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Original article

Analysing Microbial Risks Associated with Heavy Metal Levels in Groundwater Sources: A Case Study of Werdama, Libya

Hameda Moftah[1]*, Adel Mahmoud[2], Lamya El-jalel[1]

[1]*Department of Environmental Sciences, Faculty of Natural Resources and Environmental Sciences, Omar Al-Mukhtar University, Al-Bayda, Libya*
[2]*Department of Forestry and Rangeland, Faculty of Natural Resources and Environmental Science, Omar Al-Mukhtar University, Al-Bayda, Libya*
Email. *hameda.adam@oum.edu.ly*

ABSTRACT
Groundwater is the main source of drinking water for rural and peri-urban communities across the Al-Jabal Al-Akhdar region of Libya, yet its quality has rarely been documented in any systematic way. In this study we assessed the physicochemical and microbiological quality of groundwater drawn from three wells (W1–W3) in the Werdama area, Al-Bayda, Libya, focusing in particular on four priority heavy metals: lead (Pb), cadmium (Cd), chromium (Cr), and iron (Fe), benchmarked against World Health Organization (WHO) maximum contaminant levels (MCL). Physicochemical parameters (electrical conductivity, temperature, pH, dissolved oxygen, turbidity, and total dissolved solids) were measured both in situ and in the laboratory, and metal concentrations were quantified by atomic absorption spectrophotometry following acid digestion; calibration curves showed excellent linearity throughout (R^2 = 0.9984–0.9999). Pb and Fe exceeded WHO limits in all three wells, Cd exceeded the WHO limit but stayed below the U.S. EPA threshold, and Cr remained within internationally accepted limits. The computed Heavy Metal Pollution Index (HPI) came to 97.99, 120.40, and 128.50 for W1, W2, and W3, placing W2 and W3 in the high-pollution category. Culture-based screening on Nutrient Agar, MacConkey Agar, and Eosin Methylene Blue Agar detected Escherichia coli in all three wells, with colony counts pointing to a low-to-moderate contamination risk (5–40 CFU/100 mL). Together, these results show that Werdama groundwater carries both geogenic heavy-metal enrichment and low-level faecal contamination, and is therefore not consistently safe for direct consumption without treatment. We argue that routine groundwater surveillance, targeted remediation, and better sanitation infrastructure are needed in this water-scarce region.
Keywords: Heavy Metal Pollution, Heavy Metal Pollution Index, Groundwater Quality, Escherichia Coli.

Introduction

Access to safely managed water is still one of the harder problems in global development. Sustainable Development Goal 6 (SDG 6) calls for universal access to safe, affordable drinking water, and progress on it feeds into almost everything else, from public health to food security [1]. Libya makes this problem more acute than most places. Its climate is arid, it has essentially no perennial rivers, and rainfall is limited to short winter storms, all of which put it among the countries with the highest water-poverty index in the world (48.6) [2-5]. As a result, wells and boreholes have taken on a growing share of the country's water supply, and population growth, urban expansion, and agricultural development have pushed already-limited aquifers harder than they were designed to handle. For roughly four decades now, the shortage of safe, potable water has remained one of the country's most persistent development problems. Organic and inorganic pollutants, heavy metals, and other emerging contaminants add further pressure on these groundwater resources [2,3,5,6,7]. Heavy metals are usually defined as elements with atomic weights between 63.6 and 200.6 and specific gravities above 4.0, at least five times that of water. Depending on their chemical form, they can occur in water as colloidal, particulate, or dissolved fractions, coming from both natural processes (sediment leaching, rock weathering) and human activity such as industrial effluents, domestic wastewater, and solid-waste disposal [8-10].

Calcium, magnesium, potassium, and sodium are alkali and alkaline-earth macroelements rather than heavy metals, and along with trace amounts of cobalt, copper, iron, manganese, molybdenum, and zinc, they are essential to biological function and act as enzymatic cofactors; even so, chronic exposure to non-essential metals or excess loads of essential ones is harmful. It's the combination of persistence, bioaccumulation, and toxicity that makes heavy-metal contamination of aquatic systems such a serious environmental concern [11,12]. Chronic lead exposure, for instance, has been tied to immune dysfunction in infants, elevated cardiovascular risk, impaired fertility, and neurobehavioral effects [13,14]. Cadmium is a recognised carcinogen linked to kidney dysfunction, skeletal demineralisation, and gastrointestinal problems, and hexavalent chromium (Cr(VI)) can be lethal at acute doses of 50–150 mg/kg body weight. Because lead builds up in the body with no known safe threshold, the U.S. EPA has adopted a zero-tolerance goal for lead in drinking water [15,16]. None of this can be managed well without careful measurement, so quantifying metal loads in water is a necessary first step toward understanding contamination severity and planning any mitigation.

Natural sources of heavy metals include rock weathering, biological decomposition, and atmospheric deposition, while mining, mineral processing, agricultural runoff, industrial discharge, and improper solid- and biomedical-waste disposal are the main human-driven pathways. Given how limited Libya's surface water is, groundwater accessed through shallow wells and boreholes has become an essential resource for development [17-20]. Water quality is typically judged against the WHO Maximum Contaminant Level (MCL) for heavy metals [21,22]. In Libya's case, water scarcity compounds the heavy-metal problem, since it pushes people toward wells and boreholes that are themselves exposed to industrial and

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solid-waste contamination. Protecting public health therefore depends on continuous monitoring of heavy-metal concentrations against MCL thresholds [23-28]. Alongside chemical contamination, waterborne microbial pathogens pose an equally serious public-health risk, one that grows with population growth, urbanisation, and poor sanitation [29]. Escherichia coli, a facultative anaerobe that lives naturally in the gut of warm-blooded animals, is the indicator organism most widely used for faecal contamination of water and food [30-33].
Finding E. coli doesn't prove that pathogens are present, but it does signal a real chance that faecal-borne organisms such as Salmonella spp. and hepatitis A virus are also there [30-35], which is why regulators worldwide rely on E. coli counts to judge microbiological water safety. Even though the risks from heavy metals and from microbial contaminants are each well documented on their own, studies that look at both together for Libyan groundwater are still hard to find. This is the gap we try to address here: a fairly complete look at the Werdama wells that combines physicochemical characterisation, quantitative heavy-metal analysis against WHO/EPA standards, HPI computation, and culture-based microbial risk assessment, to produce evidence that local water managers can actually use.

Materials and Methods

Study area and sampling

Groundwater samples were collected from three wells (W1, W2, W3) distributed across the Werdama area, Al-Bayda, Al-Jabal Al-Akhdar, Libya. From each well, three independent 250 mL aliquots were collected in polyethene bottles fitted with Teflon-lined caps, immediately after purging stagnant water from the well column. Bottles were rinsed three times on-site with sample water to collection and transported to the laboratory under cool, dark conditions to minimise microbial die-off and metal speciation changes prior to analysis. Samples designated for heavy-metal analysis were pre-filtered through cellulose Millipore membranes.

Physicochemical Analysis

Electrical conductivity (EC), temperature, pH, dissolved oxygen (DO), and total dissolved solids (TDS) were measured using a handheld multiparameter meter (HANNA HI 9828, USA). Turbidity was determined spectrophotometrically, while sodium and potassium concentrations were quantified by flame photometry.

Sample Digestion

Organic matter was removed for metal quantification by acid digestion: water samples were treated with concentrated nitric acid at a 4:1 (sample: acid) ratio in a 250 mL conical flask, heated on a hot plate until the volume was reduced by approximately half, then cooled and filtered.

Standard preparation

A 1000 mg/L multi-element stock solution of chromium, cadmium, iron, and lead was prepared in a 1 L volumetric flask using 5% nitric acid as the matrix. Working calibration standards were prepared from this stock by serial dilution.

Instrumental analysis

Digested samples were analysed for lead, cadmium, chromium, and iron using a Thermo Fisher M-Series atomic absorption spectrophotometer (Thermo Fisher Scientific, USA). Ultrapure water (18.2 MΩ·cm), produced by an ADVANTEC RFS342NC purification system (Advantec Co., Ltd., Tokyo, Japan), was used throughout the analytical procedure. Metal concentrations were extrapolated from linear calibration curves constructed from the standard series (Section 2.4), and the instrument reported final concentrations in mg/L (or µg/L, as tabulated).

Heavy Metal Pollution Index (HPI)

The HPI provides a composite measure of the cumulative impact of the target heavy metals on overall water quality. Following Mohan et al. [36], the HPI is inversely weighted by the standard permissible value (Si) of each parameter, such that metals with lower permissible thresholds contribute proportionally more to the index when exceeded. Composite HPI scores were classified as low (< 50), medium (50–100), or high (> 100) pollution risk.

Microbiological analysis

Water samples were collected in sterile containers and transported to the laboratory under refrigeration. Following centrifugation to concentrate particulate and microbial matter, sediments were plated using the streak-plate technique on three selective/differential media — Nutrient Agar, MacConkey Agar, and Eosin Methylene Blue (E.M.B) Agar — and incubated at 37 °C for 24–48 h. Colony counts were recorded as colony-forming units per 100 mL (CFU/100 mL) and interpreted against WHO risk categories: 0 CFU/100 mL (safe), 1–10 CFU/100 mL (low risk), and 11–100 CFU/100 mL (moderate risk) [37].

Results and Discussion

Physicochemical Characteristics of Groundwater

Table 1 lays out the physicochemical profile of the three wells against a distilled-water blank. All three wells produced water that was tasteless and odourless, a good baseline sign for a potable supply. Measured pH values (7.09–7.34) sat

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comfortably within the WHO-acceptable range of 6.5–8.5, with W3 closest to the upper edge. Electrical conductivity was much higher than the blank (557–668 µS/cm vs. 0.214 µS/cm), pointing to a substantial dissolved ionic load, most likely of geogenic origin given how deep the wells are and how long the water has been in contact with rock. Water temperatures (25.8–27.3 °C) didn't deviate much from the blank and stayed within a normal ambient range. Turbidity was slightly higher across all wells (2.15–2.76 NTU vs. 1.30 NTU in the blank), suggesting a modest suspended-particulate load, though not enough to affect the water's sensory quality. Total dissolved solids (TDS) were far higher in the well water (266–308 mg/L) than in the blank (0.715 mg/L), reflecting the same dissolved mineral load behind the elevated EC readings; at these levels, TDS starts to matter for both taste and long-term health if it persists.
Dissolved oxygen was fairly similar across wells (16–18%), with W3 showing the lowest aeration, which can in turn affect how redox-sensitive metals behave. Sodium was undetectable in the blank but ranged from 0.224–0.254 mg/L in the wells, and potassium was likewise higher than the blank, with W1 running roughly eight times higher than W2 or W3. Overall, the physicochemical picture (Figures 1–2) suggests W2 has the most favourable profile of the three for potable use, though none of them would qualify as optimal without further treatment. These numbers line up reasonably well with other physicochemical surveys done within Al-Bayda itself. A ten-site assessment of municipal wells in the city reported pH of 7.63–7.92, EC of 593–865 µS/cm, and TDS of 285–493 mg/L, all in the same range as what we found at Werdama, and that study also traced the variability to local terrain, geology, and soil composition rather than any single point source [38]. Two independent datasets from the same city landing in similar territory make it more likely that the elevated EC and TDS at Werdama reflect a broader geogenic pattern across the city's aquifer, rather than something specific to this site.

Table 1. Mean physicochemical parameters of groundwater samples from the Werdama wells (mean ± SD, n = 3).

Parameter	Blank	W1	W2	W3
Taste	Tasteless	Tasteless	Tasteless	Tasteless
Odor	Odorless	Odorless	Odorless	Odorless
pH	7.020 ± 0.07	7.250 ± 0.04	7.090 ± 0.05	7.340 ± 0.03
EC (µS/cm)	0.214 ± 0.10	668.0 ± 25.85	593.0 ± 52.12	557.0 ± 38.21
Temperature (°C)	25.80 ± 1.02	26.50 ± 0.60	27.34 ± 0.67	26.82 ± 0.82
Turbidity (NTU)	1.300 ± 0.94	2.150 ± 1.20	2.340 ± 1.04	2.760 ± 1.17
TDS (mg/L)	0.715 ± 0.30	308.0 ± 40.56	281.0 ± 25.87	266.0 ± 35.98
DO (%)	19.00 ± 3.32	18.00 ± 3.67	18.00 ± 4.95	16.00 ± 3.65
Na^+ (mg/L)	ND*	0.254 ± 0.14	0.239 ± 0.12	0.224 ± 0.10
K^+ (mg/L)	0.003 ± 0.00	0.891 ± 0.26	0.183 ± 0.19	0.372 ± 0.21
Fe (mg/L)	0.005 ± 0.00	0.987 ± 0.25	1.080 ± 0.37	1.032 ± 0.36

**ND = Not detected.*

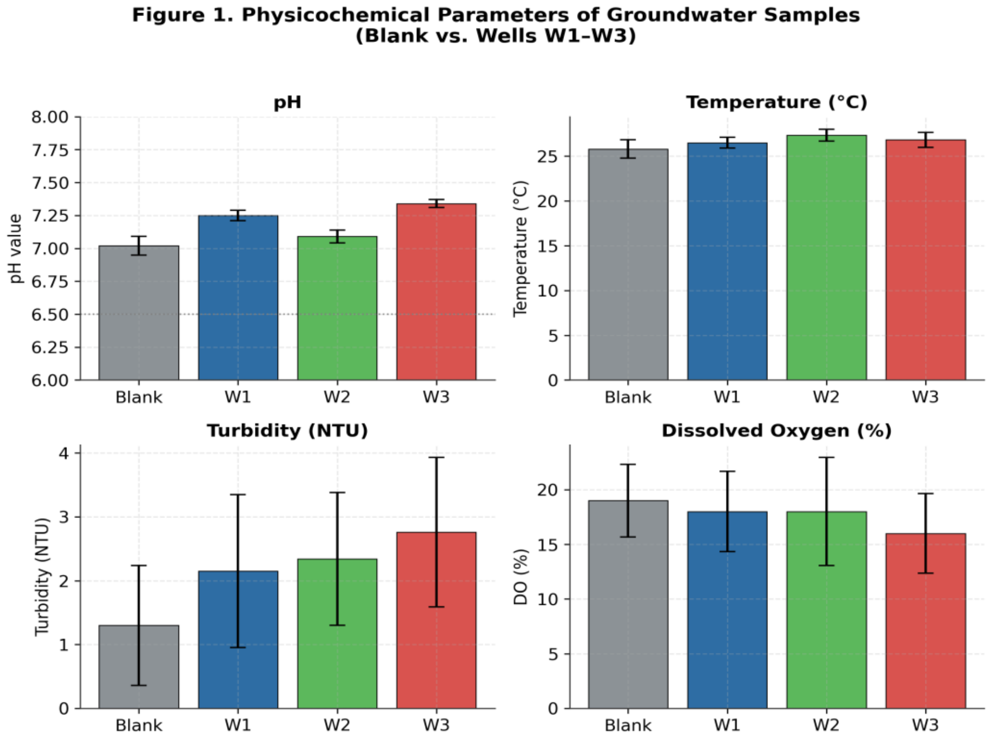

Figure 1. Physicochemical parameters (pH, temperature, turbidity, and dissolved oxygen) of groundwater samples from the Blank and Wells W1–W3. Error bars represent ± SD; dotted lines in the pH panel denote the WHO-acceptable range (6.5–8.5).

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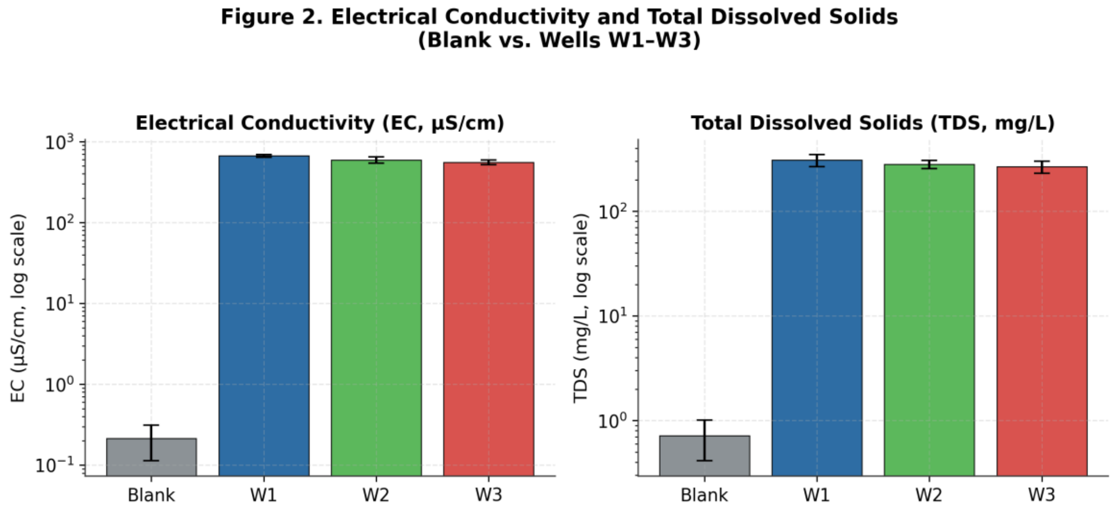

Figure 2. Electrical conductivity (EC) and total dissolved solids (TDS) of groundwater samples, shown on a logarithmic scale to accommodate the large contrast between the distilled-water blank and the well samples.

Calibration Performance and Heavy-Metal Concentrations

We built linear calibration curves for Pb, Cd, and Cr from serial dilutions of the multi-element standard (Table 2, Figure 3). The resulting regression coefficients, R^2 = 0.9984, 0.9999, and 0.9987 for Pb, Cd, and Cr, confirm the instrument was behaving linearly across the tested range, which gives us confidence in the trace-metal readings from the digested well-water samples. No measurable heavy metals turned up in the distilled-water blank, which rules out analytical or procedural contamination as an explanation for what follows.

Table 2. Calibration regression equations and coefficients of determination for lead, cadmium, and chromium (atomic absorption spectrophotometry).

Heavy metal	Regression equation	Regression coefficient (R^2)
Lead (Pb)	y = 0.0100x + 0.0004	0.9984
Cadmium (Cd)	y = 0.0665x + 0.0027	0.9999
Chromium (Cr)	y = 0.3562x + 0.0089	0.9987

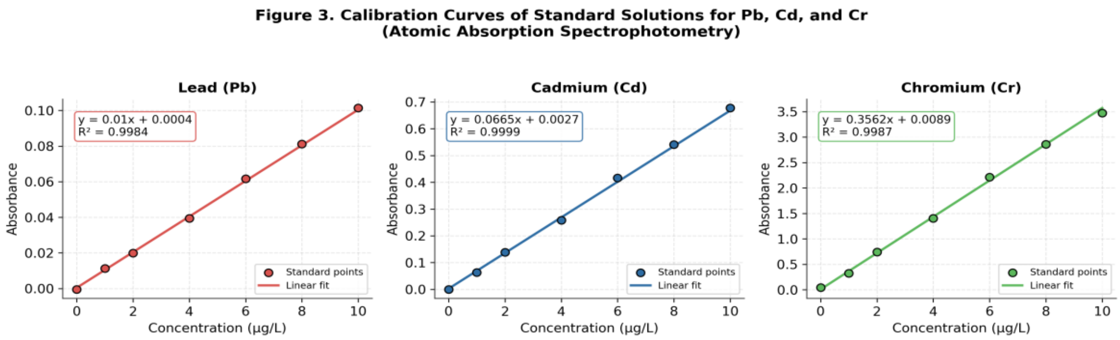

Figure 3. Calibration curves of standard solutions for lead, cadmium, and chromium by atomic absorption spectrophotometry, showing regression equations and coefficients of determination (R^2).

Regulatory bodies including the WHO and U.S. EPA set maximum contaminant levels for heavy metals in drinking water specifically to protect public health [39,40,41]. Table 3 lines up the measured concentrations in the Werdama wells against the WHO MCL for each metal.

Table 3. Concentrations of lead, cadmium, chromium, and iron in the three Werdama wells, compared with WHO maximum contaminant levels (MCL).

Heavy metal	W1 (µg/L)	W2 (µg/L)	W3 (µg/L)	WHO MCL (µg/L)
Lead (Pb)	5.3	45.0	9.1	15
Cadmium (Cd)	5.6	6.4	6.1	5
Chromium (Cr)	1.4	2.1	1.1	50
Iron (Fe)	986.0	1080.2	1031.8	300

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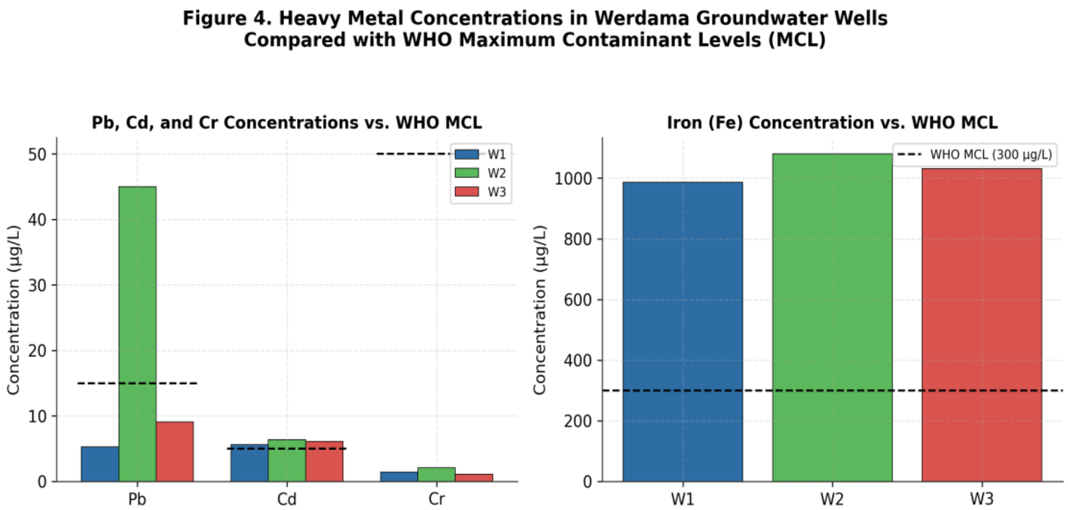

Figure 4. Heavy metal concentrations (Pb, Cd, Cr, and Fe) in Werdama groundwater wells compared with WHO maximum contaminant levels (dashed lines).

Lead is a highly toxic, non-essential metal that affects nearly every organ system once it's absorbed, mainly through its interference with sulfhydryl-dependent enzymes and its tendency to displace essential coenzyme metals. Chronic exposure has been linked to miscarriage, stillbirth, and congenital abnormalities, which is why the WHO limit is set as low as 15 µg/L. Measured Pb in the Werdama wells ranged from 5.3 µg/L (W1) to 45 µg/L (W2); W1 and W3 (5.3 and 9.1 µg/L) stayed within the WHO limit, but W2 came in roughly three times over it, the single largest exceedance we recorded in this study. A similar heavy-metal survey of groundwater wells in El-Beida city also found measurable lead enrichment above WHO guidance in some of its wells, and the authors pointed to a mix of natural mineralisation and encroaching human activity around the wellheads as the likely cause [42]. Seeing Pb exceedances turn up again in an independent well network within the same city supports the idea that localised contamination sources, not uniform city-wide geology, are what's driving the sharp gap between W2 and its neighbouring wells here. The WHO maximum contaminant levels for iron, cadmium, and chromium are 300, 5, and 50 µg/L [22]. Iron came in at 986.0, 1080.2, and 1031.8 µg/L in W1, W2, and W3, roughly three times the WHO limit in every well, most likely from well depth and long contact with iron-bearing rock. Cadmium (5.6–6.4 µg/L) exceeded the WHO limit but stayed below the more permissive EPA threshold of 7 µg/L, a borderline signal that's worth continuing to watch. Chromium (1.1–2.1 µg/L) was comfortably within internationally accepted limits in all three wells (Table 6).

Heavy Metal Pollution Index (HPI)

The HPI folds the concentrations of multiple heavy metals into one composite score reflecting overall pollution burden; higher values mean worse contamination. Using the WHO classification (low, < 50; medium, 50–100; high, > 100), we calculated HPI values of 97.99, 120.40, and 128.50 for W1, W2, and W3 (Table 4, Figure 5).

Table 4. Heavy Metal Pollution Index (HPI) values and risk classification for the Werdama wells.

Well	HPI value	Classification (WHO scale)	Risk category
W1	97.99	50–100	Medium (borderline high)
W2	120.40	>100	High
W3	128.50	>100	High

W1's HPI of 97.99 sits right at the upper edge of the medium-risk band, meaning a moderate but real long-term health risk if the water is drunk untreated; it's worth keeping an eye on in case it climbs further. W2 (120.40) and W3 (128.50), on the other hand, both land in the high-risk category. In practical terms, that means the water isn't fit for direct consumption as-is, and some form of corrective treatment- household- or community-scale reverse osmosis or ion exchange- would be needed, along with tracking down and controlling whatever is driving the contamination. These HPI numbers are in the same ballpark as, though a bit higher than, what's been reported for comparable semi-arid, agricultural aquifers elsewhere in the region. In the Mnasra area of Morocco's Gharb Plain, HPI ranged from 20.23 to 128.60 across an agricultural aquifer, and the upper end of that range lines up closely with what we see at W2 and W3 [43]. A similar assessment of the Saïs Plain aquifer in Morocco, using essentially the same AAS methodology for Pb, Cd, Cr, and Fe, also flagged iron and chromium as locally important, though at lower absolute levels than at Werdama [44]. Groundwater from the Wadi Al-Hamd basin in northwest Saudi Arabia tells a different story: an arid setting with less agricultural and human pressure returned much lower HPI values (0.15–10.07), more consistent with a mostly geogenic, low-pollution signature [45]. Set against that spread, the elevated HPI at Werdama points to local factors, quite possibly well depth, aquifer lithology, or nearby waste disposal, pushing heavy-metal levels beyond what geogenic weathering alone would produce in a similarly arid setting.

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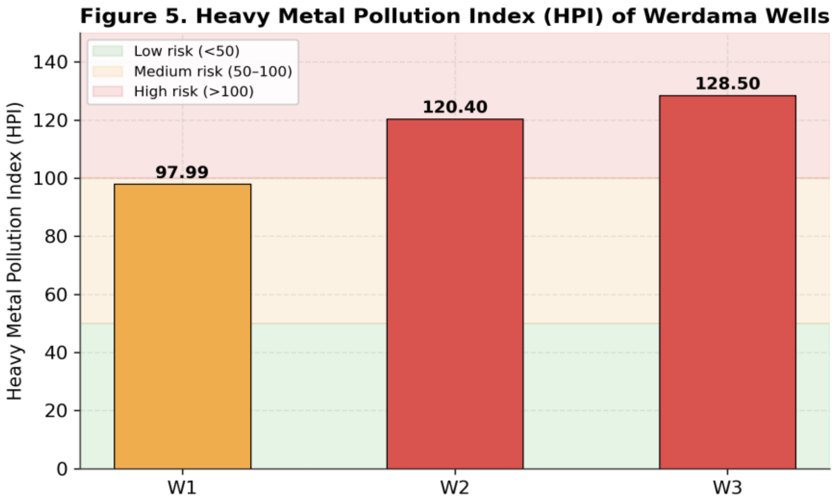

Figure 5. Heavy Metal Pollution Index (HPI) of the Werdama wells, shaded by WHO risk category (low < 50; medium 50–100; high > 100).

Microbial risk assessment

We used Nutrient Agar, MacConkey Agar, and E.M.B Agar together to get a multi-media read on the microbial load in each well (Table 5, Figure 6), and interpreted the counts against WHO risk thresholds for drinking water [37].

Table 5. Microbial colony counts (CFU/100 mL) across three culture media and corresponding water-quality classification for the Werdama wells.

Well	Nutrient Agar (CFU/100 mL)	MacConkey Agar (CFU/100 mL)	E.M.B Agar (CFU/100 mL)	Water quality rating
W1	5	8	7	Low risk / exceptionally pure
W2	24	30	32	Moderate risk / moderately pure
W3	27	40	35	Moderate risk / moderately pure

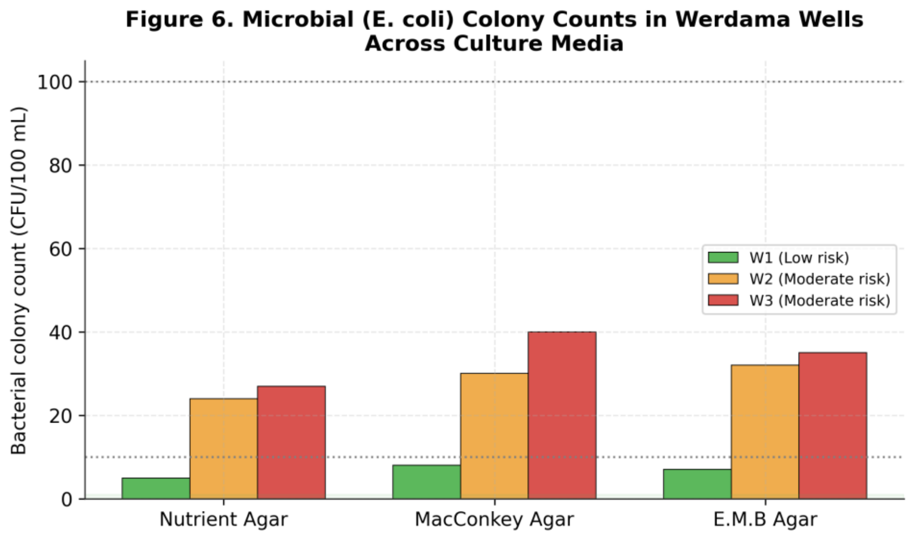

Figure 6. Bacterial colony counts (E. coli-consistent) across Nutrient Agar, MacConkey Agar, and E.M.B Agar for Wells W1–W3, relative to the WHO low-risk (1–10 CFU/100 mL) and moderate-risk (11–100 CFU/100 mL) thresholds (dotted lines).

W1 had the lowest colony counts on all three media (5–8 CFU/100 mL), putting it in the low-risk, exceptionally pure category. W2 and W3 came in much higher (24–40 CFU/100 mL), consistent with moderate contamination risk. The fact that neighbouring wells differ this much in microbial loading points to spatial heterogeneity, probably tied to differences in well construction, depth, or how close each well sits to contamination sources like septic systems or surface runoff. Growth on MacConkey Agar confirmed Gram-negative bacteria were present [46], and the E.M.B Agar results told the same story, together supporting the conclusion that faecal-associated Gram-negative organisms, E. coli among them, are present at low-to-moderate levels in all three wells [47]. Finding E. coli doesn't confirm actual pathogens are present, but it's a recognised proxy for faecal contamination, and it's reasonable to treat the water as a precaution before drinking it [31]. This kind of uneven microbial loading between neighbouring wells shows up elsewhere too. A ten-well survey in Tobruk, in eastern Libya, found E. coli in most of the untreated wells but not all, and conventional treatment eliminated it from every well afterwards [48]. That before-and-after comparison has a practical takeaway for Werdama: routine disinfection or

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filtration at the wellhead would probably be enough to clear the low-to-moderate E. coli loads seen here, even though the heavy-metal contamination would still need its own, separate fix.

Integrated risk perspective
Putting the physicochemical, heavy-metal, and microbiological data side by side, they tell a consistent story: groundwater quality at Werdama is compromised both by geogenic heavy-metal enrichment (Pb and Fe especially, Cd to a lesser degree) and by low-level microbial contamination pointing to faecal input. Table 6 pulls the heavy-metal exceedance profile together for quick reference. None of the three wells can really be called consistently safe for direct, untreated consumption, and W2 and W3 are the two with the most combined chemical and microbial concern. This kind of dual vulnerability, heavy metals and microbial contamination showing up in the same source, has turned up before in a comparative hygienic survey of unprotected dug wells across Libyan and Egyptian districts, which similarly found elevated lead, cadmium, and hardness alongside poorly protected wellheads, and concluded that the lack of a unified, routine monitoring framework was a key structural reason behind the problem [49]. Seeing this same chemical-microbial pattern crop up in independent studies across the region suggests Werdama isn't an isolated case, but one instance of a wider regional challenge in how groundwater is managed.

Table 6. Summary of mean heavy-metal concentrations relative to WHO maximum contaminant levels, with associated health concerns.

Heavy metal	WHO MCL (µg/L)	Mean well concentration (µg/L)	Exceedance vs. WHO MCL	Health concern
Lead (Pb)	15	19.8	+32%	Neurotoxic; developmental and cardiovascular effects
Cadmium (Cd)	5	6.0	+21%	Nephrotoxic; bone demineralisation
Chromium (Cr)	50	1.5	Within limit	Toxic only at high Cr(VI) doses
Iron (Fe)	300	1032.7	+244%	Aesthetic/taste issues; GI irritation at excess

Conclusion
What we found is that groundwater in the Werdama area of Al-Bayda, Libya, is affected by both heavy-metal contamination and a low-to-moderate microbial risk, which speaks to the wider challenge of protecting water quality in a water-scarce, developing-country setting. Lead and iron exceeded WHO maximum contaminant levels in most of the sampled wells, cadmium exceeded WHO but not EPA thresholds, and chromium stayed within accepted limits. The Heavy Metal Pollution Index put two of the three wells in the high-risk category and the third at the borderline of medium-risk, values that sit toward the upper end of what's been reported for comparable agricultural and semi-arid aquifers elsewhere in North Africa [43,44], and well above the more geogenically stable, low-pressure aquifer of Wadi Al-Hamd in Saudi Arabia [45]. At the same time, E. coli counts confirmed faecal-indicator organisms at low-to-moderate levels across all wells, a pattern similar to, though somewhat less severe than, faecal contamination documented in untreated wells elsewhere in Libya before treatment was applied [48]. Taken with comparable physicochemical and heavy-metal surveys done elsewhere in Al-Bayda city [38,42], these results tell us the Werdama groundwater sources aren't consistently safe for untreated consumption. Addressing this will take several things working together: better solid-waste and wastewater management to cut anthropogenic loading, protective well construction and sanitary buffers to keep fecal contamination out, targeted treatment (ion exchange or reverse osmosis, for instance) wherever heavy-metal exceedances are confirmed, and, importantly, a regular groundwater-monitoring program to catch trends early and trigger action in time, in line with what's been recommended for comparable Libyan and regional aquifers [38,49]. Future work should widen the sampling in both space and time, bring in isotopic or geochemical source-apportionment methods to separate geogenic from anthropogenic metal sources, and extend the microbial work to molecular confirmation of E. coli and any associated pathogens.

Statements and Declarations
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Author Contributions
All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Hameda A. Moftah and Lamya F. A. El-jalel. The manuscript was written and edited by Hameda A. Moftah and Adel M. A. Mahmoud. All authors read and approved the final manuscript.

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Data Availability
The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing Interests
The authors declare that they have no known competing financial or non-financial interests that could have influenced the work reported in this paper.

Ethics Approval
This study did not involve human participants or animals; therefore, ethical approval was not required.

Consent to Participate
Not applicable.

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