

Article

Microbial and Chemical Contamination in Springs of Northern and Central Lithuania

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Abstract

Groundwater springs are an important source of drinking water in Lithuania; however, they are highly sensitive to microbial and chemical contamination. The aim of this study was to assess microbial and chemical contamination in springs from different regions of Lithuania. Five springs were analyzed: Kučgaliai, Smardonė, Salomėja and Žalsvoji (Biržai and Pasvalys districts, Northern Lithuania) and Svilė (Kelmė district, Central Lithuania). Water samples were collected four times a year—during spring, summer, autumn, and winter—and analyzed according to international standards. Chemical parameters included pH, nitrites, nitrates, chlorides, sulfates, and permanganate index, while microbiological analysis targeted *Escherichia coli*, *Enterococcus* spp., and coliform bacteria. The results revealed substantial differences between karst and groundwater-fed springs. Karst springs were more vulnerable to fluctuations in contamination, with Smardonė exhibiting extremely high sulfate concentrations and significant microbial loads. In contrast, Kučgaliai, although located in a karst region, was covered and protected, and its water fully complied with hygiene standards. Groundwater-fed springs showed less variability but were still affected by surface sources. The highest microbial contamination was recorded in autumn and winter, coinciding with increased rainfall and reduced dilution capacity.

Keywords: springs; groundwater; Lithuania; karst; microbial contamination; chemical contamination; water quality; seasonality



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1. Introduction

A spring is a natural outflow of groundwater to the Earth's surface, serving as an essential source of drinking water, irrigation, and industrial use, while also playing an important role in groundwater circulation processes [1]. The quality of spring water is therefore of high environmental and public health importance, as it reflects both natural geological processes and anthropogenic impacts, directly influencing the safety of drinking water resources. Previous studies have shown that freshwater ecosystems, including springs, are particularly vulnerable to contamination and thus require continuous monitoring to protect both the environment and human health [2]. The formation of springs is determined by various geological and hydrological conditions, such as rock composition, soil porosity, terrain slope, and precipitation levels [3]. The quality of groundwater is closely linked to its susceptibility to contamination, which can be both chemical and microbial in nature [4–6]. Chemical pollutants most commonly originate from agricultural activities (nitrogen and phosphorus compounds), transportation (road salt), industry (petroleum products, heavy metals), or the infiltration of domestic wastewater [4,6]. Microbial contamination is

another major concern, as pathogenic microorganisms such as *Escherichia coli*, *Enterococcus* spp., *Salmonella* spp., *Vibrio cholerae*, or *Shigella* spp. may enter groundwater through the infiltration of fecal wastewater, livestock farms, or contaminated fields [5,7–9]. These microorganisms are key indicators of fecal contamination, and their presence poses a direct risk to human health. Waterborne diseases cause more than 3.4 million deaths worldwide each year [10]. Studies have shown that *E. coli* and intestinal enterococci most frequently exceed permissible limits in karst regions, where groundwater circulates rapidly there without natural filtration barriers [7]. Seasonal factors also have a significant influence on microbial and chemical contamination. Research indicates that during spring and autumn, when rainfall is more abundant, pathogens more easily infiltrate groundwater, whereas during summer droughts, their concentrations may increase due to reduced dilution [11]. In addition, extreme weather events—such as floods or sudden heavy rainfall—can facilitate the entry of pathogens into groundwater from surface sources, including wastewater discharge sites or livestock facilities [5]. In the Apuseni Mountains of Romania, an analysis of six karst springs throughout all seasons revealed that most exceeded the EU Directive 98/83/EC limits for *E. coli*, total coliforms, and enterococci, indicating a high health risk [7]. It has also been found that karst springs microbiomes are sensitive to anthropogenic pollution, and antimicrobial resistance genes have been identified [12]. In spring, intensive use of mineral fertilizers often leads to nitrate infiltration into groundwater, while in summer their concentration may increase due to limited dilution [6]. During winter, chemical contamination is commonly exacerbated by chloride-containing salts used on roads, which infiltrate the soil and reach aquifers as snow melts [12–15]. Other studies examining seasonal hydrochemical dynamics have reported that the concentrations of nitrates, heavy metals, and organic compounds fluctuate depending on the season and precipitation levels [6].

In Lithuania, karst and groundwater-fed springs are the most widespread types, both highly sensitive to anthropogenic pollution [15–17]. Karst springs are found exclusively in the karst zone of Northern Lithuania, namely in the Biržai and Pasvalys districts, where gypsum and anhydrite layers predominate. Groundwater in these areas is characterized by particularly high sulfate concentrations (600–1200 mg/L) and mineralization up to 2.3 g/L, exceeding drinking water standards [16,17]. A distinctive feature of karst springs is rapid water circulation without natural filtration barriers, making them extremely vulnerable to both chemical and microbial contamination.

In contrast, in Central Lithuania, such as the Kelmė district, groundwater-fed springs prevail. These springs form from infiltrating precipitation and are characterized by slower circulation. Although somewhat more resilient, they remain sensitive to the effects of intensive agriculture and other anthropogenic pressures. Due to natural conditions, elevated levels of sulfates, chlorides, and fluoride are also recorded in Lithuania, and in some areas even arsenic has been detected [17,18]. In recent years, monitoring of pharmaceutical substances (drug residues) has also been initiated in Lithuania, with carbamazepine, diazepam, benzotriazole, ibuprofen, and diclofenac detected in different locations [17]. These studies indicate that groundwater can be affected by both natural geological processes and human activities. In Lithuania, as well as globally, groundwater is highly susceptible to pollution, as reflected in both chemical (sulfates, nitrates, pharmaceuticals, heavy metals) and microbial (fecal indicator microorganisms, pathogens) parameters. The distribution and concentrations of these contaminants fluctuate seasonally, highlighting the need for comprehensive investigations of springs across different seasons and for identifying the most significant pollution sources. This issue is particularly relevant in the Biržai and Pasvalys districts, where karst geological conditions promote the rapid infiltration of con-

taminants into groundwater, and in the Kelmė district, where groundwater-fed springs prevail, which are also sensitive to anthropogenic pollution.

Despite extensive research on groundwater quality in Europe, systematic studies jointly assessing microbial and chemical contamination in Lithuanian springs remain limited. Most available data focus either on hydrochemistry or on microbial quality, with limited integration of both approaches. Furthermore, little is known about how seasonal variability and geological settings (karst and non-karst) jointly influence contamination risks.

Therefore, it is crucial to investigate spring water quality, as microbial and chemical pollution directly affect drinking water safety, especially in regions where springs remain an important source for local communities. Understanding how geological settings and seasonal factors shape contamination helps identify the most vulnerable water sources and periods of increased risk. Although this study focuses on springs in Lithuania, the observed patterns are relevant to many other regions worldwide facing similar challenges in karst and groundwater-fed systems. By combining microbial and chemical assessments across seasons, this study provides new insights that may support improved groundwater monitoring and protection strategies internationally.

The aim of this study is to assess microbial and chemical contamination in springs from different regions of Lithuania.

2. Materials and Methods

2.1. Sample Collection

Samples for the study were collected in three regions of Lithuania: the Biržai, Pasvalys, and Kelmė districts. In the Biržai district, three springs were investigated—Kučgaliai, Smardonė and Salomėja; in the Pasvalys district, the Žalsvoji spring was investigated; and in the Kelmė district, the Svilė spring was analyzed. These locations were selected due to their popularity among local residents, as the spring water is used not only as a natural source of drinking water but also for household purposes. Samples were taken four times per year—in spring, summer, autumn, and winter—to assess seasonal variations in water quality and to evaluate the characteristics of microbial and chemical contamination in different regions of Lithuania (Figure 1).



Figure 1. Locations of the investigated springs in Northern and Central Lithuania: (1) Kučgaliai, (2) Smardonė, (3) Salomėja, (4) Žalsvasis (karst springs, Northern Lithuania), and (5) Svilė (groundwater-fed spring, Central Lithuania).

Spring water analyses were conducted in accordance with international standards at the National Public Health Surveillance Laboratory in Kaunas. Water samples were collected following LST EN ISO 19458:2006 [19] and LST EN ISO 5667-11:2009 [20] standards, using sterilized glass or plastic containers. Samples were taken directly from the initial outflow of each spring to avoid secondary contamination. Prior to sampling, containers were rinsed with the spring water, and collected samples were stored in a cool environment until analysis to prevent temperature-induced changes in microbiological and chemical parameters.

2.2. Chemical Contamination Analysis

Chemical analyses were performed to evaluate the main water quality indicators, including pH, nitrite, nitrate, chloride, and sulfate concentrations, as well as the permanganate index. Water acidity (pH) was determined potentiometrically according to LST EN ISO 10523:2008 [21], using a calibrated pH meter (model 330i) with SenTIX® 21 electrode (WTW/Xylem Analytics, Weilheim, Germany). Nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were determined by colorimetric spectrophotometric method in accordance with LST ISO 6777:1998 [22] and LST ISO 7890-3:1998 [23], using a spectrophotometer (UV1200-PC, UVISON Technologies Ltd., Wrotham, UK). Nitrite color reagent and sodium salicylate ($\text{C}_7\text{H}_5\text{NaO}_3$, Labochema, Vilnius, Lithuania) for nitrate determination were used as analytical-grade reagents. Chloride (Cl^-) concentration was measured by titration with silver nitrate (AgNO_3 , Labochema, Vilnius, Lithuania) according to LST ISO 9297:1998 [24], while sulfate (SO_4^{2-}) concentration was determined spectrophotometrically following LST ISO 9280:1998 [25], using a spectrophotometer (UV1200-PC, UVISON Technologies Ltd., Wrotham, UK) and barium chloride (BaCl_2 , Labochema, Vilnius, Lithuania) as a precipitating reagent. The permanganate index, indicating the amount of oxidizable organic matter in water, was determined by titration according to LST EN ISO 8467:1993 [26]: Water sample was acidified with sulfuric acid (H_2SO_4 , Labochema, Vilnius, Lithuania), treated with a standard potassium permanganate solution (KMnO_4 , Labochema, Vilnius, Lithuania), and subsequently titrated with sodium oxalate ($\text{Na}_2\text{C}_2\text{O}_4$, Labochema, Vilnius, Lithuania). The analysis of these chemical parameters allowed not only the characterization of spring water composition but also the identification of potential pollution sources and their implications for human health.

2.3. Microbiological Analysis

Standardized microbiological methods were applied to accurately determine both the quantity and composition of microorganisms in water samples. The membrane filtration method was used according to LST EN ISO 8199:2019 [27], employing 0.45 μm pore size membrane filters, through which 100 mL of each sample was passed. The filters were transferred with sterile forceps onto selective nutrient media and incubated under optimal conditions.

Coliform bacteria, including *E. coli*, were identified following ISO 9308-1:2014(E) [28], using chromogenic coliform agar (Biometrija, Vilnius, Lithuania) and incubating samples at 36 °C for 24 h. *Enterococcus* spp. was detected in accordance with ISO 7899-2:2000 [29], using Slanetz–Bartley (Biometrija, Vilnius, Lithuania) and bile esculin azide agar (Biometrija, Vilnius, Lithuania), with incubation at 36 °C for 48 h. Colony counts (CFU/mL) were determined for each sample, and morphological analysis of the colonies was conducted.

The selection of these microorganisms was deliberate: *E. coli* serves as the primary indicator of fecal contamination, reflecting potential impacts from wastewater or livestock, while *Enterococcus* spp. are highly resistant to environmental stressors and also indicate fecal pollution. In addition, coliform bacteria provide an overall measure of water's sanitary

quality. Microbiological analysis was supplemented by seasonal assessment, enabling the evaluation of temporal variations in bacterial prevalence and the factors influencing contamination dynamics.

2.4. Statistical Analysis

All measurements for each spring and season were carried out in triplicate, and the values are presented as means. Seasonal variations within the same spring (across four seasons) were analyzed using repeated measures ANOVA, followed by Tukey's HSD post hoc test for pairwise comparisons where significant differences were detected ($p < 0.05$). Due to the non-normal distribution of microbiological data, seasonal and inter-spring differences were analyzed using the Kruskal–Wallis non-parametric test with Dunn's post hoc comparisons ($p < 0.05$).

3. Results

3.1. Assessment of Chemical Indicator Concentrations and Values

During the research in three water springs—Kučgalis (Biržai district), Žalsvasis (Pasvalys district) and Svilė (Kelmė district)—it was found that the pH values of all the studied water bodies remained slightly alkaline throughout the year. The pH values remained slightly alkaline throughout the year, fully complying with the Lithuanian drinking water quality standards. According to the World Health Organization [30] guidelines, the acceptable pH range for drinking water is 6.5–8.5, and the obtained results fall within this range, confirming that the water quality meets both national and international requirements (according to the hygiene standard HN 24:2023 [31]), therefore it allows us to state that the water from these water springs is suitable for human consumption. A slight decrease in pH was observed during the winter period in the Kučgalis and Žalsvasis springs. This can be associated with the reduced activity of photosynthetic organisms, the slowdown of organic matter decomposition processes and the lower amount of biogenic substances in winter, which determines the weaker buffering effect of carbonates and a weaker alkaline reaction (Table 1). During the autumn season, the Svilė spring exhibited the highest alkalinity. This increase can be explained by more intensive processes of decomposition of organic matter and increased concentration of biogenic elements, which is characteristic of the autumn period. Anthropogenic factors and hydrogeochemical conditions characteristic of a specific source can also have a significant influence. When analyzing seasonal variations, a general trend can be observed: pH values tend to be slightly higher in spring and autumn compared to winter and summer. Such fluctuations can be associated with seasonal biochemical processes, for example, spring plankton bloom, which increases the intensity of photosynthesis and at the same time reduces the concentration of carbon dioxide in water, thereby increasing pH values. In autumn, the activity of microorganisms may increase due to the mineralization of organic matter.

Table 1. pH—hydrogen ion concentration, permanganate index value, nitrite, nitrate, chloride concentration in spring water at different times of the year.

Indicator	Season	Water Springs				
		Svilė (Kelmė District, Central Lithuania)	Smardonė (Biržai District, Northern Lithuania)	Salomėja (Biržai District, Northern Lithuania)	Kučgalis (Biržai District, Northern Lithuania)	Žalsvoji (Pasvalys District, Northern Lithuania)
pH (6.5–9.5)	Spring	7.7	7.3	7.3	7.5	-
	Summer	7.5	7.1	7.2	7.3	7
	Autumn	7.8	7.3	7.3	7.5	7.3
	Winter	7.5	7.3	7.2	7.1	7.1

Table 1. Cont.

Indicator	Season	Water Springs				
		Svilė (Kelmė District, Central Lithuania)	Smardonė (Biržai District, Northern Lithuania)	Salomėja (Biržai District, Northern Lithuania)	Kučgalis (Biržai District, Northern Lithuania)	Žalsvoji (Pasvalys District, Northern Lithuania)
Statistical analysis		$p < 0.05^*$	ns	ns	ns	ns
NO ₂ [−] (≤0.5 mg/L)	Spring	0.03	0.025	0.009	0.01	-
	Summer	0.01	0.018	0.013	0.01	0.01
	Autumn	0.01	0.028	0.071	0.01	0.01
	Winter	0.01	0.018	0.015	0.01	0.011
Statistical analysis		ns	ns	$p < 0.05^*$	ns	ns
NO ₃ [−] (≤50 mg/L)	Spring	3.65	0.87	0.69	0.15	-
	Summer	2.96	3.55	4.79	0.8	1.74
	Autumn	2.4	1.54	2.8	0.8	1.53
	Winter	3.45	1.71	1.66	0.8	0.47
Statistical analysis		$p < 0.05^*$	ns	$p < 0.05^*$	ns	ns
Permanganate Index (≤5 mg O ₂ /L)	Spring	0.06	3.84	3.52	3.93	-
	Summer	0.5	4.6	3.7	4.5	3
	Autumn	0.54	3.84	4.13	4.6	3.3
	Winter	0.51	3.83	3.49	4.6	3.39
Statistical analysis		$p < 0.05^*$	$p < 0.001^{***}$	$p < 0.001^{***}$	$p < 0.001^{***}$	$p < 0.001^{***}$
Cl [−] (≤250 mg/L)	Spring	9.5	6.1	22.4	3.4	-
	Summer	12	23	23	6	47
	Autumn	8.17	21.8	21.1	2.72	46.3
	Winter	9.52	23.8	24.5	4.08	42.2
Statistical analysis		ns	ns	ns	ns	$p < 0.001^{***}$
SO ₄ ^{2−} (≤250 mg/L)	Spring	18.6	2555	1564	2.94	-
	Summer	33	996	1051	3	1139
	Autumn	377	1379	1403	19.6	1199
	Winter	9.52	23.8	24.5	3.52	42.2
Statistical analysis		$p < 0.05^*$	$p < 0.001^{***}$	$p < 0.001^{***}$	ns	$p < 0.001^{***}$

“-” The data for the Žalsvoji water spring regarding nitrites, nitrates, permanganate index and other indicators are inaccurate due to the mixing of river and spring water during the flood, permissible limit exceeded. ns = not significant ($p > 0.05$). * $p < 0.05$ —significant. *** $p < 0.001$ —very highly significant.

In this study, the results show that in the spring season, the NO₂[−] concentrations of all sources were extremely low, the nitrite concentrations were very low and well below the permissible limits. This is well below the permissible limits defined in the Lithuanian hygiene standard (HN 24:2023). According to the World Health Organization [30] guidelines, the maximum acceptable concentration of nitrites in drinking water is 0.5 mg/L, therefore the obtained results are well within both national and international limits. Such a low nitrite (NO₂[−]) content in spring can be associated with the active activity of the microbial community, during which further conversion of nitrites (NO₂[−]) into nitrates (NO₃[−]) occurs quickly, as well as with a higher oxygen content in water due to increased photosynthesis, which accompanies the spring growth of biological production. On the contrary, in autumn, clear spikes in NO₂[−] concentration were observed in some sources. The highest NO₂[−] concentration was recorded in autumn in the Salomėja (Biržai district) water spring—0.071 mg/L, and in the Smardonė spring—0.028 mg/L. These increases, although not exceeding the norms, indicate a seasonal change in biochemical processes, possibly related to more active decomposition of organic matter, especially plant residues entering the springs in late autumn. According to the study, the NO₃[−] concentrations in all sources did not exceed the permissible norm, but seasonal fluctuations were observed.

The World Health Organization (WHO) also sets a guideline value of 50 mg/L for NO_3^- in drinking water, confirming that all measured concentrations are within the safe limit [30]. Higher NO_3^- levels were observed in spring and summer, likely due to active agricultural activities, especially fertilization, and precipitation-induced leaching of nitrates from the soil. Lower NO_3^- concentrations were characteristic of the Kučgalis spring. This can be attributed to better protection of the spring from external pollution and hydrogeological features (Table 1). A moderate increase in NO_3^- levels was also observed in other sources during the summer NO_3^- , but all values remained good below the norm, therefore the water is considered suitable for consumption. However, seasonal changes indicate the need for continuous monitoring, especially during periods of intensive agriculture.

The permanganate index, which reflects water pollution by organic matter, was highest in the Salomėja and Kučgalis springs, while the lowest values were found in the Svilė spring. These results indicate that the Svilė water is cleaner, probably due to natural filtration through sand or gravel, while high values in other springs indicate greater interaction with organic matter. According to WHO guidelines, the recommended limit for the permanganate index in drinking water is 5 mg O_2/L , therefore all measured values are within the safe range and indicate good organic matter control [30]. No clear seasonal variation in the permanganate index was observed, which implies relatively stable organic input throughout the year (Table 1).

Chloride concentrations in all examined springs complied with the permissible drinking water standards. The highest Cl^- levels were detected in the Žalsvasis and Salomėja springs throughout the year, while the lowest were recorded in the Kučgalis spring. According to the WHO Guidelines for Drinking-water Quality, the esthetic guideline value for chlorides is 250 mg/L; thus, all the observed values are well below this threshold and do not pose any health or taste concerns [30]. The water in the Žalsvasis and Salomėja springs is of the calcium sulfate type, which is characterized by high mineralization, due to which an increased Cl^- concentration is found. The low Cl^- concentration in the water of the Kučgalis spring indicates that the spring does not have significant contact with chloride-containing minerals and is not affected by significant anthropogenic pollution sources (industrial wastewater) (Table 1).

Studies of SO_4^{2-} concentrations in water from the analyzed springs revealed both clear differences among the sources and seasonal variations. The highest SO_4^{2-} levels were found in the Smardonė and Salomėja springs in the Biržai district and in the Žalsvasis spring in the Pasvalys district, indicating greater contact with sulfate-bearing rocks or more mineralized groundwater (Table 1). The WHO guideline value for SO_4^{2-} in drinking water is 500 mg/L; therefore, the concentration measured in the Smardonė spring exceeds the recommended limit, indicating that this source may be unsuitable for direct consumption without treatment [30]. The lowest SO_4^{2-} levels were generally observed during winter, likely due to reduced mineral dissolution and weaker runoff. In contrast, the Kučgalis spring maintained very low SO_4^{2-} concentrations throughout the year, suggesting limited interaction with sulfate minerals and effective natural protection from surface contamination. A noticeable autumn increase in SO_4^{2-} concentrations in the Svilė spring points to the influence of precipitation and surface runoff. Such an increase is likely related to more intense surface runoff after precipitation, which may promote changes (leaking of SO_4^{2-} from the surface soil layers into groundwater), showing that this source is sensitive to seasonal environmental changes (Table 1).

The statistical analysis revealed that some parameters exhibited significant seasonal or spatial variation (Table 1). The pH values remained within the permissible range (6.5–9.5), with significant seasonal differences observed only in the Svilė spring ($p < 0.05$, where autumn values were higher than those in summer). Nitrite concentrations were generally low,

except in Saloméja where autumn values were significantly higher ($p < 0.05$). Nitrate levels ranged from 0.15 to 4.79 mg/L, with significant seasonal variation observed in Svilė and Saloméja ($p < 0.05$). The permanganate index differed significantly among springs ($p < 0.001$), with lower values in Svilė compared with the others. Chlorides were significantly higher in Žalsvoji (42–47 mg/L, $p < 0.001$), while seasonal variation was not significant in the other springs. Sulfates displayed both seasonal fluctuations in Svilė ($p < 0.05$) and marked spatial differences, with Smardonė and Saloméja exhibiting significantly higher values than the other springs ($p < 0.001$).

3.2. Evaluation of Microbiological Indicators

Microbiological analysis showed that the Kučgalis spring (Biržai district) provided the best drinking water quality. No *E. coli*, *Enterococcus* spp., or coliform bacteria were detected in any samples throughout the year (Table 2), indicating stable microbiological safety. Therefore, the spring water fully complies with HN 24:2023 “Drinking water safety and quality requirements” [31]. According to the WHO Guidelines for Drinking-water Quality [30], the presence of *E. coli* in 100 mL of drinking water is considered unacceptable; thus, its absence in the Kučgalis confirms high microbiological quality. In the Svilė spring, no *Escherichia coli* bacteria were detected during the study period, suggesting relatively clean water in terms of fecal contamination. However, *Enterococcus* spp. and coliform bacteria were periodically found, particularly during warmer seasons. These findings indicate a seasonal increase in microbial contamination, especially in the warm period, likely associated with intensified biological activity in the environment and surface pollution. Although WHO guidelines [30] recommend that *E. coli* and thermotolerant coliforms be completely absent in 100 mL of water intended for human consumption, occasional detection of *Enterococcus* spp. and coliforms in the Svilė spring suggests minor surface contamination and limited natural protection. Consequently, while the spring meets some microbiological parameters, its overall water quality according to HN 24:2023 [31] is limited. The Smardonė spring, by contrast, showed clear signs of microbiological contamination throughout the year. *E. coli* and *Enterococcus* spp. were frequently detected, especially in colder months, indicating persistent fecal pollution and insufficient natural filtration. Coliform bacteria were detected in all seasons, with the highest concentration in autumn. The Kruskal–Wallis test revealed significant differences in *Enterococcus* spp. occurrence among springs ($H = 9.02$, $p < 0.05$). Dunn’s post hoc test showed that the Smardonė and Svilė springs had significantly higher *Enterococcus* spp. counts than the Kučgalis and Saloméja springs. Seasonal variations were near the significance threshold ($H = 7.68$, $p = 0.053$), suggesting increased contamination during summer and winter periods, likely linked to enhanced microbial activity and runoff processes. According to WHO standards [30], any detection of *E. coli* in 100 mL of water indicates fecal contamination and renders the water unsafe for drinking purposes. Such a distribution of indicators indicates constant and significant microbiological pollution; therefore, the water of this spring clearly fails to meet the requirements of HN 24:2023 [31]. The Saloméja spring generally showed good microbiological quality. *Escherichia coli* and *Enterococcus* spp. were not detected in any samples, confirming the absence of fecal contamination. However, an increase in coliform bacteria was observed during the summer months, suggesting enhanced microbial activity under warmer conditions and possible greater human or surface influence. These results may reflect a seasonal effect of environmental pollution, possibly related to a more intense flow of visitors or surface rain runoff. Although the main pathogenic indicators (*E. coli*, *Enterococcus* spp.) were absent the significant summer increase in coliform bacteria counts indicates that the spring water only partially meets the quality requirements. The data from the Žalsvoji spring should be interpreted with caution due to external factors—after heavy

rains, the spring water was mixed with river surface flow, meaning that the samples do not fully reflect normal hydrogeological conditions. Nevertheless, traces of microbiological contamination were also detected in other seasons, indicating that microbial presence is not limited to warm periods. *E. coli* and *Enterococcus* spp. were occasionally present in colder months, while coliform bacteria were more prevalent during warmer conditions, suggesting that temperature and hydrological changes influence bacterial survival and transport. Considering the atypical hydrogeological conditions and identified microorganisms, this source does not comply with HN 24:2023 norms [31]. The Kruskal–Wallis test revealed significant differences in coliform bacteria occurrence among the studied springs ($H = 10.84$, $p < 0.05$). Dunn’s post hoc test showed that the Salomėja, Smardonė, and Svilė springs had significantly higher coliform counts than the Kučgalis spring. Seasonal variation was also statistically significant ($H = 8.57$, $p < 0.05$), with the highest contamination levels recorded in summer and autumn, highlighting the influence of surface runoff, higher temperatures, and increased biological activity during these periods. Overall, seasonal effects had a significant impact on water quality. In the warm season (spring and summer), a higher number of microorganisms, particularly coliform bacteria, were recorded in most sources. This may be related to intensified biological activity around the springs, lower water levels, longer surface residence time, and more frequent anthropogenic influence (e.g., visitor flow, animal migration). The location of each spring also plays an important role, for instance, the Kučgalis spring, situated away from settlements and less affected by human activity, exhibited the most stable microbiological quality. Meanwhile, the proximity of the Smardonė and Žalsvoji springs to surface water bodies or their shallow depth may have contributed to their sensitivity to pollution.

Table 2. Detection of *E. coli*, *Enterococcus* spp. and coliform bacteria in water springs at different times of the year (percent of all samples tested).

Microorganisms	Season	Water Springs				
		Svilė (Kelmė District, Central Lithuania)	Smardonė (Biržai District, Northern Lithuania)	Salomėja (Pasvalys Biržai District, Northern Lithuania)	Kučgalis (Biržai District, Northern Lithuania)	Žalsvoji (Pasvalys District, Northern Lithuania)
<i>E. coli</i>	Spring	-	-	-	-	inaccurate
	Summer	-	-	-	-	3%
	Autumn	-	67%	-	-	-
	Winter	-	33%	-	-	11%
<i>Enterococcus</i> spp.	Spring	33%	-	-	-	inaccurate
	Summer	67%	-	-	-	-
	Autumn	-	-	-	-	-
	Winter	-	100%	-	-	3%
Coliforms	Spring	32%	13%	6%	-	inaccurate
	Summer	18%	15%	93%	-	39%
	Autumn	39%	48%	-	-	-
	Winter	11%	24%	1%	-	2%

“-” —not detected. “inaccurate” —the data from the Žalsvoji spring are considered inaccurate due to mixing with river water after heavy rains, meaning they may not fully reflect normal hydrogeological conditions.

4. Discussion

The results of this study demonstrate that both chemical and microbial indicators varied substantially among springs in Northern and Central Lithuania, depending on hydrogeological type, environmental conditions, and seasonality. Karst springs (Smardonė, Salomėja, Kučgaliai in Biržai and Pasvalys districts, Northern Lithuania) were generally more vulnerable to fluctuations in contamination, while groundwater-fed springs (Svilė in Kelmė district, Central Lithuania) exhibited lower but more consistent pollution levels. For instance, the Kučgaliai spring, although located in a karst region, was covered and protected, resulting in the cleanest water that met hygiene standards. In contrast, the Smardonė spring exhibited extreme sulfate concentrations and high microbial activity, explained by karst hydrogeological conditions where rapid groundwater circulation without natural filtration barriers facilitates the transport of both chemical pollutants and microorganisms. Elevated sulfate concentrations in the Smardonė spring can be attributed to the natural geological composition and groundwater mineralization in Northern Lithuania's karst zone. In such areas, groundwater interacts with gypsum or anhydrite-containing rock layers, which enrich the water with sulfates. Due to these properties, Smardonė water has historically been used in sanatoriums for therapeutic purposes, though it does not meet drinking water requirements. In contrast the Kučgaliai spring had the lowest sulfate concentrations, while the Žalsvoji spring exhibited particularly high chloride levels, most likely due to road salts or wastewater infiltration. These observations align with broader European trends. Recently, Eid et al. [6] reported seasonal nitrate and heavy metal fluctuations in Polish springs due to agricultural activity, with concentrations several times higher than in our study. Comparable patterns have also been documented in Lithuania. Juodkakis and Papievis [32] analyzed spring waters in Vilnius and found that concentrations of sulfates, chlorides, and trace ions varied among springs and sometimes exceeded acceptable limits, highlighting the susceptibility of urban-proximal springs to contamination. Their findings support our interpretation that chemical variability in springs can reflect both geological and anthropogenic influences. Kitterød et al. [33] emphasized that groundwater quality in the Nordic and Baltic countries is strongly influenced by geological settings, with Quaternary aquifers typically showing lower mineralization, whereas confined bedrock aquifers are more affected by geogenic elements such as arsenic, fluoride, or radon. Importantly, unconfined Quaternary aquifers were identified as being especially vulnerable to anthropogenic pollution, particularly from agricultural nitrogen inputs. These findings are consistent with our observation that karst springs, which are hydraulically similar to unconfined aquifers, showed higher variability in sulfate, chloride, and nitrate concentrations, whereas the groundwater-fed Svilė spring was less affected by seasonal and anthropogenic influences.

Stupar et al. [7], investigating karst springs in Romania's Apuseni Mountains, found frequent exceedances of nitrate and sulfate limits, resembling the Smardonė case. Similarly, Ayeta et al. [11] documented high chloride levels in Ghana, though linked to seawater intrusion rather than road salts. Such comparisons confirm that contamination patterns in Lithuanian springs are similar to those observed internationally, while also reflecting unique local geological conditions. Furthermore, as highlighted by Rivett et al. [34], the fate of nitrates in groundwater strongly depends on biogeochemical attenuation processes (e.g., denitrification, redox conditions, availability of electron donors), which can either mitigate or exacerbate pollution risks depending on site-specific hydrogeological settings. Groundwater-fed springs, although less variable, are clearly influenced by surface activities. In the Svilė spring, nitrate levels and microbial contamination likely originated from intensive agriculture in surrounding areas, while high chloride levels in the Žalsvoji spring point to the impact of transport infrastructure and domestic wastewater. Although not karstic, these springs remain vulnerable because groundwater readily responds to surface-derived pollution.

Microbiological analyses also revealed significant variation across springs. Kučgaliai water showed no *E. coli* and *Enterococcus* spp. or coliforms, fully complying with hygiene standards, while the Svilė spring contained enterococci and coliforms, especially in autumn. The Smardonė and Žalsvoji springs were more heavily contaminated, with persistent detection of *E. coli*, enterococci, and coliforms. Seasonal trends were evident: microbial contamination peaked in autumn and winter, likely due to rainfall and reduced dilution. These findings mirror international studies. Stupar et al. [7] found that many karst springs in Romania exceeded EU Directive 98/83/EC microbial thresholds consistent with our results for Smardonė and Žalsvoji, while Bagordo et al. [5] emphasized the link between agricultural practices, wastewater infiltration, and fecal contamination of groundwater, factors likely influencing Svilė and Smardonė. Seasonal rainfall effects observed in Ghana [11] also parallel the autumn peaks in our study. Furthermore, Szekeres et al. [12] highlighted the sensitivity of karst microbiomes to anthropogenic pressures, often containing fecal indicators and even antimicrobial resistance genes.

Comparison between karst and groundwater-fed springs highlights their differing vulnerabilities: karst systems are more sensitive to rapid infiltration through fractured geology, whereas groundwater-fed springs are more affected by diffuse surface pollution. These findings are consistent with the broader literature and underline the importance of considering hydrogeological context when assessing groundwater quality [5,7]. The Kučgaliai spring was found to be the cleanest, reflecting effective protection, whereas Smardonė, Žalsvoji, and Svilė, located in intensively used environments, exhibited the highest chemical and microbial contamination. Our results therefore support international evidence that both karst and groundwater-fed springs are highly vulnerable to fecal pollution. Microbial indicators fluctuate seasonally, with the greatest risks occurring in autumn and winter, when contaminants more easily infiltrate groundwater.

5. Conclusions

This study revealed significant differences in chemical and microbiological water quality among springs in northern and central parts of Lithuania, driven by hydrogeological type and seasonality. Karst springs were most sensitive to sudden fluctuations in pollution, particularly in terms of sulfate concentration and the abundance of microorganisms, while groundwater springs were characterized by more stable water quality, but still influenced by anthropogenic factors. The results show that the springs, despite their natural occurrence, remain vulnerable to environmental conditions and human impacts. Considering that the water from these springs is widely used for household and health purposes, consistent monitoring and detailed analysis of the causes of pollution are necessary to ensure water safety and preserve these springs as valuable drinking water sources and a natural resource for future generations.

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