



Bacteriological Assessment of Natural Spring Waters in the Al Jabal Al Akhdar Region, Libya: A Field Study of Biological Indicators of Fecal Contamination and Their Suitability for Human Consumption

Twfeik Mohammed Elbagrmi¹, Issa Abdulkarim Al-Haen^{2*}, Abdulsalam Mohammed Amharb³,
Mohammed Abdulhadi Al-Haen⁴

¹Faculty of Public Health, University of Benghazi, Benghazi, Libya

^{2,3} Higher Institute of Science and Technology of Bir Al-Ashhab, Libya

⁴Department of Natural Resources, Faculty of Natural Resources and Environmental Sciences,
Tobruk University, Libya.

التقييم البكتيريولوجي لمياه العيون الطبيعية في منطقة الجبل الأخضر- ليبيا: دراسة ميدانية
للمؤشرات الحيوية للتلوث البرازي وصلاحيتها للاستهلاك البشري

توفيق محمد الباقرمي¹، عيسى عبد الكريم الهين^{2*}، عبد السلام محمد أعمار³، محمد عبد الهادي الهين⁴،

¹كلية الصحة العامة، جامعة بنغازي، بنغازي، ليبيا

^{2,3}المعهد العالي للعلوم والتقنية- بئر الأشهب، ليبيا

⁴قسم الموارد الطبيعية، كلية الموارد الطبيعية والعلوم البيئية، جامعة طبرق، ليبيا

*Corresponding author: Ysyb5219@gmail.com

Received: January 10, 2026

Accepted: February 12, 2026

Published: February 22, 2026

Copyright: © 2026 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract:

This study aims to assess the microbial quality of water from selected natural springs in the Al Jabal Al Akhdar region of eastern Libya, given the importance of these sources as a primary supply of drinking water in arid and semi-arid areas. The study was based on the analysis of water samples collected from ten springs during the autumn of 2024. The numbers of fecal coliform bacteria and *Escherichia coli* were estimated using the Most Probable Number (MPN) method in accordance with approved standard procedures. The results revealed variation in contamination levels among the study sites, with *E. coli* counts ranging from 0–1 to 7–12 MPN/100 mL, while fecal coliform counts ranged from 0–1 to 12–79 MPN/100 mL. In most springs, these values exceeded the permissible limits established by the World Health Organization and the Libyan drinking water standards. The findings also indicate that the presence of *E. coli* reflects recent fecal contamination and the potential health risks associated with it. The study confirms that some of these springs are unsuitable for direct human consumption without treatment, highlighting the need for regular monitoring, identification of contamination sources, and the implementation of preventive measures to ensure water safety and protect public health.

Keywords: Natural Springs, Microbial Contamination, Fecal Coliforms, *Escherichia Coli*, Drinking Water Quality.

الملخص

تهدف هذه الدراسة إلى تقييم الجودة الميكروبية لمياه بعض العيون الطبيعية في منطقة الجبل الأخضر شرق ليبيا، وذلك نظرًا لأهمية هذه المصادر كمورد رئيسي لمياه الشرب في المناطق الجافة وشبه الجافة. اعتمدت الدراسة على تحليل عينات مياه جُمعت من عشر عيون خلال خريف عام 2024، حيث تم تقدير أعداد بكتيريا القولون البرازية والإشريكية القولونية باستخدام طريقة العدد الأكثر احتمالاً (MPN) وفق الطرق القياسية المعتمدة. أظهرت النتائج وجود تباين في مستويات التلوث بين مواقع الدراسة، حيث تراوحت الإشريكية القولونية (*E. coli*) بين 0-1 إلى 7-12 (MPN/100 mL)، بينما تراوحت بكتيريا القولون البرازية بين 0-1 إلى 12-79 (MPN/100 mL)، وقد تجاوزت هذه القيم في أكثر العيون الحدود المسموح بها وفق معايير منظمة الصحة العالمية والمواصفات الليبية لمياه الشرب. كما تشير النتائج إلى أن وجود الإشريكية القولونية يعكس تلوثًا برازياً حديثاً وما يرتبط به من مخاطر صحية محتملة. وتؤكد الدراسة أن بعض هذه العيون غير صالحة للاستهلاك البشري المباشر دون معالجة، مما يستدعي ضرورة المراقبة الدورية، وتحديد مصادر التلوث، واتخاذ الإجراءات الوقائية لضمان سلامة المياه وحماية الصحة العامة.

الكلمات المفتاحية: العيون الطبيعية، التلوث الميكروبي، القولونيات البرازية، الإشريكية القولونية، جودة مياه الشرب

Introduction

Groundwater is considered one of the most important natural resources for securing drinking water supplies and supporting human and economic activities, particularly in arid and semi-arid regions where water resources are limited. With increasing environmental and anthropogenic pressures on these resources, water quality has become increasingly vulnerable to deterioration from various sources of pollution, raising serious public health and environmental concerns. Groundwater contamination is especially problematic because its remediation is often costly and time-consuming (Sudhir et al., 2013).

Libya is one of the countries located within the arid and semi-arid climatic zone and suffers from limited freshwater resources, making dependence on groundwater and natural springs critically important. In the Al-Jabal Al-Akhdar region in particular, many residents rely on natural spring water for various purposes because of its availability and local accessibility. Natural springs are of particular importance in water-scarce countries, where they represent a major source of water in many rural and mountainous areas (Alhendawi et al., 2009; Asma et al., 2019).

Natural spring water is characterized by unique properties due to the presence of various dissolved elements and minerals, which encourages local communities to depend on it for drinking, domestic use, and agricultural activities. However, despite their environmental and economic value, these springs are considered sensitive ecosystems that may be threatened by the interaction between surface water and groundwater, as well as by surrounding hydrogeological and environmental conditions. Therefore, continuous monitoring of their quality is essential to ensure their safety and long-term sustainability (An & Breidenbach, 2005; Al Kuisi et al., 2014).

Microbiological indicators are among the most important criteria used to assess the suitability of spring water for human consumption because they provide direct evidence of potential sanitary contamination. Microbial contamination of drinking water is associated with the transmission of serious diseases such as cholera, typhoid fever, hepatitis, and diarrheal illnesses. Accordingly, determining the microbiological characteristics of water is a fundamental step in spring resource management, identification of contamination sources, and the development of appropriate treatment and monitoring programs. Previous studies have shown that coliform bacteria are widely used as biological indicators of water pollution. The presence of fecal coliform bacteria reflects the general sanitary condition of water, whereas *Escherichia coli* is regarded as a more specific indicator of fecal contamination of human or animal origin and the associated potential health risks. Studies have also emphasized the importance of long-term monitoring of water quality in order to assess deterioration and determine its suitability for different uses (Abubaker et al., 2019; Balogun et al., 2013; Sammoudi et al., 2023).

The problem addressed in this study arises from the limited information available on the bacteriological status of some natural springs in the Al-Jabal Al-Akhdar region. Although a few previous studies have been conducted, such as the study by Abubaker et al. (2019), which investigated microbial contamination only in the Masa area of Al-Jabal Al-Akhdar, there is, to the best of the researchers' knowledge, no recent multi-site study comparing several natural springs in Al-Jabal Al-Akhdar using two standardized bacteriological indicators during the same season.

This issue is particularly important because local populations depend on these springs as a major source of drinking water and domestic use, and in some cases for agriculture and livestock watering, making their sanitary safety a matter of considerable importance. Although spring water is a valuable natural resource, it may be exposed to microbial contamination, particularly fecal contamination

resulting from human activities or surrounding environmental conditions, which may adversely affect human health. Studies by Asma et al. (2019) and Lamloum et al. (2023) reported sewage leakage into a number of springs. Therefore, the importance of this study lies in evaluating the microbial safety of these waters using two widely recognized and reliable bacterial indicators, namely fecal coliform bacteria and *Escherichia coli*, in order to determine their sanitary suitability and provide scientific data that contribute to a better understanding of the current condition of the studied springs. Such data may also provide a basis for future research and monitoring measures aimed at protecting public health.

Accordingly, this study aims to evaluate the microbial quality of selected natural springs in the Al-Jabal Al-Akhdar region by detecting fecal coliform bacteria and *Escherichia coli* as indicators of microbial contamination, in order to assess the bacteriological suitability of spring water for human consumption in accordance with World Health Organization standards.

Material and methods

Study Area: The study area is located in northeastern Libya and covers an area of approximately 2,000 km². According to the 2006 census, the population of the study region was 163,506 inhabitants. It extends from the city of Shahat in the west to the city of Derna in the east, and from the Mediterranean coast in the north to the areas of Ain Mara, Al-Qubba, and Al-Abarq in the south (Al-Rawashdeh, 2012). Geographically, the study area lies between latitudes 32°56' and 34°32' north of the Equator, and between longitudes 21°50' and 22°38' east of the Greenwich Meridian. Figure 1 illustrates the location of the study area and the sampling sites.

Sample Collection: The study was conducted during the autumn of 2024 on ten natural springs distributed across several locations in the Al-Jabal Al-Akhdar region, eastern Libya. Samples were collected from each spring using sterile 500 mL glass bottles, from the center of the spring at a depth ranging from 15 to 30 cm. The samples were then transported directly to the laboratory in a cooled container at 4°C in order to preserve their microbiological characteristics and minimize biological changes until microbiological analyses were performed. The sampling locations were also recorded using a GPS device.

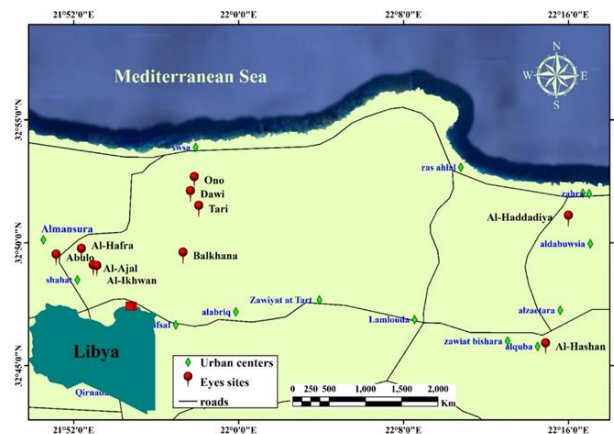


Figure (1): Location of the study area and sample collection sites.

Microbiological Analysis

Estimation of the Most Probable Number (MPN)

Determination of Fecal Coliforms and *Escherichia coli* by the Multiple-Tube Fermentation (MPN) Method According to APHA. The most probable number (MPN) of fecal coliforms and *Escherichia coli* was determined using the multiple-tube fermentation technique in accordance with Standard Methods for the Examination of Water and Wastewater, particularly Sections 9221C, 9221E, and 9221F (Baird et al., 2017). The method is based on inoculating known volumes of the water sample, or its tenfold serial dilutions, into tubes containing Lauryl Tryptose Broth (LTB), followed by incubation at $35 \pm 0.5^\circ\text{C}$ for 24–48 h. Tubes showing gas formation and/or acid production were considered positive in the presumptive test (Baird et al., 2017; National Environmental Methods Index [NEMI], n.d.). For confirmation of fecal coliforms, positive cultures were transferred to EC broth and incubated in a water bath at $44.5 \pm 0.2^\circ\text{C}$ for 24 ± 2 h, with gas production taken as evidence of a positive result (Baird et al., 2017; NEMI, n.d.). For confirmation of *Escherichia coli*, EC-MUG broth was used under the same incubation conditions, and tubes exhibiting bright blue fluorescence under ultraviolet light at a wavelength of 366 nm were considered positive for *E. coli* (Baird et al., 2017; Feng & Hartman, 1982;

NEMI, n.d.). The results were then expressed as MPN/100 mL based on the number of positive tubes at each dilution, using the APHA 9221C MPN tables (Baird et al., 2017). Samples were stored at 1–4°C, and sodium thiosulfate was added to chlorinated samples to neutralize residual chlorine. All analyses were performed as soon as possible, preferably within 6 h of sample collection.

Results and discussion

Fecal Coliform Bacteria

The results of the bacteriological examination of fecal coliforms using the most probable number method (MPN/100 mL) are presented in Table 1 and revealed variation in the level of contamination among the spring water sources in the study area. Values ranged from 0–1 to 12–79 MPN/100 mL. The highest counts were recorded in Wdouï and Al-Ajal, whereas the lowest values were observed in Al-Hafrah, Tri, and Ouno. These findings indicate that most of the studied springs showed the presence of fecal coliform bacteria, and that a considerable number of them exceeded the microbiological limits recommended for drinking water by the World Health Organization (WHO, 2017), which sets the guideline value at 0 cells/100 mL, as well as the Libyan standard specification for drinking water suitability, which sets the permissible limit at 3 cells/100 mL (National Center for Standardization and Metrology, 2015).

The presence of coliform bacteria may be attributed to contamination sources such as grazing areas adjacent to the springs or sewage wastewater. Contaminants may infiltrate groundwater through fractures and fissures associated with karstic formations, particularly during rainfall events, when runoff washes accumulated wastes and pollutants from the land surface. This increases the pollutant load carried by floodwater, and when such contaminated water passes through the bottoms of valleys located near the aquifer layers feeding the springs, the likelihood of pollutant infiltration into these water-bearing strata becomes greater. In addition, contamination from household cesspits or septic pits located near these springs cannot be excluded (Nouyang et al., 2009; Sitotaw et al., 2021). These findings are consistent with those reported by Balogun et al. (2013), who recorded high coliform counts in spring water, but differ from the results of Daghara et al. (2019), who found that only 3% of springs were contaminated. Similarly, Sammoudi et al. (2023) reported that fecal coliform contamination exceeded standard limits and was more severe during dry periods.

Table 1. Estimated counts of fecal coliform bacteria and *Escherichia coli* in spring water samples, expressed as calculated ranges using the most probable number method (MPN/100 mL).

Spring Name	Faecal coliforms MPN/100ml	<i>Escherichia coli</i> MPN/100ml
Al-Ajal	79–12	1–0
Abulo	75–9	7–2
Al-Haddadiya	71–10	7–2
Al-Ikhwan	75–9	1–0
Al-Hashan	79–12	12–7
Tari	7–2	1–0
Ono	1–0	1–0
Al-Hafra	7–2	1–0
Dawi	79–12	7–2
Balkhana	9–83	0–1

Escherichia coli (E. coli)

Escherichia coli (*E. coli*) is a Gram-negative, rod-shaped bacterium that normally inhabits the intestines of humans and animals, and its detection in water is considered an important indicator of fecal contamination (Al-Balou and Al-Numan, 2019). The results of the bacteriological examination using the most probable number method (MPN/100 mL), presented in Table 1, revealed variation in the level of contamination among the springs in the study area. *E. coli* counts ranged from 0–1 to 7–12 MPN/100 mL, with the highest value recorded at Ain Al-Hashan, while the lowest values were observed at Ouno, Al-Hafrah, Balkhana, and Al-Ajal.

The results indicate variation in the levels of microbial contamination among the springs in the study area. This variation may be attributed to differences in the environmental conditions surrounding each spring, such as proximity to residential settlements or agricultural and grazing activities, in addition to the possibility of surface runoff or sewage leakage reaching the water source. The elevated contamination observed at Ain Al-Hashan appears to be associated with its location within, or close to, inhabited areas. The presence of *E. coli* is of particular importance because it indicates

relatively recent fecal contamination. Several studies have identified sewage wastewater and wildlife as two major sources of this type of contamination (Antony and Renuga, 2012; Asma et al., 2019; Lamloum et al., 2023).

The presence of this bacterium in natural spring water suggests the potential for adverse health effects among individuals or communities exposed to such water. Moreover, fecal contamination of spring water may pose a potential public health risk if the water is used as raw drinking water without treatment (Breidenbach and An, 2005).

The treatment of infections associated with this bacterium becomes increasingly difficult over time because of its growing resistance to antibiotics (Pena, 2014). Certain strains of this bacterium are associated with a variety of diseases, including gastrointestinal infections, diarrhea, and urinary tract infections, in addition to other complications in some cases (Brandl, 2006).

The findings of this study are consistent with those of Abubaker (2019), who reported substantial microbial contamination and considered these findings to represent a real health risk to water sources in the city of Massa in Al-Jabal Al-Akhdar. They are also in agreement with the results of Al-Hamaiedeh et al. (2023), who reported microbial contamination exceeding international standard limits as a result of inadequate protective measures around springs and the spread of grazing and agricultural activities in the surrounding area.

Conclusion

This study aimed to evaluate the microbial quality of water from selected natural springs in the Al-Jabal Al-Akhdar region by detecting fecal coliform bacteria and *Escherichia coli* as indicators of contamination, in order to determine the suitability of these waters for human consumption. The results revealed varying levels of bacterial contamination among the studied springs, with microbial indicator concentrations in most sites exceeding the permissible limits according to the adopted standards, indicating that a number of these sources are unsuitable for direct use as drinking water without treatment. Despite the importance of these findings, their interpretation may be influenced by certain limitations, most notably the limited number of sampling sites and the fact that samples were collected during a single time period, which may not reflect seasonal variations in water quality. Overall, this study contributes to improving understanding of the status of microbial contamination in natural spring waters in the region and highlights the importance of continuous monitoring and the implementation of protective and treatment measures to support water resource management and safeguard public health.

References

- Al-Balou, M. O. H., & Al-Numan, A. Y. S. (2019). Detection of bacteriological contamination in drinking water on the left coast of Mosul city using the multiple-tube fermentation method [In Arabic]. *Journal of Education and Science*, 28(2), 167–184.
- Abubaker, N. A. S., Alfageih, L. M., & Hmoda, A. A. (2019). Analysis of ground water and spring water quality in Aljabal Al akhdar. *IOSR Journal of Pharmacy and Biological Sciences*, 14(3), 13–20.
- Al Kuisi, M., Mashal, K., Al-Qinna, M., Abu Hamad, A., & Margane, A. (2014). Groundwater vulnerability and hazard mapping in an arid region: Case study, Amman-Zarqa Basin (AZB)-Jordan. *Journal of Water Resource and Protection*, 6, 297–318.
- Al-Hamaiedeh, H., Al-Rfo'u, F., Al-Hamaiedeh, K. D., El-Hasan, T., & Alakayleh, Z. (2023). Springs water quality assessment for drinking purposes: A case study of Bsaira, Jordan. *Iraqi Geological Journal*, 56(2A), 48–56.
- Alhendawi, R. A., Younis Alhendawi, & Abdullhafeed Mousa. (2009). Water Scarcity in Libya. *Paper presented at the Second International Conference of Natural Resources in Africa*, Cairo University, pp. 33-39.
- Al-Rawashdeh, Z. (2012). Groundwater pollution problem in the Al-Jabal Al-Akhdar region [In Arabic]. *Journal of the Libyan Center for International Agricultural Research*, 3(2), 1369–1415.
- An, Y.-J., & Breindenbach, G. P. (2005). Monitoring *E. coli* and total coliforms in natural spring water as related to recreational mountain areas. *Environmental Monitoring and Assessment*, 102(1–3), 131–137.
- Antony, R. M., & Renuga, F. B. (2012). Microbiological analysis of drinking water quality of Ananthaner channel of kanyakumari district, Tamil Nadu, India. *Ambi-Agua*, 7(2), 42-48.
- Asma, A. R., Alkadi, F. B., Lamloum, F. B., Abdalla, K. A., Alhendawi, Y. A., Alzway, A. A., & Alhendawi, R. A. (2019). Hydro Chemical Water Quality and its Suitability for Drinking and Irrigation Purpose of the Main Springs Water in the Study Area of Jabal Al-Akhdar-Libya. *World Applied Sciences Journal*, 37(2), 98–105.
- Baird, R. B., Eaton, A. D., Rice, E. W., & Bridgewater, L. (Eds.). (2017). *Standard Methods for the Examination of Water and Wastewater* (23rd ed.). American Public Health Association (APHA).

- Balogun, S. A., Ejelonu, B. C., Lasisi, A. A., & Adeogun, A. I. (2013). Microbiological and chemical assessment of spring water from a rural setting in Ondo State Southwest, Nigeria. *African Journal of Environmental Science and Technology*, 7(6), 555–559.
- Brandl, M. T. (2006). Fitness of human enteric pathogens on plants and implications for food safety. *Annual Review of Phytopathology*, 44, 367-392.
- Daghara, A., Al-Khatib, I. A., & Al-Jabari, M. (2019). Quality of drinking water from springs in Palestine: West Bank as a case study. *Journal of Environmental and Public Health*, 2019, Article 8631732.
- Feng, P. C., & Hartman, P. A. (1982). Fluorogenic assays for immediate confirmation of *Escherichia coli*. *Applied and Environmental Microbiology*, 43(6), 1320–1329.
- Lamloum, F., Abdalmaula, G., Jaballah, A., El-Gammal, M., & Alhendawi, R. A. M. (2023). Preliminary environmental assessment of the quality of some groundwater and springs in Al-Jabel Al-Akhdar, Libya. *International Journal of Water Resources and Environmental Sciences*, 12(1), 16-30.
- National Center for Standardization and Metrology. (2015). Libyan standard for drinking water suitability (2nd ed.) [In Arabic]. Ministry of Planning, Libya.
- National Environmental Methods Index (NEMI). (n.d.). *Standard methods: 9221A,B,C,F: Coliform bacteria by multiple-tube test using lauryl-tryptose and EC-MUG broth*.
- National Environmental Methods Index (NEMI). (n.d.). *Standard methods: 9221E: Fecal coliforms by multiple tube fermentation*.
- Nouyang, M. E., Nola, M., Njine, T., Zebaze Togoutet, S. H., Djaousda, M., & Djah, M. (2009). The influence of hydrochemistry on the distribution of pathogenic strains of *Escherichia coli* in urban groundwater of Yaounde, Cameroon. *Proceedings of the Groundwater and Climate in Africa Conference*, Int. Assoc. Hydrol. Soc. Pub. No. 334.
- Pena, I., Picazo, J. J., Rodriguez-Avial, C., & Rodriguez-Avial, I. (2014). Carbapenemase-producing Enterobacteriaceae in a tertiary hospital in Madrid, Spain. *International Journal of Antimicrobial Agents*, 43(5), 460-464.
- Sammoudi, R., Chahlaoui, A., Ouarrak, K., Taha, I., & Khaffou, M. (2023). Bacteriological Quality Status of Spring Waters from the Taanzoult Plain (Morocco). *Journal of Ecological Engineering*, 24(4), 184–191.
- Shafi, S., Kamili, A. N., Shah, M. A., & Bandh, S. A. (2013). Coliform bacterial estimation: A tool for assessing water quality of Manasbal lake of Kashmir, Himalaya. *African Journal of Microbiology Research*, 7(3), 3996-4000.
- Sitotaw, B., Melkie, E., & Temesgen, D. (2021). Bacteriological and physicochemical quality of drinking water in Wegeda Town, Northwest Ethiopia. *Journal of Environmental and Public Health*, 2021, Article 6646269.
- Sudhir, S., Srivastava, A., Pandey, A., & Gautam, S. (2013). Integrated Assessment of Groundwater Influenced by a Confluence River System. *Water Resources Management*, 27, 4291-4313.
- World Health Organization (WHO). (2017). *Guidelines for drinking-water quality: Fourth edition incorporating the first addendum*. Geneva: WHO Licence.