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Seasonal Changes in Chemical and Microbiological Contamination of Rural Groundwater: A Case Study from Dalgo Pole, Bulgaria

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HIGHLIGHTS

- Despite the EU efforts many rural regions continue to drink contaminated groundwater.
- Five wells underwent assessment for water quality parameters across four seasons.
- Hydrochemical analyses indicate sewage and fertilization as main pollution sources.
- PCA shows positive NH_4^+ and microbiological relation during summer-autumn season.

Abstract: One of the main EU priority is to ensure clean water for all its residents. However, challenges persist, particularly in rural regions lacking proper infrastructure, where groundwater from individual wells is basically used. The present study examines seasonal changes and potential pollution sources in five groundwater wells G1, G2, G3, G4 and G5 in the village of Dalgo Pole, Bulgaria. The results obtained by standard methods reveal consistent exceedances of NO_3^- concentrations, particularly notable in autumn, reaching 246 mg/dm^3 in well G5. NH_4^+ concentrations exceed permissible levels only in summer, with values in wells G2 and G5 over 5 mg/dm^3. NO_2^- levels are higher than allowable for drinking water by two times in the autumn sample of well G4. The nitrate pollution index and NO_3^-:Cl^- ratio suggest sewage runoff and fertilization as predominant pollution sources, with seasonal variations. Microbial contamination persists year-round, especially mesophilic aerobic and facultative anaerobic microorganisms at 22 °C with reduced levels in autumn, and *E*. *coli* viable cells peaking in summer and autumn. Others such as *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and sulphite-reducing bacteria show highest concentrations in spring-autumn seasons. Principal component analysis indicates consistent positive relationships between NH_4^+ and microbiological concentrations during summer and autumn, particularly in wells G1, G2, and G4, highlighting seasonal trends and potential contamination sources. Hence, more efforts are needed to improve groundwater quality in vulnerable rural areas of Bulgaria, by implementing enhanced monitoring and control of well waters, mirroring practices in other EU countries.

Keywords: groundwater pollution, microbiological indicators, NO_3^-:Cl^-, PCA.

INTRODUCTION

Clean drinking water is vital for human life, agriculture, and industry [1-7]. Despite its importance, water remains poorly managed globally [5]. While surface water is prominent, groundwater is equally critical, supplying much of the world's freshwater, especially in arid regions where it serves as the primary drinking source due to its resistance to surface pollution [3,6,8-11]. Groundwater contamination threatens its sustainable use, stemming from point sources (industrial discharges, livestock operations) and diffuse sources (agricultural runoff, atmospheric deposition) [5,10,12,13]. Contamination persists for long periods, making remediation costly and complex [5,10,12]. While regulations like Directive 2010/75/EU address industrial pollution, diffuse sources remain poorly controlled [5,14]. Thus, stricter measures are needed to manage contaminated groundwater and reduce associated health risks.

Standards by the World Health Organization (WHO) and European Union (EU), particularly Directive (EU) 2020/2184, ensure the chemical and microbiological safety of drinking water by setting criteria for inorganic N forms: NO_3^-, NO_2^- and NH_4^+ [11] and hygiene indicator microorganisms, as groundwater contamination rises in Europe [2,13,15-19]. NO_3^-, often from fertilizers and septic systems, frequently exceeds the WHO limit of 50 mg/L due to its solubility and persistence, penetrating deep aquifers [5,18,20-22]. This has led to nitrate-vulnerable zones in the EU, including Bulgaria, though their delineation remains inadequate [20,23]. Regular assessments of private rural wells, especially in nitrate-vulnerable areas, are crucial as many residents rely on this water, risking illness from elevated NO_3^- [20,23]. Other contaminants include NO_2^-, carcinogenic above 0.5 mg/L, and NH_4^+, signaling fecal contamination at levels above 0.2 mg/L, traceable using NH_4^+:Cl^- and NO_3^-:Cl^- ratios [8,12,21,24-26]. Microbiological contamination, linked to 50% of global waterborne diseases, worsens with urban and agricultural non-point pollution in areas with poor sewage systems [2,22,27,28]. Routine testing is essential, as pathogens like *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Clostridium perfringens* are common in untreated well water [2,29]. While *E*. *coli* indicates fecal contamination, its reliability drops in warmer climates, favoring *Pseudomonas aeruginosa* [28-30]. Testing delays (24-48 hours) hinder timely risk identification, emphasizing the need for thorough water quality assessments in regions with seasonal pollution variability [5,30]. Therefore, relationships between groundwater contaminants, such as inorganic ions and hygienic indicator microorganisms, should be explored to enable accurate and rapid recognition of contamination. Thus, the detection of specific ions in groundwater can indicate the potential presence of hygiene-indicator microorganisms and alert rural residents about the serious health risks posed by the use of untreated water, especially considering the significant impact of seasonal variations in pollution levels.

Despite existing EU regulatory frameworks for groundwater quality and efforts to improve water supply and reduce bottled water consumption, exemptions for small water supply installations (under 50 people) present significant challenges in ensuring water purity, particularly in rural areas with limited plumbing infrastructure which prevail on the territory of Bulgaria [16,17]. As of November 2024, there are 438,467 registered private wells in the country. Due to recent droughts, strict water restrictions, and severe drinking water shortages in some villages, many people have turned back to underground water sources for relief. This practice, which has helped Bulgarians manage water scarcity for centuries, is now vital, but often lead residents to rely on untreated water from their own wells for drinking and domestic purposes. The underlying cause is that the Water Act regulates the amount of water consumed from wells, but it does not address the quality of the water, leading residents to use unprocessed well water without being aware of the potential health risks [18,31]. Thus, the need for continuous monitoring and enhanced regulation of groundwater sources, especially private wells in non-urban Bulgarian territories is essential [3,5,11,16,17].

Currently, there is no data on how seasonal changes impact pollution levels in individual water sources in the village of Dalgo Pole, Bulgaria. This knowledge deficiency necessitates a study to monitor seasonal fluctuations in groundwater quality and identify potential risks of contamination in five unregulated wells used for drinking and domestic needs in this locality. The aim of the present research is to provide insights into these seasonal variations and assess contamination risks in the selected wells. The obtained results could be an important base for raising public awareness about the potential risks of groundwater contamination and highlight the need for its future treatment before consumption.

MATERIAL AND METHODS

Materials

The studied groundwater sources are in northern Dalgo Pole village, Bulgaria, which has an aging water system fed by two tube wells [32]. Although the supplied water meets national standards, frequent interruptions of 2–4 days per week force residents to use individual wells [33,34]. The village lacks a sewage system, and a significant flood occurred in 2005 when the nearby Tikla River inundated the entire area [32,35].

Five wells (designated as G1, G2, G3, G4, and G5) were analyzed. Figure 1 and Figure 2 illustrate the study area, including the georeferenced locations of the wells and the Land Use and Land Cover (LULC) map, respectively. Land potentially influencing the studied zone includes pastures, complex cultivation patterns, and agricultural land with significant natural vegetation. The depth of groundwater is 12 m for well G1, 14 m – for G2, 12 m – for G3, 13 m – for G4 and 11 m for G5, respectively. About 10 m from G1 and 6 m from G3 there is a septic tank, abandoned more than 10 years ago. About 10 m from G5 there is still a usable septic tank and animals are raised around.

The groundwater samples scheduled for analysis have been collected since November 2022 to September 2023 to cover the four seasons: autumn (September-December), winter (December-March), spring (March-June), summer (June-September). The results were evaluated based on the water quality requirements for drinking and domestic activities defined by [36].

Methods

Map generation methods

Map of the study area and a LULC map for the village of Dalgo Pole are generated using Geographic Information System (GIS). The CORINE Land Cover 2018 dataset is used to visualize the LULC types within the village boundaries [37].

Chemical and microbiological indicators

Standard analysis procedures are used in the study for determination of groundwater chemical and microbiological indicators. Photometric test series Spectroquant®, Merck Millipore, USA were applied for assessment of NH_4^+, NO_2^-, NO_3^-, Cl^- concentrations by application of ammonia test [38], nitrite test [39], nitrate test [40] and chloride test [41], respectively. The number of viable cells of *Pseudomonas aeruginosa*, are determined following the ISO 16266:2008 [42]. Detection and enumeration of faecal enterococci was performed in accordance with ISO 7899-2:2003 [43]. The total number of mesophilic aerobic and facultative anaerobic microorganisms was conducted by application of ISO 6222:2002 [44] at 22°C and at 37°C, respectively, while sulphite-reducing bacteria – by using the ISO 6461-2:1986 [45].

Nitrate pollution index and NO_3^-:Cl^- ratio

Following the procedure, described by [46] nitrate pollution index is calculated by Equation (1):

$$NPI = (C_s - HAV)/HAV \tag{1}$$

where: NPI is nitrate pollution index; C_s is real NO_3^- concentration determined in the groundwater sample; HAV – human affected value - threshold value of human related activities taken as 20 mg/dm^3 [47].

Groundwater quality is categorized based on NPI into five groups: clean (unpolluted): NPI < 0; slightly polluted: 0 < NPI < 1; moderately polluted: 1 < NPI < 2; significantly polluted: 2 < NPI < 3; very significantly polluted: NPI > 3.

NO_3^-:Cl^- ratio analysis is interpreted based on the methodology of [48].

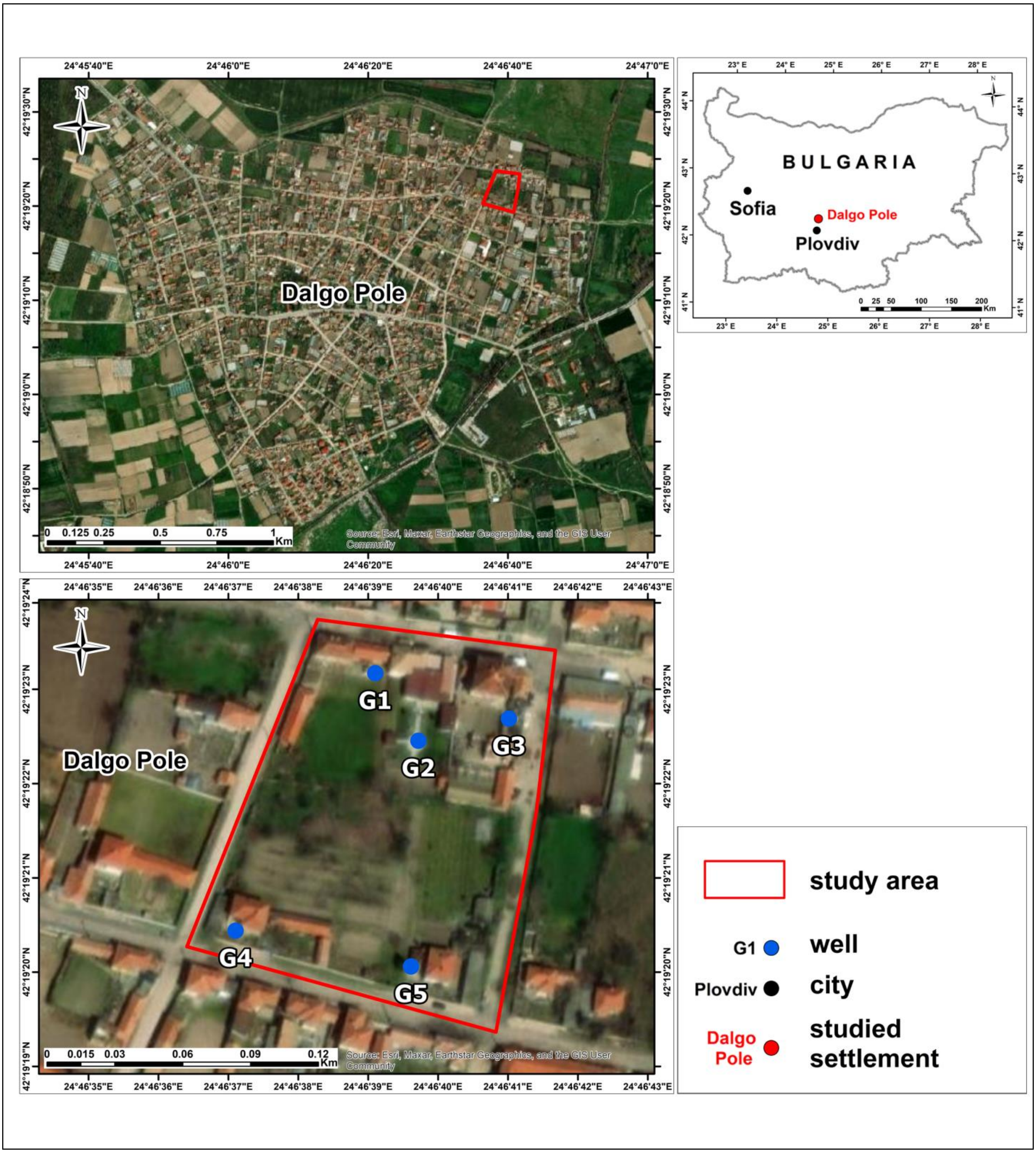

Figure 1. Georeferenced representation of the study area with locations of the five wells

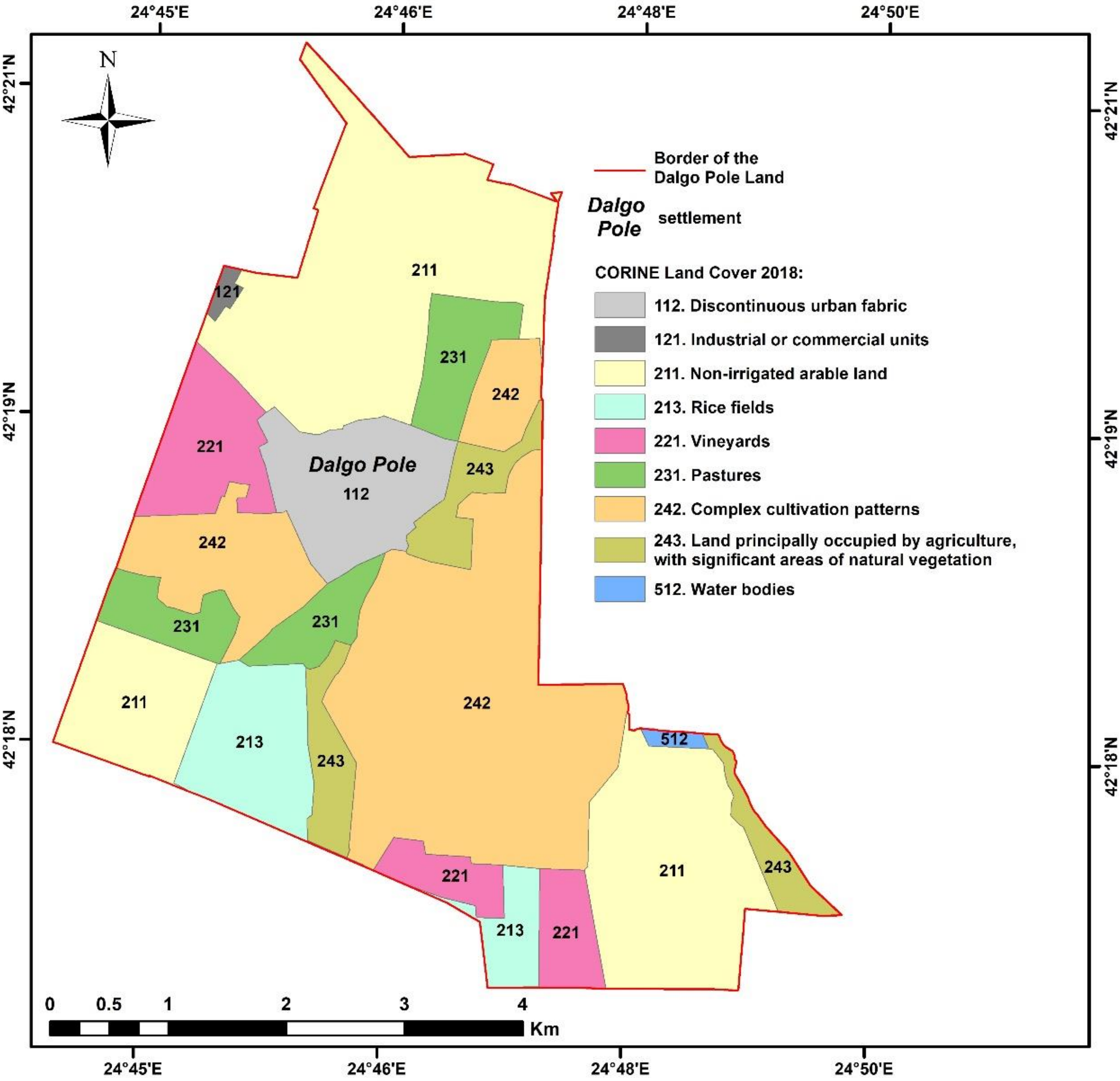

Figure 2. Land use/Land cover map of the village Dalgo pole, Bulgaria

Data analysis and statistical methods used

In accordance with the aims of the research Pearson's correlation coefficient was calculated using MS Office Excel®, 2010. Principal component analysis (PCA) was performed on the collected data to obtain correlations between hydrochemical parameters and microbiological indicators of the groundwater samples [49]. Friedman's test was conducted to identify significant differences in groundwater parameters. Statistical analysis was performed using software XLSTAT (Addinsoft©).

RESULTS AND DISCUSSION

Seasonal changes in NO_3^-, NO_2^-, NH_4^+ and Cl^- concentrations in groundwater from wells G1-G5

The EU has prioritized providing clean water since the UN recognized it as a universal human right in 2010 [50]. However, small settlements still face challenges in accessing safe water, often relying on self-extraction methods, which are further complicated by the prevalence of groundwater pollutants such as inorganic N compounds and Cl^- [5,10]. Figure 3 presents the concentrations of the investigated ions NO_3^-, NO_2^-, NH_4^+ and Cl^- in groundwater from wells G1-G5. The results are categorized by seasons and include the maximum allowable concentration for each ion based on Bulgaria's national water quality standards for drinking and domestic use [36].

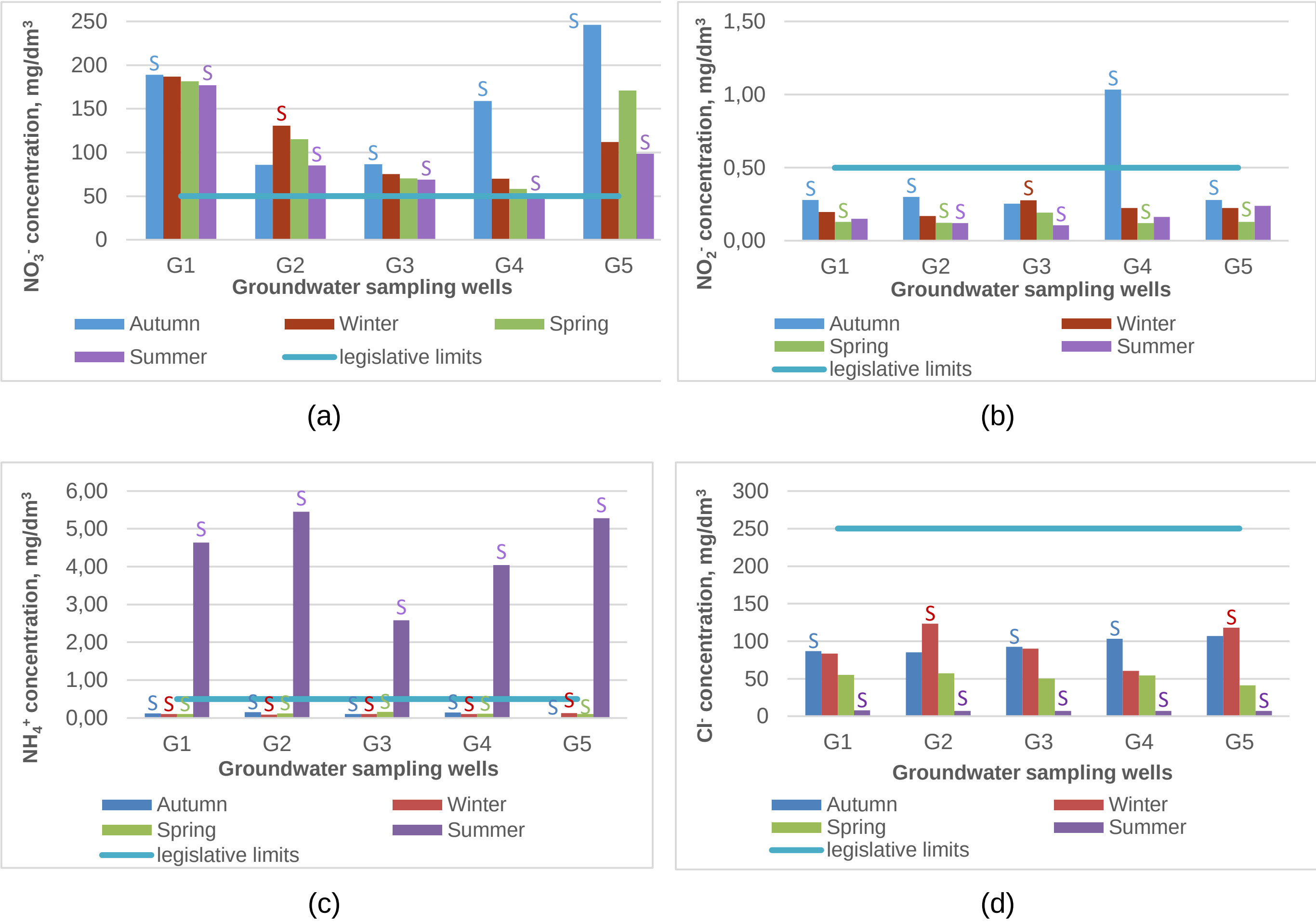

Figure 3. Concentrations of studied ions in groundwater from wells G1-G5: (a) NO_3^-; (b) NO_2^-; (c) NH_4^+; (d) Cl^-Legend: S - denotes seasonal significant differences in the groundwater concentrations of NO_3^-, NO_2^-, NH_4^+, Cl^- for the corresponding well.

The monitoring shows significant seasonal variations in hydrochemical indicators across specific wells, with exceedances of allowed levels for inorganic N. The most indicative results pertain to NO_3^-, with concentrations frequently surpassing the legally established limit of 50 mg/dm³, underscoring the pervasive issue of nitrate contamination in groundwater across small settlements within the EU, as documented by multiple studies [5,6,16,51-53]. Substantial seasonal changes, according to Friedman's test, are observed in wells G1, G3, G4, and G5, with decreasing NO_3^- values from summer to autumn, while well G2 shows notable differences between spring and summer. The septic tank near well G1 causes consistently high NO_3^- levels year-round. Putra and coauthors, 2007 noted higher NO_3^- leakage rates in older villages compared to those with newer sewage systems [54]. Despite being unused for 10 years, G1 shows concentrations of 177-189 mg/dm³. Well G5, near an active septic tank, exhibits significant seasonal NO_3^- variations due to continuous pollution and its shallow depth, with peaks in autumn (246 mg/dm³) and less in summer (99 mg/dm³). Similar findings by Sasakova and coauthors, 2018 in Slovakia [5] suggest these fluctuations result from anoxic zones, where high summer temperatures, low oxygen, and microbial activity drive denitrification [55]. NO_3^- concentrations decrease sharply, particularly in shallow wells [10]. The lowest levels are in G3 (69–87 mg/dm³) and G4 (52–159 mg/dm³). Wells G1, G3, and G4 show a decreasing trend from autumn to summer. Autumn NO_3^- increases are likely due to agricultural activity and rainfall. At G2, concentrations reach 131 mg/dm³ in winter and 115 mg/dm³ in summer, dropping to 85–86 mg/dm³ in other seasons, a pattern similar to G5 but during autumn-spring (Figure 3a). At G5, rainfall and wastewater filtrates may drive NO_3^- increases, while in G2, mixing of groundwater flows likely plays a role [56-58]. Though high, NO_3^- levels (443–885 mg/dm³) are not extreme for healthy adults but pose risks for infants and young children if consumed untreated [5].

NO_2^- and NH_4^+ are unstable and easily oxidized to NO_3^- [13]. Groundwater NO_2^- levels generally meet standards (0.12–0.28 mg/dm³) [36], except in autumn at well G4, which shows 1.03 mg/dm³ (Figure 3b). This exceeds the permitted limit (0.5 mg/dm³) and poses a significant carcinogenic risk to G4 users [25]. The EU threshold values for NH_4^+ in groundwater vary from 0.084 mg/dm³ to 5 mg/dm³, at 0.5 mg/dm³ for drinking waters [36,59]. Higher concentrations indicate potential fecal contamination or extensive use of artificial or unstabilized natural fertilizers [8,60]. Summer well samples show significant differences with other seasons in NH_4^+ concentrations, reaching levels 5-10 times above the country's permissible drinking water limits (Figure 3c). In the hot season, soil microbes degrade organic compounds into NH_4^+, which quickly enters groundwater and converts to NO_3^- [10]. The highest concentration is at G2 (5.45 mg/dm³), likely due to intensive nitrogen fertilizer use [4,6]. G5 (5.28 mg/dm³) and G1 (4.64 mg/dm³) follow, likely influenced by fecal matter from latrines and agricultural practices [29]. The lowest levels are at G4 (4.04 mg/dm³) and G3 (2.58 mg/dm³). In summer, it is advised to avoid drinking groundwater from these wells, as NH_4^+ can disrupt metabolism, affect acid-base balance, impair glucose tolerance, and reduce insulin sensitivity [55].

Only Cl^- meet the regulatory requirements for groundwater samples (Figure 3d) [36].

Seasonal distribution of nitrate pollution index for groundwater from wells G1-G5

The Nitrate Pollution Index (NPI) is a common tool for assessing groundwater quality related to human activities [46,47,61-63]. NO_3^- levels exceeding 20 mg/dm³ indicate that human impact is the primary pollution factor [64]. Groundwater analysis results are presented in Table 1. The data for each well are analyzed seasonally with an annual summary also provided.

Table 1. Nitrate pollution index in investigated wells

Sample	NPI				
	Autumn	Winter	Spring	Summer	Anual
G1	8.45	8.33	8.07	7.85	8.17
G2	3.29	5.54	4.76	3.25	4.21
G3	3.33	2.76	2.53	2.44	2.76
G4	6.95	2.50	1.92	1.60	3.24
G5	11.31	4.60	7.55	3.93	6.84

Legend: NPI – nitrate pollution index; G1-G5 – investigated wells. NPI value groups: - unpolluted: NPI < 0; slightly polluted: 0 < NPI < 1; moderately polluted: 1 < NPI < 2; significantly polluted: 2 < NPI < 3; very significantly polluted: NPI > 3.

The annual NPI indicates very significant contamination (NPI > 3) in most wells, with the highest value at G1 (8.17). Groundwater NO_3^- pollution is higher in areas with porous, well-aerated soils, such as the alluvial layers in Dulgo Pole [65], where N compounds quickly oxidize to NO_3^- and infiltrate groundwater [66]. Only G3 shows significant pollution, with an NPI of 2.76. The highest NPI, 11.31, is found in G5 autumn samples, likely due to the nearby septic pit. The lowest NPI, 1.92 and 1.60, is in G4 for spring and summer, respectively. Agricultural fertilization in Bulgaria runs from February to August, with the 2023 season starting amid low groundwater levels during the hottest years on record [67-69]. N compounds are absorbed by crops in spring and summer, and dry weather limits NO_3^- infiltration. However, autumn rains may increase NPI values by promoting NO_3^- penetration into groundwater [6].

Variations in groundwater NO_3^-:Cl^- ratio for wells G1-G5

Anthropogenic factors, such as untreated sewage runoff and agriculture, impact NO_3^- groundwater pollution [70]. The NO_3^-:Cl^- ratio is a reliable indicator for identifying pollution sources, widely recommended for detecting groundwater contamination [54,71,72]. Annual and seasonal NO_3^-:Cl^- variations for wells G1–G5 are shown in Figure 4. The well locations suggest that point sources from sanitary-domestic activities are the main contributors to contamination [6], which is confirmed by the results. Annual values show the NO_3^-:Cl^- ratio in all wells ranges from 1:1 to 1:8 indicating that NO_3^- origin is likely fecal contamination [48,70]. Ratha and coauthors, 2019 claim that human sewage has a NO_3^-:Cl^- ratio of 2-2.5:1 [10]. The most impacted is the G5 well with a corresponding ratio NO_3^-:Cl^- – 2.3:1, where the probable point source of fecal contamination is a functioning septic tank. The greatest impact of untreated wastewater on pollution with NO_3^- was found in a well G1 with NO_3^-:Cl^- ratio 3.14:1, influenced by decomposing waste in an abandoned pit. Old septic tanks and similar point sources of pollution can cause high NO_3^- in rural groundwater [6]. At G3 it is the lowest – 2.14:1.

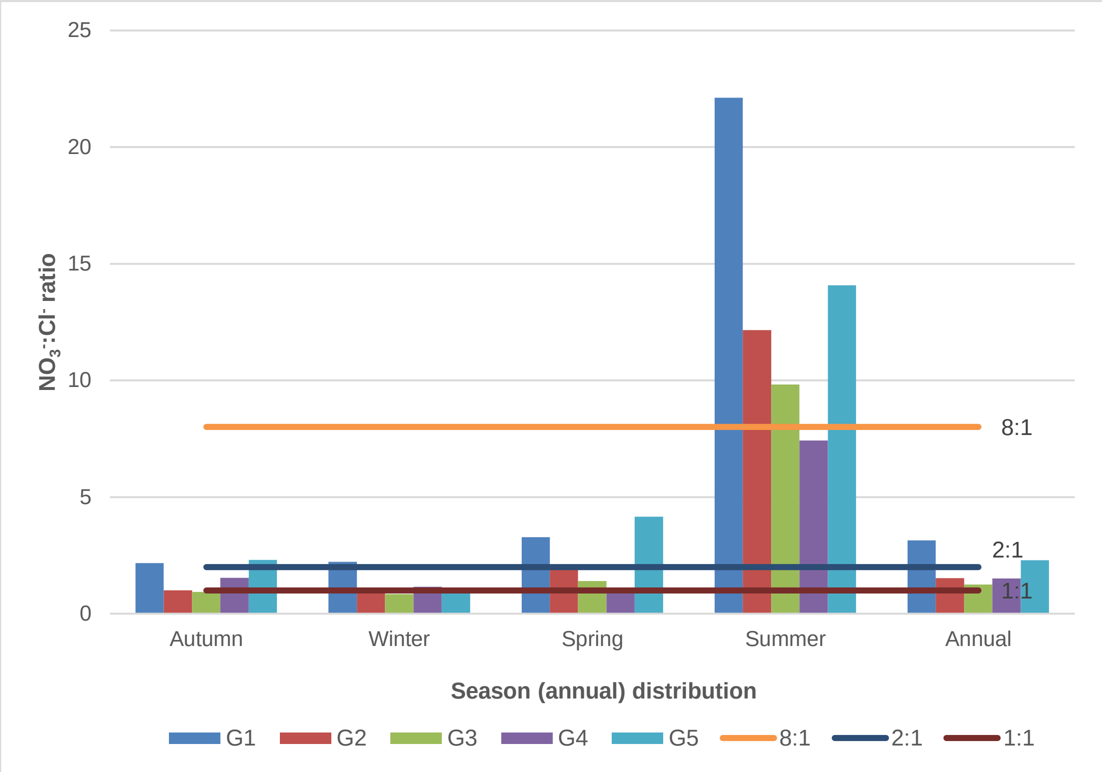

Figure 4. Groundwater NO_3^-:Cl^- ratio of seasonal samples from wells G1-G5

Throughout the year, Figure 4 shows a summer increase in NO_3^-:Cl^- ratios, shifting from 1:1–8:1 to higher levels, indicating that not all NO_3^- from local sewage reaches groundwater. Agricultural fertilization, a non-point source, dominates over point sources like domestic activities [10,73]. This effect is most evident at well G1 (22.12:1), followed by G5 (14.07:1), G2 (12.15:1), and G3 (9.83:1). At G4, summer NO_3^- pollution stems mainly from domestic effluents (7.42:1). Autumn and winter show the lowest ratios, with G3 and G5 values dropping below 1:1 (0.94:1, 0.84:1, and 0.95:1), likely indicating fecal contamination in populated areas. The most significant changes in the NO_3^-:Cl^- ratio occur in autumn and winter, when Cl^- concentrations peak, probably due to seasonal chlorination of drinking water. This factor may disrupt the NO_3^-:Cl^- balance in subsequent seasons [74].

Microbiological evaluation of investigated groundwater from wells G1-G5

The microbiological safety of groundwater is crucial for ensuring its suitability for drinking and domestic use [3]. Table 1 summarizes the results of the microbiological quality of groundwater from wells G1-G5. Exceeded permissible values are described in bold. Significant differences in the groundwater concentrations of the indicator microorganism between each season for the respective well are shown in brackets. All wells show high microbial contamination. The total microbial count (HPC-22) ranges from $1.01*10^2$ cfu/cm³ at G1 in autumn-winter to $3*10^3$ cfu/cm³ at G2 in summer. Only the autumn sample from G4 meets legal limits: 1 cfu/100 cm³ [34]. In summer, HPC-22 counts are significantly higher at G1, G2, and G4, reaching $2.1*10^3$, $3*10^3$, and $2.5*10^3$ cfu/100 cm³, respectively. A Friedman test identifies significant seasonal variations in HPC-22 values, notably between autumn and summer (Table 2), alongside inversely proportional NO_3^- concentrations in wells G1, G3, G4, and G5 (Figure 3). A comparable pattern emerges during the spring-summer period for well G2. HPC-22 assesses general water pollution, while HPC-37, measuring mesophilic microorganisms at 37°C, is more significant for detecting contamination from warm-blooded animals [75]. Only two samples meet the norm of 20 cfu/100 cm³: G4 in autumn (18 cfu/100 cm³) and G1 in winter (9 cfu/100 cm³). In winter and spring, HPC-37 reaches the 2nd order, rising to the 3rd order in summer for wells G1 and G4. A resemblance in seasonal trends is noted between HPC-37 viable cell counts and NO_3^- concentrations during the autumn-summer period for wells G2 and G5 (Figure 3). The results corroborate Sasakova and coauthors findings, 2018 that groundwater from individual sources in small settlements consistently exhibits high contamination levels year-round, indicating potential deterioration of water quality [5,30].

Table 2. Microbiological groundwater quality in analyzed wells G1-G5

Season	Sample	*E. coli*	*Ent. faecalis*	*Ps. aeruginosa*	SRM	HPC-22	HPC-37
		Legislative limits					
		0 cfu/100 cm^3	0 cfu/100 cm^3	0 cfu/100 cm^3	0 cfu/50 cm^3	$<1 \ast 10^2$ cfu/cm^3	<20 cfu/cm^3
Autumn	**G1**	0 (S_{G1})	**2**	0	**$5 \ast 10^2$** (S_{G1})	**$1.03 \ast 10^2$** (S_{G1})	21
	G2	0 (S_{G2})	**$1 \ast 10^3$** (S_{G2})	0	0 (S_{G2})	**$1.04 \ast 10^2$** (S_{G2})	22 (S_{G2})
	G3	**$1.5 \ast 10^2$** (S_{G3})	**13**	**18**	**$1.2 \ast 10^2$** (S_{G3})	**$1.06 \ast 10^2$** (S_{G3})	**$1.5 \ast 10^2$**
	G4	**$1.1 \ast 10^2$** (S_{G4})	0 (S_{G4})	0	0 (S_{G4})	1 (S_{G4})	18
	G5	0 (S_{G5})	**17**	0	**$1 \ast 10^3$** (S_{G5})	**$1.01 \ast 10^2$** (S_{G5})	21 (S_{G5})
Winter	**G1**	**5**	0	0	**2**	**$3.85 \ast 10^2$**	9 (S_{G1})
	G2	**3**	**2** (S_{G2})	0	0 (S_{G2})	**$1.5 \ast 10^2$**	**$2.05 \ast 10^2$**
	G3	**1**	0 (S_{G3})	0 (S_{G3})	**3** (S_{G3})	**$2 \ast 10^2$** (S_{G3})	**$1.37 \ast 10^2$**
	G4	**10**	0 (S_{G4})	0	**21**	**$9 \ast 10^2$**	**$4.5 \ast 10^2$**
	G5	**61**	**6** (S_{G5})	0	**46** (S_{G5})	**$8.5 \ast 10^2$**	**$1 \ast 10^2$**
Spring	**G1**	0 (S_{G1})	**3**	0	**3**	**$1.03 \ast 10^2$** (S_{G1})	**$1.07 \ast 10^2$**
	G2	**3**	**$1.05 \ast 10^2$**	0	**$1.03 \ast 10^2$** (S_{G2})	**$1.03 \ast 10^2$** (S_{G2})	**$1.05 \ast 10^2$**
	G3	0 (S_{G3})	**$1.02 \ast 10^2$** (S_{G3})	**52** (S_{G3})	**51**	**$1.07 \ast 10^2$**	**$1.07 \ast 10^2$** (S_{G3})
	G4	0 (S_{G4})	**52** (S_{G4})	**$1.07 \ast 10^2$**	**$1.06 \ast 10^2$** (S_{G4})	**$1.03 \ast 10^2$**	**$1.08 \ast 10^2$**
	G5	0	**$1.04 \ast 10^2$** (S_{G5})	0	**$1.07 \ast 10^2$**	**$1.07 \ast 10^2$**	**$1.02 \ast 10^2$**
Summer	**G1**	**50** (S_{G1})	0	**$1.5 \ast 10^2$**	0 (S_{G1})	**$2.1 \ast 10^3$** (S_{G1})	**$1.5 \ast 10^3$** (S_{G1})
	G2	**26** (S_{G2})	**25**	0	**50**	**$3 \ast 10^3$** (S_{G2})	**$3.5 \ast 10^2$** (S_{G2})
	G3	**1**	**52**	**50** (S_{G3})	**58**	**$2 \ast 10^2$** (S_{G3})	**$1.6 \ast 10^2$** (S_{G3})
	G4	0 (S_{G4})	**3**	0	**50**	**$2.5 \ast 10^3$** (S_{G4})	**$1 \ast 10^3$**
	G5	**$1 \ast 10^2$** (S_{G5})	**16**	0	**54**	**$2 \ast 10^2$** (S_{G5})	**$8.5 \ast 10^2$** (S_{G5})

Legend: G1-G5 – analyzed groundwater wells; SRM – sulphate reducing microorganisms; HPC-22 – total number of mesophilic aerobic and facultative anaerobic microorganisms at 22 °C; HPC-37 – total number of mesophilic aerobic and facultative anaerobic microorganisms at 37 °C; cfu – colony forming units; S_{G1}, S_{G2}, S_{G3}, S_{G4}, S_{G5} – denote seasonal significant differences in the groundwater concentrations of the indicator microorganism for the corresponding well.

According to WHO, *E. coli* is a key indicator of fecal contamination and should not be present in 100 cm^3 of water [24,36,76]. In spring, only well G2 meets this standard with 3 cfu/100 cm^3. During winter, *E. coli* contamination was found in all wells, with the highest counts in G4 and G5 (10 and 61 cfu/100 cm^3, respectively). In autumn, *E. coli* was present in wells G3 ($1.5*10^2$ cfu/100 cm^3) and G4 ($1.1*10^2$ cfu/100 cm^3). In summer, only G4 met the regulatory limits, while G5 had $1*10^2$ cfu/100 cm^3. Seasonal variations are linked to sources like an abandoned septic tank at G3 and possibly unstabilized manure at G4 [29]. Autumn rains reduce soil resistance, allowing *E. coli* from manure to infiltrate groundwater, where it can survive in cold temperatures [28]. The spring-autumn transition showed significant differences in *E. coli* concentrations, with a reduction in G3 and G4 by spring, and in G5 during summer due to an active septic tank. In G1, a former septic tank continues to contribute to bacterial contamination, albeit at lower levels [2].

Ent. faecalis, typically a harmless bacterium found in mucosal tracts, can act as an opportunistic pathogen in immunocompromised individuals [77]. As a hygiene indicator microorganism, its presence is prohibited in 100 cm^3 of drinking water [34]. A significant difference is observed in winter-summer shift for G3, G4, G5 when high concentrations of *Ent. faecalis* are reported in the groundwater. For wells G2, G3 and G5 they are above $1*10^2$ cfu/100 cm^3. For wells G2 and G3 the likely cause lies in leachate from manure used to grow crops, and for G5 – mixing of underground and contaminated wastewater from uninsulated outdoor toilets [78,79]. However, the concentration is the highest in autumn – in a well G2 – $1*10^3$ cfu/100 cm^3 (Table 2).

Several authors have reported *Ps. aeruginosa* in groundwater, affecting its color, taste, and turbidity. Rural areas with poor hygiene are particularly vulnerable, as the microorganism readily forms resilient biofilms in groundwater systems [3,17,75]. Among the studied well samples in the spring and summer months, high concentrations of this bacterium were found at G1 reaching $1.5*10^2$ cfu/100 cm^3 in the summer and at G4 $1.07*10^2$ cfu/100 cm^3 in the spring, which poses health risks for people with a poorly functioning immune system [75]. However, a Friedman's test significant variations can be found in winter-summer period. In cold months only in well G3 there are counted 18 cfu/100 cm^3 for autumn groundwater samples. They are found often together with *E. coli* in a stable symbiosis in drinking water sewage systems [17]. Such a relationship was observed in well G3 in autumn, but was more pronounced in summer for wells G1 and G3.

Sulfite-reducing bacteria (SRM), known for their resistance to adverse conditions, are conservative hygiene indicators [80,81]. Table 2 suggests widespread SRM pollution during rainy seasons, with values exceeding $1*10^2$ cfu/50 cm^3 in spring. Significant variations, according to Friedman's test, are observed in the autumn-spring period for wells G2 and G4. A corresponding trend is noted for *Ent. faecalis*, whose presence indicates point sources of contamination from fecal waste of ruminants [82,83]. The highest concentration of SRM is from the autumn groundwater G5 – $1*10^3$ cfu/50 cm^3.

Analysis of the main components of the investigated chemical and microbiological indicators in groundwater samples from wells G1-G5

Principal component analysis was used to examine the relationship between seasonal changes in indicators, identifying three components with eigenvalues >1 that cumulatively explained 69.18% of the variance. Table 3 shows component formation, with bolded features indicating component loading.

Table 3. PCA factor loadings of the hydrochemical and microbiological indicators in groundwater from wells G1-G5

Groundwater Indicators	Factor loadings		
	F1	F2	F3
NO_3^-	-0.403	0.419	**0.638**
NO_2^-	-0.386	**0.656**	-0.404
Cl^-	**-0.866**	0.178	-0.103
NH_4^+	**0.912**	0.193	0.074
Escherichia coli	0.113	**0.758**	-0.464
SRM	-0.379	0.214	**0.727**
Enterococcus faecalis	-0.176	**-0.487**	-0.368
Pseudomonas aeruginosa	**0.458**	-0.084	0.152
HPC-22	**0.869**	0.188	0.051
TPC-37	**0.866**	0.212	0.108

Legend: PCA – Principal component analysis; F1-F3 – Factor 1 to 3, respectively; SRM – sulphate reducing microorganisms; HPC-22 – total number of mesophilic aerobic and facultative anaerobic microorganisms at 22 °C; HPC-37 – total number of mesophilic aerobic and facultative anaerobic microorganisms at 37 °C.

Although the formation of ions in groundwater is complex, the data can be grouped by component according to the main formation steps of inorganic pollutants, including ammonification (Component 1) and nitrification-denitrification (Components 2 and 3). The first principal component (PC) is primarily composed of five indicators: NH_4^+ – with very high positive PC loading (0.912); HPC-22 and HPC-37, both with high positive PC loadings – (0.869 and 0.866, respectively). *Ps*. *aeruginosa* has medium positive PC loading (0.458). The second PC consists of three basic indicators: *E*. *coli* and NO_2^- with high (0.785) and medium (0.656) PC loadings, respectively, and *Ent*. *faecalis* with low-positive PC loading (-0.487). The third PC is basically described by indicators with medium positive PC loadings: SRM (0.727) and NO_3^- (0.638). In other words, the first PC can be used to find a positive relationship between NH_4^+, HPC-22 and HPC-37: associated with uncontrolled animal waste degradation with NH_4^+ release in groundwater; the second – for predominant denitrification processes – as there is a positive correlation between NO_2^- and *E*. *coli* and the third one – with anaerobic N stabilization: with a positive PC values of SRM and NO_3^-.

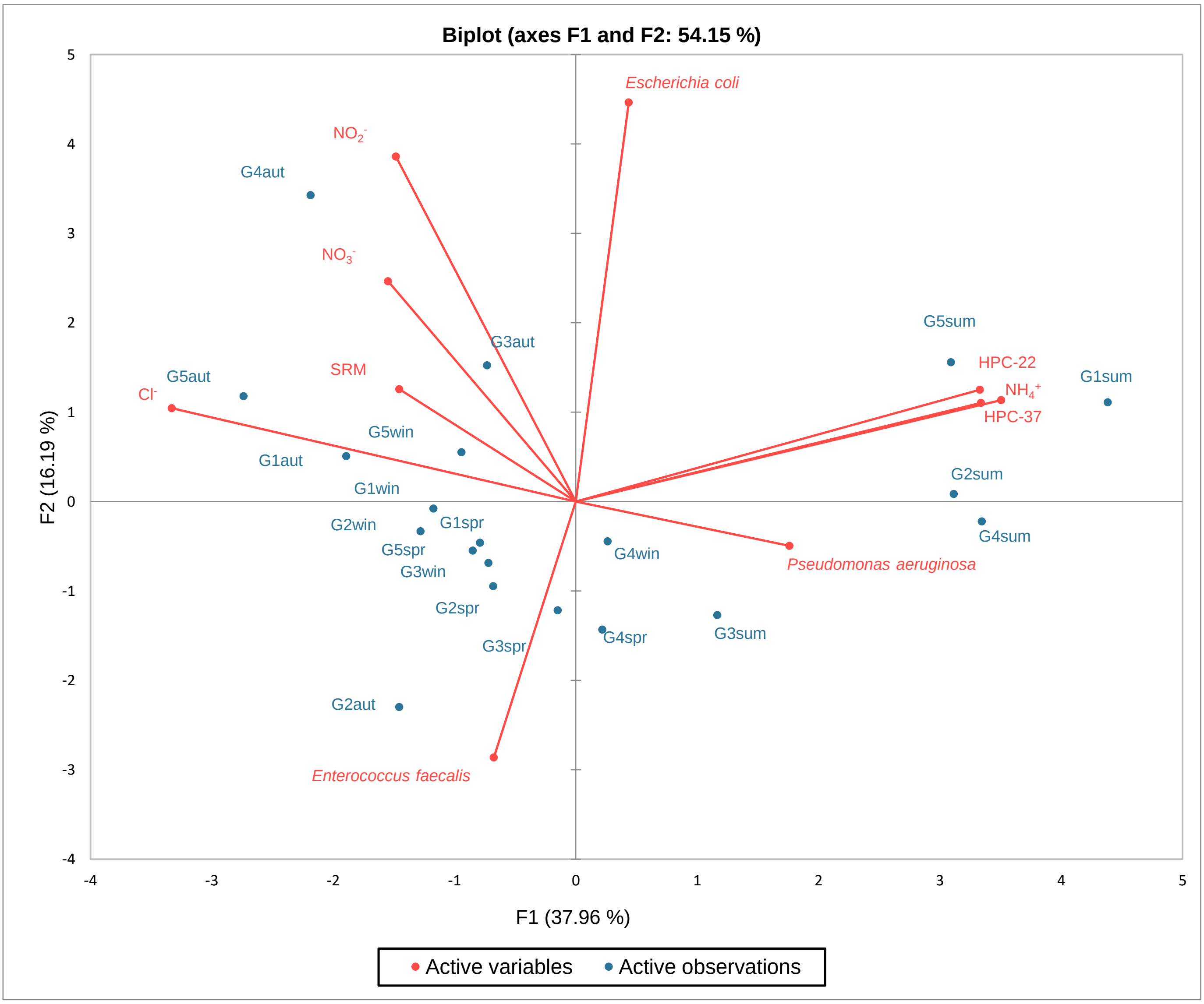

Figure 5. Projection of two-dimensional principal component analysis of the investigated chemical and microbiological indicators in groundwater samples taken during the four seasons in wells G1-G5
Legend: G1aut-G5aut, G1win-G5win, G1spr-G5spr, G1sum-G5sum – seasonal groundwater samples from the respective wells.

Figure 5 is a two-dimensional projection of the computer analysis for the first two components, in which it is possible to see how the different seasons of the groundwater samples are described by the investigated chemical and microbiological indicators. The total contribution of the two components is 54.15%. The left side of the figure shows that summer groundwater samples from wells G1, G2 and G4 have a high concentration of HPC-22, HPC-37 и NH_4^+, relatively high concentration of *Ps. aeruginosa*. However, the samples G1aut and G5aut have low of HPC-22, HPC-37 and NH_4^+. Sample G2aut has a high concentration of *Ent. faecalis*, but low of *E. coli*. In fact, very few viable *E. coli* cells were detected in the samples, and in some cases they were completely absent. The remaining samples, centered on the graph, show balanced indicator levels. This suggests that principal component analysis could be considered indicative for groundwater pollution from human-related sources, particularly during summer and autumn [83].

CONCLUSION

In Dalgo Pole, Bulgaria, a rural area reliant on untreated private wells, significant groundwater contamination is observed, with high concentrations of inorganic pollutants (mainly NO_3^-) and microbiological indicators across seasons. NO_3^- levels exceed safe limits throughout the year, peaking at 246 mg/dm^3 in autumn at well G5, while NH_4^+ is detected above 5 mg/dm^3 only in summer at wells G2 and G5. The NPI and NO_3^-:Cl^- ratios indicate a dominant influence from point sources like septic tank runoff and agricultural fertilization. High microbial contamination is present year-round, with peaks in *Escherichia coli* in summer and autumn, and increases in *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and sulphite-reducing bacteria in spring and autumn. Principal component analysis shows stable relationships between NH_4^+, HPC-22, and HPC-37 in summer and autumn at wells G1, G2, and G4. These findings can aid in mapping sewage infrastructure, identifying critical areas impacting groundwater quality, and enhancing public awareness. Future studies should cover a wider region to improve the understanding of groundwater contamination.

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