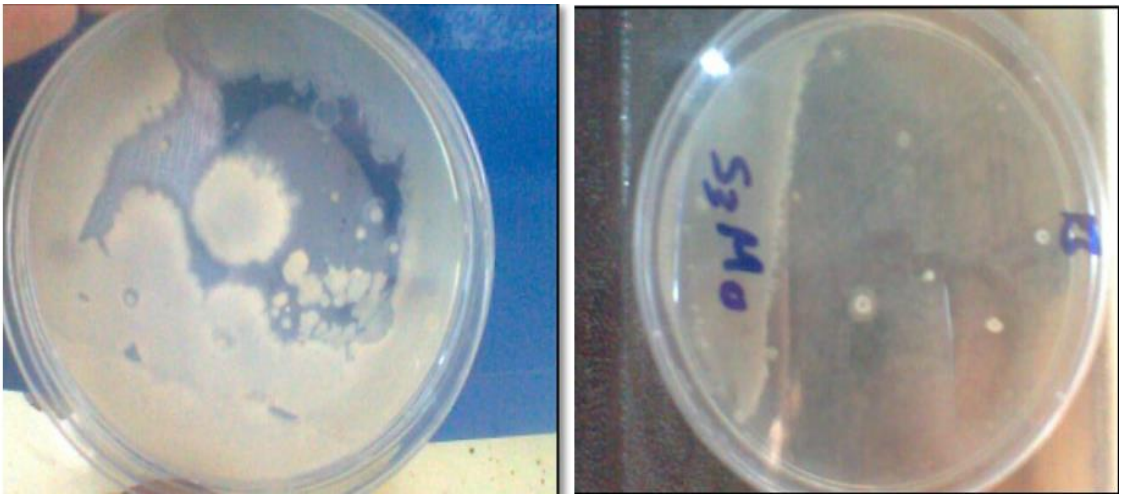

Fig. 1. The bacterial colonies belonging to *P. aeruginosa* bacteria isolated from the water of a well

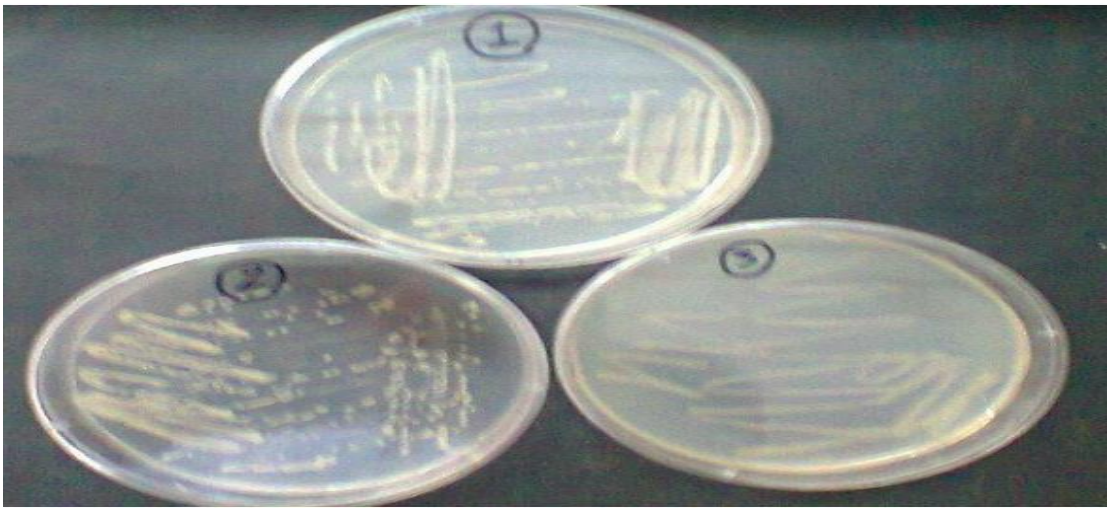

Fig. 2. The planning process for bacterial isolation for purification

Diagnosis of *Pseudomonas aeruginosa* bacteria

Microscopic examination of bacterial isolation

The results of the microscopic examination of the bacterial isolate using an optical microscope showed that it was a single Gram-negative bacillus bacterium. It appeared on the nutrient culture medium with a colonial shape and irregular convex edges capable of producing the yellowish-green dye pyocyanin. Table (3) shows the results of the biochemical tests, confirming that the bacterial isolate belongs to the bacteria *P. aeruginosa*. The diagnostic results are consistent with that of **Holt *et al.* (1994)**.

Table 3. Morphological and biochemical tests for the bacterial isolate *P. aeruginosa*

Colony shape	Cram formula	Production of the pigment pyocyanin	Growth at 4°C	Growth at 42°C	oxidase test	Hemolysin	Citrate consumption	Borase enzyme	Gelatin hydrolysis	Red instance	Fermentation of lactose	Fermentation of maltose	Glucose fermentation
Convex irregular edges	-	+	-	+	+	+	+	+	+	+	-	-	+

(-) The test result is negative.
(+) The test result is negative.

The results showed that the well water is contaminated with *P. aeruginosa* bacteria, despite the variation in the most likely count rate of bacteria in the well water. This variation may be caused by the proximity or distance of these wells to the sources of pollution (e.g., wastewater or sewage), in addition to the fact that the soil is a major repository for living things. Microscopic bacteria, including pathogenic and non-pathogenic bacteria such as *P. aeruginosa*, were found in a wide range of soil, water, plants, animals, and humans, causing various diseases in living organisms **(Jawetz *et al.*, 2007; Abdullah *et al.*, 2020; Okereafor *et al.*, 2024)**. The highest rate of the most likely count of these bacteria in well water was 100/520ml, while the lowest rate of the most likely count was 100/155ml of the well water. This variation in the numbers of *P. aeruginosa* bacteria may be due to the difference in the concentrations of available organic and inorganic materials. These results are consistent with that of **Morais *et al.* (1997)**, specifically regarding the ability of the bacteria *P. aeruginosa* to grow and reproduce in water and low-concentration nutrients. The presence of *P. aeruginosa* bacteria is evidence of the existence of other opportunistic pathogens in the water, due to the possibility of these bacteria to remain in water with natural minerals and with a low level of insoluble solids **(Stover *et al.*, 2000; Abdullah *et al.*, 2020; Okereafor *et al.*, 2024)**.

Concentration of toxic elements in well water

The concentrations of nitrate (No^{-3}), boron (B^{+3}), lead (Pb^{+2}), and cadmium (Cd^{+2}) ions in the water of the wells were measured for the purpose of determining whether or not there is contamination of these available ions, i.e., by comparing the calculated concentrations with international specifications and the permissible limits of these ions as set by the World Health Organisation. The results indicated that all concentrations of these toxic ions were lower than the permissible levels according to the World Health Organisation's specifications (Tables 4, 5).

Table 4. Concentrations of toxic ions in the well water

Well number	**Milligram. Liter^{-1}**				**Well number**	**Milligram. Liter^{-1}**			
	No^{-3}	**B^{+3}**	**Pb^{+2}**	**Cd^{+2}**		**No^{-3}**	**B^{+3}**	**Pb^{+2}**	**Cd^{+2}**
1	1.04	0.25	0.02	0.001	**6**	1.29	0.22	0.02	0.002
2	0.90	0.15	0.03	0.003	**7**	0.88	0.20	0.01	0.004
3	0.97	0.19	0.01	0.001	**8**	0.91	0.16	0.02	0.003
4	1.11	0.21	0.01	0.002	**9**	1.02	0.21	0.03	0.001
5	1.01	0.35	0.04	0.001	**10**	1.09	0.26	0.03	0.002

Table 5. WHO specifications for drinking water

Dissolved ions, **Milligram. Liter^{-1}**			
No^{-3}	**B^{+3}**	**Pb^{+2}**	**Cd^{+2}**
25-50	1-2	0.05	0.005

CONCLUSION

The results show the contamination of the well water with *P. aeruginosa* bacteria, despite the variation in the most likely count rate of bacteria in the well water. It is believed that this variation is caused by the proximity or distance of these wells to the sources of pollution (e.g., wastewater or sewage), in addition to the fact that soil is a major repository for living things. Microscopic bacteria, including pathogenic and non-

pathogenic bacteria such as *P. aeruginosa*, are found in a wide range of soil, water, plants, animals, and humans, causing various diseases to living organisms. The highest rate of the most likely count of these bacteria in well water was 100/520ml, while the lowest rate of the most likely count was 100/155ml of the well water. This variation in the numbers of *P. aeruginosa* bacteria may be due to the difference in the concentrations of available organic and inorganic materials. The concentrations of nitrate (No^{-3}), boron (B^{+3}), lead (Pb^{+2}), and cadmium (Cd^{+2}) ions in the water of the wells were measured for the purpose of determining whether or not there is contamination in the well water by these available ions, i.e., by comparing the calculated concentrations with international specifications and the permissible limits of these ions as set by the World Health Organisation. The results indicate that all concentrations of these toxic ions were lower than the permissible levels set by the World Health Organisation.

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