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Review

Microbial Communities in Natural Mineral Waters of Bulgaria: Diversity and Biotechnological Potential

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Abstract

Mineral waters represent unique limnological ecosystems with stable physicochemical conditions and specialised microbial communities adapted to extreme environments. Bulgarian mineral waters remain comparatively underexplored despite their considerable ecological and biotechnological significance. This review analyses current knowledge on the diversity, ecological functions, and biotechnological potential of microbial communities from Bulgarian mineral springs. A comprehensive literature survey covers studies published between 1990 and 2026. The study integrates hydrogeological, limnological, microbiological, and biotechnological data and encompasses both culture-dependent methods and molecular approaches. The available evidence demonstrates that microbial communities in Bulgarian mineral waters include diverse bacteria, archaea, cyanobacteria, microalgae that adapt to broad thermal and geochemical gradients. These microorganisms actively participate in element cycles, form complex biofilms, and show numerous physiological adaptations to oligotrophic and extreme conditions. Many taxa produce thermostable enzymes, antimicrobial compounds, exopolysaccharides with potential applications in medicine, industrial biotechnology, environmental remediation, and cosmeceutical technologies. The review identifies significant research gaps and emphasises the importance of integrated multi-omics approaches for future exploration of Bulgarian mineral water ecosystems.

Keywords: Bulgarian mineral waters; microbial diversity; extremozymes; EPS; antimicrobial compounds; biotechnology

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1. Introduction

Natural mineral waters represent unique limnological environments characterised by stable geochemical conditions, elevated mineralisation, and, in many cases, extreme temperatures [1,7–10]. These systems occur worldwide and have considerable ecological and biogeochemical importance, as they support specialised microbial communities adapted to oligotrophic and physicochemically extreme conditions [11–21]. In addition, they can be regarded as subsurface aquatic systems with distinct hydrogeological isolation and long-term environmental stability. Such environments are increasingly recognised as natural laboratories that enable the study of microbial adaptation, ecosystem functioning, and the evolution of metabolic diversity [12,17,22–25].

Microorganisms play a central role in the ecological functioning of mineral water systems, as their metabolic activity regulate key biogeochemical cycles of C, N, S, Fe, and Si [21,26–28]. As a result, they exert a strong influence on water chemistry, mineral precipitation, and overall ecosystem stability. Microbial communities also form complex biofilms and microbial mats, which enhance survival under stress conditions and enable functional interactions between phototrophic and heterotrophic populations [17,20,28–32]. These adaptations are important under conditions of low oxygen availability, high temperature, and elevated concentrations of dissolved gases, which are typical for many mineral and thermal waters [7–10,17–19]. Furthermore, natural environmental habitat of mineral waters often possesses unique physiological and metabolic capabilities with recognised potential for applications in biotechnology, environmental processes, and industry [14,17,22–24,33–35].

Despite their ecological and applied significance, Bulgarian mineral waters remain relatively underexplored in a global context [11,22,26,36–39]. Existing studies demonstrate considerable microbial diversity and biotechnological potential; however, research remains fragmented and geographically uneven, and it rarely integrates ecological and functional perspectives [13,14,18,22–24,26,27,40–42]. Cold springs and less-studied regions are particularly insufficiently characterised, which limits a comprehensive understanding of microbial distribution, community dynamics, and metabolic activity [11,26,41,43]. In addition, most investigations have focused primarily on sanitary microbiology and do not provide detailed taxonomic or functional analyses of indigenous microbial communities [11,44–50]. In the context of the growing interest in extremophilic microorganisms and sustainable biotechnological resources, the need for a systematic synthesis of available data has become increasingly evident.

This review aims to analyse current knowledge on the diversity, ecological roles, and biotechnological potential of microbial communities in Bulgarian mineral waters. It emphasises their functional significance in limnological systems and examines the relationships between environmental conditions and microbial composition. The review also outlines perspectives for future research in this field.

2. Materials and Methods

This review is based on a comprehensive analysis of published scientific literature that addresses microbial diversity and functional characteristics of microorganisms in mineral and thermal waters, with particular emphasis on Bulgaria. Data were collected from major scientific databases Scopus, Web of Science, and Google Scholar. The analysis covers studies published between 1990 and 2026. Additional sources, monographs, regional microbiological surveys, and technical reports, were also included when necessary. These sources ensured adequate representation of Bulgarian studies that are not widely indexed.

The literature search relied on combinations of keywords related to mineral and thermal waters, extremophilic microorganisms, algae, cyanobacteria, microbial mats, biofilms, biogeochemical cycles, and biotechnology. The selection of studies followed criteria based on relevance to microbial ecology, taxonomic identification, functional activity, and biotechnological potential of microorganisms isolated from inland aquatic systems. Only peer-reviewed publications and validated scientific reports were included in the analysis. The available datasets show substantial heterogeneity. For this reason, the study applies a qualitative comparative approach. The extracted information is organised according to ecological context, microbial groups, and functional roles. This structure allows comparison of microbial diversity patterns and ecosystem functions across different Bulgarian administrative provinces. Particular attention is given to studies that report microbial communities in Bulgarian mineral springs, including thermal, hyperthermal, and cold waters. The analysis also considers limitations of culture-dependent methodologies. Therefore, it includes studies that apply molecular techniques such as 16S/18S rRNA gene sequencing, metagenomics, and bioinformatic analyses. These methods provide access to both cultivable and non-cultivable microorganisms and allow a more complete representation of ecosystem structure and function.

This methodological framework provides an integrative synthesis of ecological and biotechnological knowledge related to microbial communities in mineral waters. It also identifies existing knowledge gaps and outlines insufficiently explored research directions. The appendices present a comprehensive compilation of physicochemical parameters for Bulgarian mineral water sources included in this review. This dataset serves as a reference resource that supports data transparency and reproducibility, while the main text focuses on the synthesis of hydrogeological, limnological, and microbiological patterns at the system level.

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3. Hydroecological and Limnological Framework of Bulgarian Mineral Waters

2.1. Hydroecological Setting and Geological Control of Microbial Habitats

Mineral waters in Bulgaria originate from complex hydrogeological and geothermal systems controlled by regional tectonics and deep groundwater circulation [28,37,38,46]. Meteoric waters infiltrate through fractured rock formations, porous media, and fault zones. During their downward migration, they undergo heating along the geothermal gradient, interact with host lithologies, and become enriched in dissolved minerals and gases such as CO_2, H_2S, CH_4, and Rn [28,36–38]. These processes determine the physicochemical characteristics of mineral waters and establish the environmental framework for microbial colonisation and adaptation [10,26,36]. Fault-controlled ascent pathways regulate the emergence of cold, warm, and hyperthermal springs and ensure prolonged residence times within subsurface reservoirs [28,37,38]. This extended isolation contributes to chemical stability, geochemical equilibrium, and a high degree of natural purity, with minimal anthropogenic influence [28,38]. The tectonic configuration and the presence of an active fault network further account for the remarkable diversity of mineral waters observed within the country [26,37].

The pronounced geological heterogeneity of Bulgaria represents a key factor that controls hydrochemical variability and microbial habitat differentiation. The country may be broadly divided into two major hydrogeological regions: a northern region, associated with the Moesian Platform, and a southern region, characterised by complex tectonic and geothermal structures [4,26,37]. These contrasting geological settings determine not only water composition and temperature regimes but also influence microbial community assembly, metabolic potential, and ecosystem functionality [17–21,27,28]. To provide a hydrogeochemical and functional context for the studied ecosystems, Bulgarian mineral waters can be classified according to their therapeutic mineral composition and basic physicochemical properties. This classification describes the diversity of hydrothermal systems and serves as a basis for interpretation of associated microbial assemblages (Figure 1), while detailed physicochemical characteristics of individual water sources are provided in Appendices A and B.

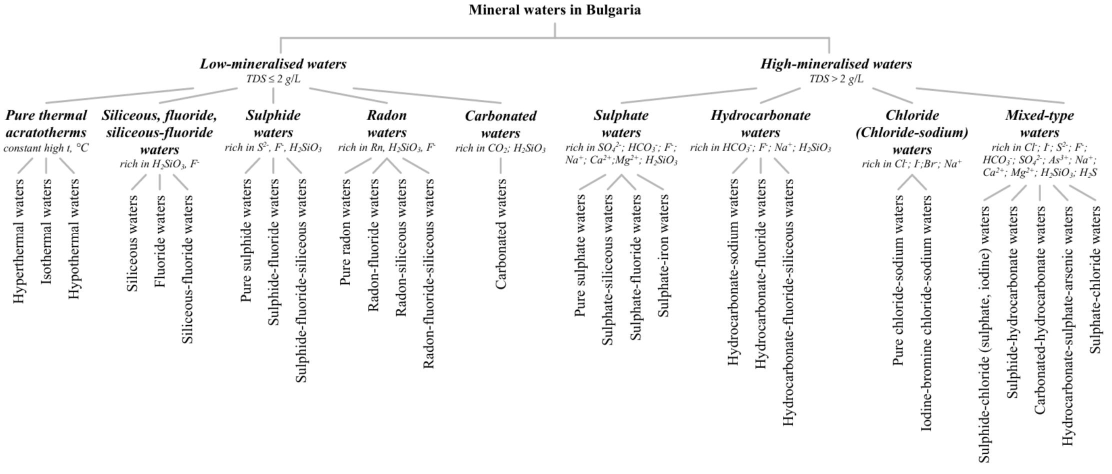

Figure 1. Classification of mineral waters in Bulgaria according to their theurapeutic mineral composition [10].

Northern Bulgaria is characterised by stratified artesian systems developed within the Moesian Platform. These systems are associated with Paleogene sandy-clay deposits and Mesozoic carbonate aquifers. The waters are predominantly extracted through boreholes and are typically characterised by relatively low temperatures and elevated mineralisation, with total dissolved solids (TDS) in range from 1 to 150 g/L [4,36]. In contrast, southern Bulgaria is dominated by active fault systems and geological massifs composed of metamorphic, volcano-sedimentary, and sedimentary rocks. These conditions favour the development of hydrothermal mineral springs, located in the Sofia and Rila-

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Rhodope regions, where thermo-mineral waters exhibit relatively low mineralisation (TDS < 1 g/L) and elevated temperatures [36,39]. Extreme geothermal conditions are observed in Sapareva Banya in southwestern Bulgaria, where the hottest geyser in continental Europe has been recorded. Water temperatures at this site reach 101-103 °C [11,51]. Such environments provide unique ecological niches for thermophilic and extremophilic microorganisms and contribute to the overall diversity of microbial habitats in Bulgarian mineral waters [14,17,18,22–24]. These contrasting hydrogeological regimes create distinct ecological templates that shape microbial community structure, functional diversity, and metabolic specialisation. The interaction between geological setting, hydrochemistry, and physicochemical conditions thus represents a fundamental control on the distribution and activity [14,17,18,22–24of microbial communities in mineral and thermal waters [10,17–21,27,28,52–55].

2.2. Physicochemical Control on Microbial Distribution

Physicochemical parameters represent a primary control on the organisation, distribution, and functionality of microbial communities in mineral water systems. Temperature, pH, mineralisation, redox potential, and gas composition exert a direct influence on microbial metabolic activity, physiological adaptation, and species differentiation [10,26,54]. These factors determine the ecological niches available for microbial colonisation and regulate the structure and stability of microbial assemblages in subsurface aquatic environments [17,21,27].

Temperature constitutes one of the most critical determinants of microbial life. It controls the rate of biochemical reactions and defines the physiological limits of different microbial groups. In thermal and hyperthermal springs, temperatures may exceed 60-70 °C, which creates favourable conditions for the development of thermophilic and hyperthermophilic communities of bacteria and archaea [13,17,26]. At intermediate temperatures, microbial diversity tends to increase due to the coexistence of mesophilic and moderately thermophilic taxa [17]. Of particular importance are akratotherms, in which relatively constant temperatures above 20 °C are maintained throughout the year. Such conditions promote the formation of stable and well-structured microbial consortia (Tables A1–A3) [10,56].

The pH of mineral waters further regulates microbial distribution and activity. Most mineral waters in Bulgaria are characterised by neutral to weakly alkaline conditions, which result from the presence of Ca^{2+}, Mg^{2+}, and HCO_3^- ions [10,45]. These components contribute to the stability of microbial cellular structures and support enzymatic processes. However, acidic environments also occur locally. In the Pernik administrative province of western Bulgaria, mineral springs with pH values between 2.6 and 3.5 have been documented. These conditions are associated with elevated concentrations of dissolved H_2S (Table A20) [10]. CO_2 also promotes the development of acidophilic and autotrophic microorganisms, whereas H_2S acts as a strong reducing agent. As a result, anaerobic conditions develop, which favour S-oxidising and SO_4-reducing microbial communities [10,36].

Mineralisation reflects the total concentration of dissolved salts and represents another key ecological factor. Elevated TDS impose osmotic stress on microbial cells and restricts survival to organisms that possess specific adaptations to high ionic strength [10,23]. In contrast, many mineral waters are oligotrophic and contain low concentrations of organic matter. These conditions favour microorganisms with highly efficient nutrient uptake

systems and specialised metabolic pathways. They also promote syntrophic interactions between different microbial taxa, which enhances ecosystem stability under nutrient-limited conditions [17,21,27].

The redox state of mineral waters is closely related to the availability of dissolved O_2 and reduced chemical species. Deep groundwater systems are typically isolated from atmospheric O_2, which results in low redox potential and the accumulation of reduced forms of Fe^{2+}, Mn^{2+}, and S^{2-} [26]. These conditions support anaerobic and anoxic environments and stimulate the activity of chemolithotrophic microorganisms. Such metabolic processes play a central role in Fe, S, Mn, C cycles and contribute to the formation of redox-active compounds within subsurface ecosystems [21,26,43].

The combined influence of these physicochemical parameters establishes strong environmental gradients that determine microbial distribution, metabolic specialisation, and ecosystem functioning in mineral and thermal waters.

2.3. Ecological Organisation and Microbial Structuring Mechanisms

Microbial communities in mineral waters are structured by interacting abiotic gradients (temperature, light, redox potential) and biotic processes (competition, symbiosis, and biofilm formation) [49]. Thermal gradients in Bulgarian mineral waters, with an average geothermal gradient of approximately 4.5 °C per 100 m of depth [36], induce pronounced vertical and spatial stratification of microbial taxa [25,42]. Thermophiles and archaea dominate high-temperature zones [13,26,27], while phototrophic microorganisms are typically restricted to cooler, illuminated surface layers [51]. However, cyanobacteria have been reported even in systems exceeding 80 °C under sufficient irradiance [12,51], which demonstrate the adaptive potential of phototrophic lineages in extreme thermal conditions. Light availability further contributes to functional compartmentalization and support photosynthetic microzones within otherwise thermally extreme systems [17]. The functional stratification of microbial communities results from the combined influence of temperature and light and lead to the organisation of microorganisms into distinct ecological layers according to optimal environmental conditions [17].

Hydrological dynamics based on seasonal recharge, flow variability, and mixing with surface waters, influence microbial population turnover and community composition [2,42]. Seasonal shifts have been associated with transient detection of opportunistic taxa *Pseudomonas* spp. in some Bulgarian springs [2]. These processes collectively affect nutrient availability, redox-active compound distribution, and overall microbial activity.

Microbial interactions like syntrophy, competition, and biofilm formation, contribute to ecosystem stability and enhance metabolic efficiency [56]. Biofilms also facilitate the retention of nutrients and support complex microbial consortia capable of producing bioactive compounds [30]. The presence of redox-active elements S and Fe further supports chemolithotrophic metabolism and shapes biogeochemical cycling within these systems [56].

Bulgarian mineral waters represent geologically controlled limnological systems in which subsurface geological architecture determines physicochemical conditions, which in turn structure microbial community composition and functional potential [56,57]. Geological heterogeneity acts as the primary driver of microbial niche differentiation which link hydrogeological processes with microbial ecology. The interaction between lithology, hydrodynamics, and geochemistry ultimately governs microbial diversity patterns, metabolic pathways, and biogeochemical cycling in these systems [56]. This integrated framework is essential for the interpretation of microbial distribution patterns and the identification of environments with high biotechnological potential.

4. Microbial Communities in Mineral Waters of Bulgaria

Mineral waters and thermal springs in Bulgaria constitute unique microbial ecosystems inhabited by bacteria, archaea, and phototrophic microorganisms that are adapted to a wide range of chemical, mineralogical, and thermal conditions [1,25,58].

3.1. Bacteria and Archaea

Bacterial and archaeal communities in Bulgarian mineral waters display marked ecological and functional heterogeneity, shaped primarily by temperature, mineralisation, oxygen availability, and geological setting [17,19]. These systems span a continuum from cold, moderately mineralised springs to hyperthermal environments exceeding 70 °C [10,13,26,27], which results in strong environmental filtration of microbial assemblages and pronounced niche differentiation [30]. In the mineral waters and thermal springs of Bulgaria, representatives of multiple bacterial phyla have been identified: *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Chloroflexi* [2,55]. It is noteworthy that the number of isolated microorganisms does not always correspond to the number of investigated springs [11].

In lowland and moderately warm springs, heterotrophic bacteria dominate and are primarily represented by metabolically versatile taxa: *Bacillus*, *Pseudomonas*, and *Aeromonas* [2,11,25,44]. These organisms are central to organic matter decomposition, nutrient utilisation, and redox balancing processes. They actively contribe to the closure of C, N, and S biogeochemical cycles [1,2,12,17,27]. Their enzymatic flexibility enables adaptation to fluctuating physicochemical conditions like seasonal hydrological variability and mineral composition shifts, and supports ecosystem stability in nutrient-limited environments. In northern and central Bulgaria, cold mineral springs like those in the Barzia region near Mount Kom show the presence of *Pseudomonas* spp. and psychrophilic bacteria with seasonal changes in spring and autumn, without evidence of faecal contamination [2]. However, the species-level diversity of these microorganisms has not yet been investigated but their ecological plasticity and ability to persist under oligotrophic conditions should be investigated in future. *Aeromonas hydrophila* has also been detected in cold waters (21–22 °C) from the villages of Hadzhi Dimitar and Gunchov izvor in the Sliven administrative province [44]. From isothermal and thermal mineral springs in the Lovech, the facultative anaerobe *Bacillus licheniformis* and the aerobic species *Bacillus amyloliquefaciens* and *Bacillus subtilis* have been isolated [11]. Thermal and hyperthermal springs, in contrast, are characterised by a shift toward thermophilic and chemolithotrophic microorganisms [13,18,27]. The genera *Brevibacillus*, *Geobacillus*, *Anoxybacillus*, *Thermus*, *Thermotoga*, and *Caldicellulosiruptor* dominate these habitats [11,17,20,25,44]. They participate in high-temperature C turnover, S transformations, and fermentation-based metabolic pathways [1,8,12,17,27]. In the Sofia region, isolates from species *Geobacillus stearothermophilus* and *Bacillus licheniformis* have been reported, alongside the thermophilic *Anoxybacillus bogrovensis* from Dolni Bogrov [11,20]. In Velingrad, *Geobacillus tepidamans* has been identified, which further demonstrate the diversity of thermophilic bacilli in Bulgarian geothermal systems [63]. Extremely diverse *Bacillus* sp.-dominated communities have been documented across multiple administrative provinces in the country: Varna, Lovech, Stara Zagora, Plovdiv, Pazardzhik, Haskovo, Sliven, Burgas, and Yambol, with frequent detection of *Bacillus subtilis*, *B. licheniformis*, B. *amyloliquefaciens*, *B. vallismortis*, and members of the *Bacillus cereus* group [11,44,59]. In the Rupite hyperthermal springs, additional taxa *Pseudomonas fluorescens*, *Stenotrophomonas maltophilia*, *Aeribacillus pallidus*, and *Anoxybacillus rupiensis* have been identified, which shows both phylogenetic and functional diversity in extreme environments [14,32]. Novel strain *Chloracidobacterium validum* sp. nov., capable of growth at 45 °C under low light conditions, further demonstrate the presence of previously uncharacterised thermophilic and microaerophilic bacteria in Bulgarian geothermal systems [60].

Metabolically distinct functional groups are also present in hot springs. Lithotrophic taxa *Aquifex* and *Hydrogenobacter* and heterotrophic *Thermus* spp., which jointly contribute to energy flow and redox cycling under low organic C availability [17]. The genera *Thermovorax*, *Hydrogenophilus*, and *Roseiflexus* participate in both lithotrophic and organotrophic processes and connect microbial metabolism to S and H cycles in mineral waters [1,8,12,17,27].

Archaeal communities remain less extensively characterised but are consistently detected across thermal gradients in Bulgarian mineral waters [13,18]. Representatives affiliated with *Euryarchaeota* and *Crenarchaeota* are particularly associated with high-temperature and anaerobic niches [13,18], where they may reach temperatures above 80 °C [13,18]. In two geographically distinct areas – hyperthermal springs near the villages of Levunovo and Vetren Dol, archaeal diversity varies significantly. Spring near Levunovo shows higher richness and dominance of *Thaumarchaeota*-related lineages and *Methanosarcinales*, while this in Vetren Dol is dominated by thermophilic crenarchaeal groups Hot Water Crenarchaeotic Group (HWCG) [13].

Metagenomic studies of Rupite springs indicate low archaeal abundance compared to bacteria, yet reveal a substantial proportion of novel operational taxonomic units with low similarity to known taxa [28]. In the Varvara thermal spring, archaeal diversity includes representatives of *Crenarchaeota*, *Euryarchaeota*, and *Korarchaeota*, along with several unassigned lineages, some of which represent entirely new phylogenetic subgroups [18]. These archaea are probably involved in S oxidation and anaerobic CO_2 respiration, consistent with the high concentrations of SO_4^{2-} and $S_2O_3^{2-}$ in the water [13]. Additional evidence suggests their participation in methanogenesis and other redox-sensitive processes in O_2-depleted microenvironments [12,13,17].

3.3. Cyanobacteria and Microalgae

Phototrophic microbial communities constitute a fundamental component of Bulgarian mineral water ecosystems, connected to surface-exposed and moderately illuminated thermal springs [12,49]. These communities form dense biofilms and multilayered microbial mats that contribute significantly to primary production and ecosystem structuring [12,25].

Cyanobacteria are the dominant phototrophic group in thermal and hyperthermal environments, where they exhibit exceptional physiological adaptability to elevated temperatures and variable mineral conditions [12,42]. Genera *Leptolyngbya*, *Oscillatoria*, *Synechococcus*, *Phormidium*, and *Geitlerinema* are frequently encountered in Bulgarian thermal systems in Rupite, Sapareva Banya, and Sofia-region springs. They can sustaine photosynthetic activity under temperatures arount 60-70 °C [12,41,42]. In hyperthermal springs (>70 °C), thermophilic genera *Chlorogloeopsis* (dominant at ~68 °C), *Thermoleptolyngbya*, *Desertifilum*, and *Oculatella* are also reported, while *Leptolyngbya geysericola* and *Geitlerinema splendidum* occur at slightly lower thermal ranges (>50 °C) [12,41]. Species *Nostoc muscorum*, *Calothrix thermalis*, *Symploca thermalis*, *Microcoleus autumnalis*, and *Cyanobacterium aponinum* contribute to the structural and functional complexity of microbial mats across Bulgarian geothermal systems. Mineral springs in Pazardzhik are rich in *Leptolyngbya tenerrima* and *Oscillatoria arachnoidea*. *Spirulina subtilissima* also occur and are adapted to montane conditions. In the Lovech region, dominant representatives include *Kamptonema okenii*, *Microcoleus amoenus*, and *Phormidium favosum*. In the Pernik region, *Lyngbya martensiana* has been recorded. Black sea coastal zone (Varna and Burgas) are characterised by marine and brackish cyanobacteria such as *Lyngbya aestuarii*, *Lyngbya major*, *Phormidium chalybeum*, and the widely distributed species *Merismopedia tranquilla* [34]. Novel thermophilic microalgae for the country include *Gloeocapsa gelatinosa*, *Leibleinia epiphytica*, *Phormidesmis molle*, *Phormidium corium*, and *Symploca thermalis* [41]. In several Bulgarian regions Rupite, Sapareva Banya, and Kazichene-Pancharevo geothermal zones, thick stratified mats are formed where cyanobacteria interact with thermophilic chemolithotrophs *Hydrogenobacter*, *Hydrogenophilus*, *Roseiflexus*, *Thermotoga*, and *Thermus*, which utilise O_2 and organic compounds produced by phototrophs [12,42]. This syntrophic coupling between phototrophic and chemolithotrophic microorganisms is a key factor in ecosystem productivity under extreme thermal conditions [12,17,27].

Microalgae in Bulgarian mineral springs include representatives of *Chlorophyta*, *Bacillariophyta*, *Charophyta*, and *Rhodophyta*. They are more prevalent in cooler and moderately mineralised springs [41,42]. Diatoms *Navicula nobilis*, *Pinnularia viridis*, *Surirella minuta*, *Amphora affinis*, *Diploneis elliptica*, *Gomphonema* spp., and *Pleurosigma spenceri* play an important role in Si cycling through biomineralisation processes. Green algae *Chlorella vulgaris*, *Draparnaldia acuta*, *Oedogonium capillare*, *Cladophora glomerata*, *Scenedesmus bijugatus*, *Hydrodictyon reticulatum*, *Oedogonium cardiacum*, *Ulothrix zonata*, and *Spirogyra* spp., contribute to O_2 production and organic C fixation in both inland and thermal systems. Charophytes *Chara braunii* and *Chara fragilis* further enhance structural complexity in macrophyte-influenced environments in Plovdiv, Hisarya, Narechenski Bani, and Karlovski Bani mineral waters. Red algae *Batrachospermum moniliforme*, *Thorea hispida*, and *Hildenbrandia rivularis*, are also present in selected spring systems [41].

3.4. Yeasts and Molds

Mineral waters are generally considered to be microbiologically clean with respect to filamentous fungi and yeasts. On rare occasions, thermophilic yeasts may coexist with bacteria in mixed microbial communities [61]. The presence of yeasts *Debaryomyces* and *Candida*, as well as filamentous fungi *Aspergillus* spp. and *Penicillium* spp., is mainly reported in bottled mineral waters

[62–64]. In Bulgaria, mineral waters typically exhibit a minimal occurrence of filamentous fungi and yeasts within permissible regulatory limits. In samples from Kom, isolated green colonies with conidia characteristic of the genus *Penicillium* have been observed, whereas in Gorna Banya and Bankya, single fungal colonies have been detected only during spring and autumn. This suggests that fungal presence is strongly regulated by temperature shifts, nutrient pulses, and hydrological dynamics rather than stable ecological establishment [2]. These organisms are typically not permanent members of native microbial communities but rather transient colonisers introduced through environmental deposition, aerosols, or processing pathways.

Beyond taxonomic group-specific observations, the available data allow an integrated interpretation of spatial microbial distribution patterns across Bulgaria. Figure 2 presents the distribution of recorded microbial taxonomic units (species, genera, and higher taxa) and available information about mineral springs across administrative provinces of Bulgaria [1,2,10,11,25,41,59,65–69]. The data reveal pronounced spatial microbial heterogeneity driven by geological setting, hydrological connectivity, and mineral water temperature. The highest taxonomic richness is observed in the Blagoevgrad, followed by Sofia, Pazardzhik, Sofia City, and Plovdiv, which correspond to geothermal hotspots with extensive *Bacillus*-, cyanobacteria-, and algae-rich systems [11,41,42]. Intermediate diversity is recorded in Haskovo, Burgas, Varna, and Montana, while the lowest values are observed in Lovech, Stara Zagora, Sliven, Yambol, and Kyustendil [24,49,60]. The observed distribution suggests strong research bias toward well-studied geothermal systems in southwestern Bulgaria: Rupite, Sandanski, Bansko, and Dobrinishte, where diverse bacterial (*Bacillus*, *Geobacillus*, *Anoxybacillus*) and phototrophic communities co-occur [11,12,34,63]. In contrast, northern Bulgarian geothermal systems (Pleven, Veliko Tarnovo, Vidin) remain underexplored despite their potential to host thermophilic taxa. The Black Sea coastal administrative provinces (Varna and Burgas districts) is characterised by mixed marine-terrestrial microbial assemblages like *Pseudomonas aeruginosa*, *Brevibacillus* spp., *Lyngbya aestuarii*, and *Cladophora fracta* which reflects complex environmental inputs [11,41].

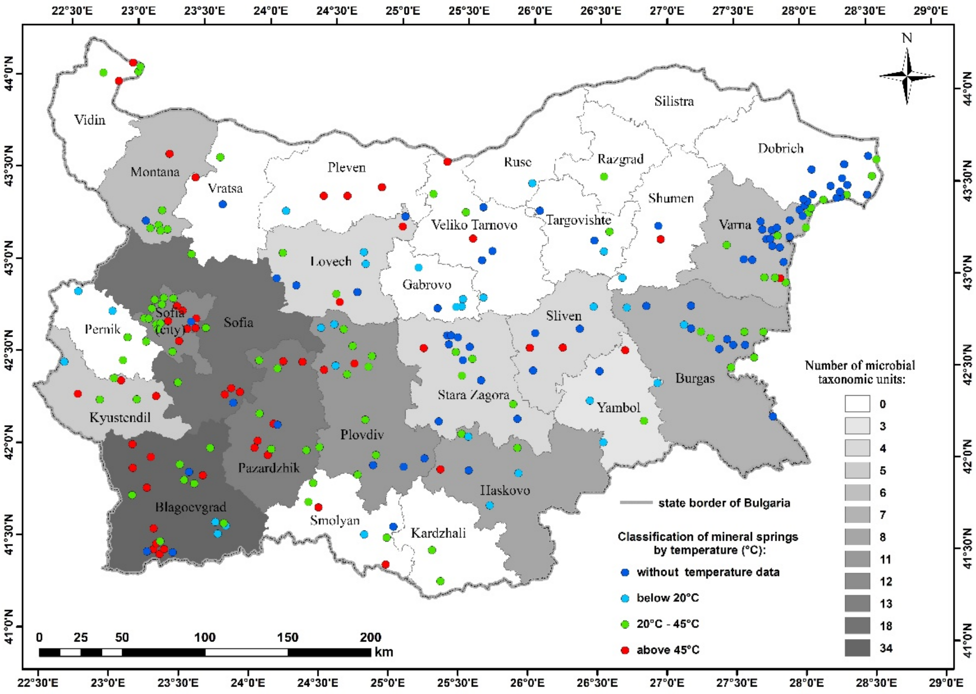

Figure 2. Distribution of microbial taxonomic units (species, genera, and higher taxa) in Bulgarian mineral waters across administrative provinces.

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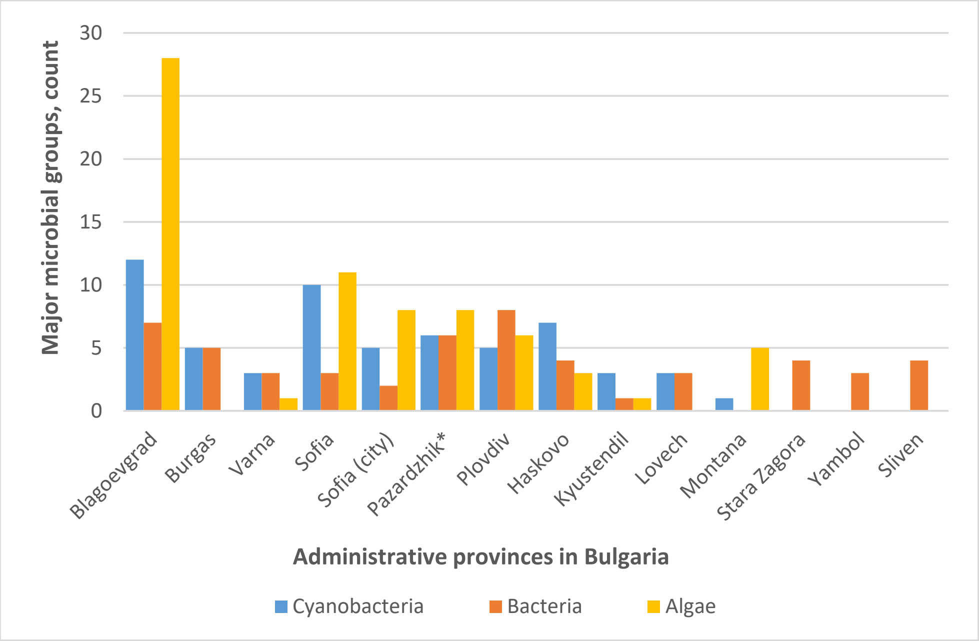
It is also important to note that temperature data are unavailable for a substantial proportion of springs, which limits the robust ecological comparison and necessitate improved physicochemical characterisation in future studies [34,59]. Cold springs in Bulgaria remain particularly underrepresented, despite their potential to harbour novel psychrophilic and psychrotolerant microorganisms [2].

Figure 3 integrates data from multiple studies on microbial communities across Bulgarian administrative provinces and demonstrate pronounced variability in bacterial, archaeal, cyanobacterial, and algal communities [1,2,12,17,25,27,41,59]. Mountainous and western administrative provinces (Blagoevgrad, Sofia) are characterised by co-dominance of cyanobacteria and algae: *Leptolyngbya*, *Oscillatoria*, *Chlorogloeopsis*, *Navicula*, and *Closterium*, which suppose freshwater and alpine geothermal influence [41,42]. Coastal administrative provinces (Varna, Burgas) exhibit balanced distributions of cyanobacteria and heterotrophic bacteria: *Lyngbya*, *Phormidium*, *Pseudomonas*, and *Bacillus*, indicative of mixed marine–terrestrial inputs [11,41]. Urban systems (Sofia city) demonstrate reduced diversity and more structured microbial assemblages dominated by opportunistic bacteria *Staphylococcus xylosus*, *Klebsiella oxytoca*, and *Aeromonas hydrophila*, along with limited algal presence [41,44]. Lowland administrative provinces (Plovdiv, Stara Zagora, Yambol, Sliven) are dominated by heterotrophic *Bacillus* species (*B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*, *B. cereus* group), which reflect simplified nutrient-driven microbial systems [11,41,42]. Certain administrative provinces exhibit distinctive microbial signatures: Montana shows increased algal representation (*Cladophora*, *Spirogyra*, *Cosmarium*), while Haskovo and Kyustendil represent transitional systems with mixed cyanobacterial-bacterial-algal communities [41]. Pazardzhik is notable for balanced microbial composition. Thermophiles (*Bacillus* sp., *Geobacillus stearothermophilus*), cyanobacteria (*Oscillatoria* spp.), and diverse archaeal groups affiliated with *Crenarchaeota* and *Euryarchaeota* have been found [13,18].



Legend: Pazardzhik* – Potential archaeal hotspot.

Figure 3. Functional microbial composition across administrative provinces in Bulgaria.

The distribution of microbial diversity across Bulgarian administrative provinces, expressed as the number of identified species, genera, and higher taxa, reveals pronounced spatial heterogeneity in the depth of taxonomic resolution (Figure 4) [2,11,41,42].

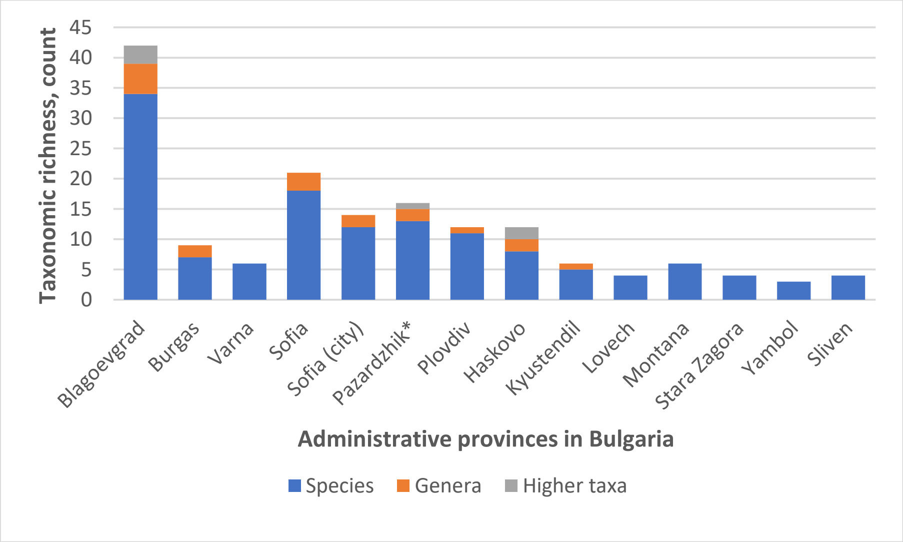


Figure 4. Spatial variation in microbial taxonomic richness (species, genera and higher taxa) across Bulgarian administrative provinces.

Blagoevgrad exhibits the highest overall diversity, with a marked predominance of species-level richness (34 species), accompanied by detectable diversity at both genus (5) and higher taxonomic levels (3), which indicates a complex and well-resolved microbial assemblage shaped by geothermal activity and diverse hydrochemical conditions [11,25,41]. In contrast, coastal and urban-influenced administrative provinces Burgas, Varna, and Sofia (city) show substantially reduced species richness and limited representation at higher taxonomic levels, which suggests comparatively simplified or less extensively studied microbial communities [11,41,44]. These areas are characterised by mixed anthropogenic and natural inputs. The presence of opportunistic bacteria *Pseudomonas aeruginosa*, *Brevibacillus* spp., and *Aeromonas hydrophila*, and marine-associated phototrophs *Lyngbya aestuarii* and *Phormidium chalybeum* [11,41]. Sofia retains moderate diversity across species (18) and genera (3), but doesn’t have higher taxonomic resolution. Geothermal springs Pancharevo, Zheleznitsa, and Kazichene, where thermophilic bacteria (*Bacillus subtilis*, *Geobacillus stearothermophilus*) and cyanobacteria (*Nostoc muscorum*, *Calothrix thermalis*, *Geitlerinema splendidum*) are commonly recorded [11,41,42]. The coexistence of bacterial and phototrophic taxa in these systems reflects intermediate thermal and nutrient conditions. Intermediate diversity patterns are observed in Pazardzhik and Haskovo, where both genera and higher taxa are present, which can be suggested as structurally more complex microbial communities compared to low-diversity administrative provinces [11,25]. These areas host a mixture of thermophilic bacilli (*Bacillus licheniformis*, *B. amyloliquefaciens*, *Geobacillus stearothermophilus*) and phototrophic organisms (*Oscillatoria*, *Leptolyngbya*, *Scenedesmus quadricauda*), which thrive in transitional ecological conditions between thermal and mesophilic systems [41]. Kyustendil and Plovdiv display moderate species richness but limited higher-level taxonomic differentiation. The dominant taxa include *Bacillus cereus* group members, *Aeromonas sobria*, *Klebsiella oxytoca*, and cyanobacteria *Oscillatoria proboscidea* and *Gloeocapsa kuetzingiana*, and green algae (*Cladophora glomerata*, *Spirogyra reticulata*) [25,41]. Lowland and northern administrative provinces Lovech, Montana, Stara Zagora, Yambol, and Sliven demonstrate minimal microbial diversity, primarily restricted to species-level observations with little or no representation of genera or higher taxa. This is probably associated with simplified community structures or limited taxonomic depth in available datasets [24,49,60]. These systems are often dominated by a narrow range of *Bacillus* species (*B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*) and occasional phototrophic taxa *Microcoleus*, *Phormidium*, and *Spirulina subtilissima*, which represents reduced ecological complexity [11,70].

5. Functional Role of Microbial Communities

Mineral water microbial communities are important for aquatic system function, biogeochemical cycles and to maintain stability under oligotrophic and extreme conditions. [1–8,12–16,20]. Their metabolic versatility enables the transformation of inorganic compounds into bioavailable forms and sustain ecosystem productivity, and regulate chemical equilibrium within subterranean and surface water systems [3–9,14–18,21–27].

5.1. Participation in Biogeochemical Cycles

In oligotrophic mineral waters, microbial communities act as primary drivers of geochemical transformations due to the limited availability of organic substrates [2–6,10–14,18–21,46–49]. Key bacterial groups *Bacillus*, *Pseudomonas*, *Aeromonas*, and *Klebsiella*, together with chemolithotrophic taxa *Aquifex*, *Hydrogenobacter*, *Hydrogenophilus*, *Thermus*, *Thermotoga*, and *Roseiflexus*, archaea from the phyla *Euryarchaeota* and *Crenarchaeota*, are actively involved in these processes [5–12,15–23].

Carbon cycle is strongly influenced by chemolithoautotrophic microorganisms. *Aquifex*, *Hydrogenobacter*, and *Hydrogenophilus* fix CO_2 in deep, aphotic environments [13]. In contrast, surface zones are dominated by photosynthetic microorganisms, where cyanobacteria and microalgae act as primary producers. They contribute to organic matter formation and local pH increase, which promotes carbonate precipitation [22,41,71]. Heterotrophic bacteria *Bacillus*, *Pseudomonas*, and *Aeromonas* dominate oxygen-rich microenvironments, while anaerobic degradation occurs in sediment layers [10–16,20,25]. Thermophilic bacteria *Caldicellulosiruptor* spp. are involved in the mineralisation of organic matter to CO_2 and CH_4, as observed in thermal springs in Rupite [17]. Methanogenic archaea, members of *Methanosarcinales* and Candidate *Thaumarchaeota*, are implicated in CH_4 formation at high temperatures (Levunovo spring, ~68 °C) [13].

Nitrogen cycle is mediated by both cyanobacteria and bacteria. Nitrogen-fixing cyanobacteria *Leptolyngbya* spp., *Mastigocladus*, *Nostoc* spp., *Calothrix thermalis*, and *Anabaena* spp. contribute significantly to atmospheric N_2 fixation [12,42], a process also supported by *Bacillus subtilis* [72]. Nitrification and denitrification processes are carried out by *Pseudomonas*, *Bacillus*, and *Aeromonas*, while NH_3 oxidation is associated with *Thaumarchaeota* archaea [73].

Sulfur cycle is driven by sulfur-oxidising bacteria *Aquifex*, *Hydrogenobacter*, *Thermotoga*, and *Beggiatoa alba*, which convert reduced sulfur compounds into SO_4^{2-}. *Crenarchaeota* archaea contribute to sulfur reduction processes in thermophilic environments. Anaerobic bacteria *Caldicellulosiruptor* participate in assimilatory sulfate reduction for amino acid biosynthesis [74].

Iron cycle is mediated through microbial redox reactions, where *Bacillus* spp. reduce Fe(III) to Fe(II). *Pseudomonas fluorescens* enhances FeO and MnO_2 mineral dissolution through redox-active phenazines [6]. Silicon cycle is influenced by diatoms *Navicula*, *Pinnularia*, *Surirella*, *Gomphonema*, and *Amphora*, whose frustules are composed of biogenic SiO_2 [34].

5.2. Microbial Interactions and Biofilms

In mineral waters, microorganisms exist predominantly in structured multispecies communities known as biofilms rather than as planktonic cells. These biofilms represent highly organised, metabolically integrated systems formed by bacteria, archaea, cyanobacteria, and algae under oligotrophic conditions [30].

Biofilm formation begins with reversible cell adhesion to mineral or organic surfaces, followed by irreversible attachment and extracellular polymeric substance (EPS) production. EPS matrices composed of polysaccharides, proteins, and extracellular DNA provide structural stability and protection against environmental stressors like temperature fluctuations, pH variation, and toxic compounds. As biofilms mature, internal channel systems develop and facilitate nutrient and gas exchange. Strong chemical gradients emerge, and lead to spatial stratification of microbial populations. Phototrophic microorganisms occupy surface layers, while heterotrophic and anaerobic taxa dominate deeper zones. Quorum sensing mechanisms regulate gene expression related to EPS synthesis, metabolic coordination, and stress adaptation [30]. In thermal springs, biofilms develop into microbial mats with distinct vertical stratification. Cyanobacteria *Chlorogloeopsis*, *Leptolyngbya*, *Synechococcus*, and *Mastigocladus* dominate the upper layers, where they perform oxygenic photosynthesis and CO_2 fixation. This oxygen production supports aerobic heterotrophic bacteria *Bacillaceae* [13–19,23–29,32–38,41–47]. *Bacillus megaterium* contribute to carbonate biomineralisation and structural stabilisation of microbial mats [71]. Deeper layers of biofilms become anaerobic due to oxygen depletion, where archaea drive methanogenesis and sulfur cycling processes [27,28]. The slow growth rates of archaeal populations contribute to heterogeneous structural development and periodic detachment and renewal of biofilm layers.

5.3. Adaptation to Extreme Conditions

Microbial communities in mineral waters exhibit a wide range of physiological and molecular adaptations that enable survival under extreme environmental conditions. Structural adaptations include endospore formation in *Bacillus* spp., which ensures long-term survival under stress conditions, particularly during nutrient limitation and physicochemical fluctuations [57]. EPS production by *Pseudomonas*, *Leptolyngbya*, and *Chlorogloeopsis* forms protective barriers against UV radiation, desiccation, and toxic compounds, and stabilise biofilm formation [57,75,76]. Filamentous cyanobacteria *Mastigocladus* and *Leptolyngbya* exhibit heterocyst differentiation, which enhance N_2 fixation efficiency and allow survival under nitrogen-limited conditions [75].

Membrane and enzymatic adaptations are important in thermophiles [9–16,20–26,29–35,38–44,47–53]. Species *Geobacillus stearothermophilus*, *Anoxybacillus*, and *Brevibacillus* possess membranes enriched in saturated and branched-chain fatty acids, increasing thermal stability by reducing membrane fluidity. These organisms produce thermostable enzymes and heat-shock proteins that maintain proper protein folding, enzymatic activity, and cellular integrity at temperatures exceeding 60-70 °C [77,78]. In contrast, psychrophilic adaptations involve increased membrane fluidity through unsaturated and short-chain fatty acids, combined with the production of antifreeze proteins that inhibit ice crystal formation and prevent cellular damage [79].

Metabolic adaptations include efficient ion transport systems, compatible solute accumulation (e.g., betaine, glycerol, trehalose), and detoxification mechanisms. *Pseudomonas* spp. demonstrate high tolerance to metal ions and osmotic stress. These systems support survival in highly mineralised environments and facilitate horizontal gene transfer of adaptive traits, often mediated by plasmid-associated genes [80]. Anaerobic adaptations involve alternative metabolic pathways like fermentation, sulfate reduction, and methanogenesis, which allow conservation under oxygen-limited conditions in deeper layers of mineral water systems [81].

At the same time, cyanobacteria *Synechococcus*, *Mastigocladus*, *Leptolyngbya*, and *Chlorogloeopsis* exhibit photosynthetic adaptations which involve structural and functional modifications of the photosynthetic apparatus. These include increased thermal stability of Photosystem II and light-harvesting complexes, and alterations in pigment composition. The synthesis of chlorophyll *f* enables efficient utilisation of low-intensity or filtered light within dense biofilms, enhancing CO_2 fixation under suboptimal light conditions [82].

At the molecular level, thermotolerant microorganisms synthesise heat-shock proteins (molecular chaperones) that prevent protein denaturation and assist in proper folding under stress. DNA stability is further enhanced by enzyme reverse gyrase, which introduces positive supercoils into DNA and protect it at high temperatures. In addition, species of *Geobacillus* and related thermophiles possess CRISPR-Cas defence systems that provide adaptive immunity against viral infections and contribute to genome stability in extreme environments [83,84].

6. Biologically Active Microbial Compounds

Microorganisms from mineral waters represent a significant source of structurally diverse bioactive compounds. Extreme physicochemical conditions drive the evolution of specialised metabolic pathways that result in the production of antimicrobial, enzymatic, antioxidant, and other biologically active molecules. As a consequence, these ecosystems are increasingly recognised as promising reservoirs for novel biotechnological and pharmaceutical agents [1,4,36,55].

6.1. Antimicrobial Substances

The synthesis of antimicrobial compounds is a common adaptive strategy among microorganisms in limited nutrient availability. Members of the genus *Bacillus* are among the most important producers of bacteriocins and antimicrobial peptides. Strains isolated from hot springs in Haskovo and Plovdiv administrative provinces *B. cereus*, *B. thuringiensis*, *B. amyloliquefaciens*, and *B. subtilis*, demonstrate pronounced inhibitory activity against Gram-negative bacteria and saprophytic fungi *Aspergillus*, *Fusarium*, *Penicillium*, and *Rhizopus* species [11,59,85]. Similar antimicrobial potential has been reported in *Bacillus* strains from geothermal systems in other regions, which confirm their broad biotechnological relevance [19,23,53,86,87]. These microorganisms produce a wide range of bioactive peptides: subtilin, subtilosin A, cereins, and thuricins, which exhibit activity against Gram-positive pathogens and fungal contaminants [59]. *Bacillus* sp. isolates from mineral waters in Stara Zagora, Sliven, Sofia and Blagoevgrad also show strain-specific antimicrobial spectra. Some demonstrate strong antifungal activity but limited antibacterial effects, which is probably due to functional diversity within the genus [11,49].

Beyond *Bacillus*, *Pseudomonas fluorescens* contributes to antimicrobial activity through the production of phenazine derivatives [88]. *Caldicellulosiruptor* spp. genomes encode potential bacteriocin transport systems which suggests hidden antimicrobial capabilities [89]. *C. owensensis*, contain homologues of subtilosin A, a bacteriocin active against certain Gram-positive bacteria [90]. The facultative anaerobe *Staphylococcus xylosus* may be effective against the ochratoxin-producing fungus *Penicillium nordicum* [91]. Archaeal microorganisms, although less studied, are potential producers of archaeocins – glutamate-rich compounds with properties analogous to polymyxin B [92]. Halophilic archaea synthesise halocins with broad-spectrum antibacterial activity [93]. Sulfolobicins constitute a novel class of antimicrobial proteins produced by members of the genus *Sulfolobus* (*Crenarchaeota*) mainly against closely related archaeal strains [94].

Cyanobacteria are key producers of bioactive secondary metabolites with antibacterial, antiviral, and antifungal properties. Species *Leptolyngbya boryana* and *Leptolyngbya* sp. HNBGU 002 synthesise bioactive hydrocarbons, δ-lactam derivatives, and phenolic compounds with activity against vancomycin-resistant *Staphylococcus aureus*, and antiviral effects against Coxsackievirus B3 and rotavirus [95–98]. The thermophilic cyanobacterium *Mastigocladus laminosus* produces capsular polysaccharides with antimicrobial, anti-inflammatory, and cytotoxic properties, while *Gloeocapsa* sp. exhibits broad-spectrum antimicrobial activity against bacterial and fungal pathogens at low inhibitory concentrations [99]. *Phormidium* spp. can syntetise eugenol and fatty acids under nutrient-limited conditions, which shows the metabolic plasticity of cyanobacteria. Extremophilic genera *Mastigocladus*, *Chlorogloeopsis*, *Leptolyngbya*, *Oscillatoria*, and *Phormidium* can produce peptides, alkaloids, and phenolic compounds that inhibit competing microorganisms. Nitrogen-fixing cyanobacteria (*Nostoc*, *Calothrix*, *Symploca*) contribute through cyclic peptide production involved in chemical defense [100]. Green algae (*Chlorella*, *Scenedesmus*, *Cladophora*, *Spirogyra*) and diatoms (*Navicula*, *Pinnularia*) produce phenolics and fatty acids with antimicrobial and allelopathic activity [101–103].

6.2. Industrially Oriented Enzymes

Microorganisms from mineral waters are an important source of extremozymes adapted to harsh environmental conditions [25,104–108]. The genera *Bacillus* and *Pseudomonas* dominate in enzymatic production due to their metabolic flexibility, ecological resilience, and ability to synthesise diverse hydrolytic enzymes with industrial relevance [49,105–112]. The presence of Ca^{2+} and Mg^{2+} ions in thermal waters further enhances enzymatic activity, particularly during the growth of *Bacillus subtilis* [25]. Microbial isolates from Bulgarian mineral springs from the administrative provinces of Pazardzhik, Sofia and Blagoevgrad exhibit combined amylolytic, proteolytic and lipolytic activities.

Representatives of *B. subtilis*, *B. amyloliquefaciens* and *B. Licheniformis* and thermophiles *Geobacillus stearothermophilus* demonstrate strong hydrolytic activity, while other strains *B. cereus*, *B. thuringiensis*, *B. methylotrophicus* show more specialised profiles: phosphatase and glucosidase activities [11]. Thermophilic representatives of *Bacillus*-related genera (*Anoxybacillus*, *Geobacillus*, *Brevibacillus)*, isolated from 18 mineral springs across different regions of Bulgaria, synthesise a wide range of thermostable enzymes: amylases, xylanases, and β-glucanases, which are essential for lignocellulosic biomass degradation [13,20,113]. Species from thermal springs *Brevibacillus thermoruber* southwestern Bulgaria and *Anoxybacillus gonensis* are specialised in xylan degradation and starch hydrolysis, respectively, while *Geobacillus pallidus* and *Thermoactinomyces* sp., produce thermostable enzymes of industrial relevance [20]. Additionally, thermophilic and alkali-tolerant bacteria with xylanolytic activity have been isolated from hyperthermal springs in southwestern Bulgaria and the Sofia (42-96 °C), while *Anoxybacillus bogrovensis*, isolated from a geothermal spring in Dolni Bogrov (Sofia), is characterised by a broad substrate spectrum and pronounced amylolytic activity [13,113]. The Gram-negative bacterium *Pseudomonas aeruginosa*, isolated from mineral waters in the Burgas administrative province, represents a potential producer of industrially relevant hydrolases. These include alkaline proteases with optimal activity at pH ~9.5, as well as lipases and esterases. Some strains of this species are also reported to produce enzymes involved in plastic degradation [109,110]. The metabolic versatility of *Pseudomonas fluorescens* is linked to extracellular protease and lipase production [111,112]. *Aeromonas sobria* from therapeutic springs in Narechenski baths (Plovdiv) may also be explored for amylase activity [114,115]. Although no direct evidence is found with strains from Bulgarian mineral springs, the facultative anaerobe *Staphylococcus xylosus*, identified in waters from the Pazardzhik administrative province, may possess enzymatic potential for the production of superoxide dismutase, nitric oxide synthase and naringinase. These biocatalisators can improve flavour characteristics in fruit juices and fermented foods [116,117].

Hot springs in the Rupite region constitute a natural habitat for diverse extremophilic bacteria of the genera *Thermus*, *Thermotoga*, *Hydrogenobacter*, *Aquifex* and *Roseiflexus*, as well as archaea (*Euryarchaeota*, *Crenarchaeota*, *Korarchaeota*) with significant potential for thermophilic enzyme production. These organisms are expected to produce DNA polymerases, proteases, amylases, cellulases and glycosidases [13,14,29,118,119]. The biotechnological potential of archaea remains poorly explored; however, they are presumed to exhibit hydrolase, proteolytic and lipolytic activities [120–122].

Extremophilic cyanobacteria *Chlorogloeopsis*, *Mastigocladus*, *Leptolyngbya* and *Phormidium* are valuable producers of thermostable proteases and lipases [123]. Nitrogen-fixing genera such as *Nostoc* and *Calothrix* are potential sources of hydrogenase enzymes [124,125]. Green algae *Chlamydomonas reinhardtii*, *Chlorella* spp. are also potential sources of enzymes involved in lipid metabolism and associated with carotenoid and other high-value bioactive compound biosynthesis [126]. Diatoms (*Navicula*, *Pinnularia*) are characterised by enzymes connected with biomineralisation and biosilicification, with applications in nanotechnology. Desmids, red algae and charophytes represent potential sources of enzymes involved in the degradation of complex organic compounds and mineral-related processes [127]. To date, there is no literature data on enzyme production from algae and cyanobacteria isolated from Bulgarian mineral springs.

5.3. Microbial Exopolysaccharides and Biosurfactants

Many microorganisms from mineral waters synthesise extracellular polymeric substances (EPS), which are essential for biofilm formation and microbial adaptation to extreme environments [128]. Numerous representatives of the family *Bacillaceae* produce EPS [125]. It has been shown that biopolymers synthesised by *B. subtilis* and *B. licheniformis* may reach concentrations of up to 12.6 mg/L [129,130]. *Geobacillus tepidamans* V264 is found in a hot spring in the village of Mizinka (Pazardzhik) has a promising EPS production potential [32]. In studies of thermophilic microorganisms from Bulgarian hot springs (43 to 85 °C) EPS-producing strains were selected [32,60]. Among them, *Brevibacillus thermotuber* was the most widespread species. It has been detected in hot springs in

Rupite, Levunovo, Dolno Osenovo (Blagoevgrad), Trebich (Sofia), and Gradeshnitsa (Vratsa). No clear correlation was observed between spring temperature and the amount of produced exopolysaccharides. *Anoxybacillus kestanbolensis* 415 from Mizinka mineral spring (85 °C), produces up to 25.3 mg/L EPS. *Geobacillus toebii* 419 and *Aeribacillus pallidus* 418 (from Rupite hot springs), show maximum yields of 50 mg/L and 53 mg/L, respectively [60,131]. EPS production potential depends on cultivation conditions rather than temperature alone [20,38,41,63]. Optimisation in bioreactor systems substantially enhances EPS biosynthesis and reach considerably higher concentrations under controlled pH, temperature and nutrient regimes [20,29]. Maximum EPS production up to ~170-180 mg/L in continuous processes) is achieved at 60 °C, pH 7.0. For *Brevibacillus thermoruber* 423 from a hot mineral spring in Gradeshnitsa, EPS production reaches up to 897 mg/L in bioreactor conditions at 55 °C and pH 6.5 [29].

Members of the genus *Pseudomonas* also contribute to EPS matrix formation and biofilm development [132,133]. Strains *Pseudomonas fluorescens* and psychrotrophic *Pseudomonas* spp. produce EPS over a broad range of environmental conditions, with yields strongly influenced by nutrient composition and trace elements Mn^{2+} and Zn^{2+} [134,135]. However, there are no scientific studies on EPS biosynthesis by *Pseudomonas* strains isolated from Bulgarian mineral waters. Archaea from the phyla *Euryarchaeota* and *Crenarchaeota* are also able to synthetise EPS as a key adaptation mechanism to extreme environments [136,137]. Cyanobacteria are *Leptolyngbya*, *Synechococcus* and *Phormidium* are considered potential EPS producers. EPS production in *Phormidium corium* depends on CO_2 and Ca^{2+} limitation [138,139]. No published literature for EPS production by archaea and cyanobacteria isolated from Bulgarian thermal waters is reported.

Biosurfactants produced by microorganisms facilitate adaptation to mineral-rich environments and reduce surface tension and enhance cell interactions [140,141]. *Bacillus* and *Pseudomonas* are of major importance, which make strains isolated from mineral springs promising sources of such compounds. *Bacillus subtilis* synthesises several classes of lipopeptide biosurfactants: surfactins, iturins and fengycins [142–146] while *Geobacillus stearothermophilus* and *Pseudomonas* spp. can produce various glycolipid biosurfactants [147–150]. A promising approach for future research is the co-cultivation of diverse algae and bacteria isolated from Bulgarian mineral springs, as microalgal-bacterial consortia have been shown to enhance biosurfactant production in a 34-fold increase in co-culture with *Desmodesmus perforates* [151]. Despite their importance, data on EPS and biosurfactant production by Bulgarian mineral water isolates remain a significant research gap.

6.3. Pigments and Antioxidants

Many microorganisms from mineral springs are potential producers of pigments with protective function: carotenoids, melanins, and chlorophylls [152]. Carotenoids are important due to their strong antioxidant capacity against UV radiation and oxidative stress. Within *Bacillus*, pigment production includes cerein in *B. cereus* with antimicrobial activity [153], carotenoids in *B. amyloliquefaciens* UCM B-5113 [154,155], and zeaxanthin synthesis in several species. *Bacillus licheniformis* and *B. megaterium* produce diverse C30 carotenoids: bacterioruberin derivatives and diapocarotenoids, with expression in *B. megaterium* linked to UV exposure [156–158]. Spore-associated melanins in thermoresistant *B. thuringiensis*, *B. pumilus*, and *B. altitudinis*, provide additional UV protection, while *B. subtilis* spores contain melanin-like pigments and related brown compounds [159–162]. Pigment production is also characteristic of *Pseudomonas*, where *P. aeruginosa* synthesises the redox-active pigment pyocyanin and *P. fluorescens* produces pyoverdine, involved in iron acquisition and antimicrobial interactions [163–168].

Cyanobacteria from Bulgarian mineral waters represent an additional important source of stable pigments. Genera S*ynechococcus*, *Oscillatoria*, and *Phormidium* are able to synthetise phycobiliproteins with strong antioxidant activity [169]. Thermophilic taxa (*Thermoleptolyngbya*, *Mastigocladus*, *Leptolyngbya*) produce thermostable phycobilins, carotenoids, and UV-protective compounds scytonemin [127,170–174]. EPS-associated pigment stabilisation further enhances their functional persistence in capsule-forming genera (*Gloeocapsa*, *Aphanothece*, *Chroococcus*) [175]. Algae from mineral and aquatic environments also contribute significantly to pigment diversity. Green algae (*Chlorella*, *Scenedesmus*, *Cladophora*) are major sources of β-carotene and lutein, while diatoms synthesise fucoxanthin and desmids accumulate phenolic compounds with antioxidant properties [176–179]. Red algae and charophytes further expand pigment diversity through phycobilins, carotenoids, and sulphated polysaccharides with antioxidant activity [180,181].

Mineral waters in Bulgaria represent a valuable source of microorganisms with notable biotechnological potential. Table 1 summarises the distribution of microorganisms with studied (in bold) or potential biotechnological activity across Bulgarian mineral water regions, together with their associated bioactive metabolites. The highest diversity of biotechnologically relevant microorganisms is observed in the geothermal regions of south-western Bulgaria: Rupite, Sapareva Banya and Velingrad, where thermophilic *Bacillus*-related taxa predominate. These findings underline the importance of Bulgarian thermal waters as a promising source of novel microbial resources for biotechnology, medicine and industrial applications.

Table 1. Microorganisms with potential for production of bioactive metabolites from Bulgarian mineral waters.

Administrative province	Bacteria	Cyanobacteria	Algae
Blagoevgrad	***Geobacillus*, *Aeribacillus*, *Brevibacillus*, *Bacillus* spp., *Pseudomonas*, *Stenotrophomonas*** [11,14,17,20] thermostable enzymes (lipase, xylanase), EPS, antimicrobial peptides, biosurfactants	*Thermoleptolyngbya*, *Leptolyngbya*, *Oscillatoria*, *Phormidium*, *Nostoc*, *Synechococcus*, *Geitlerinema* [12,41] phycobiliproteins, antioxidants, EPS, pigments	*Scenedesmus*, *Pediastrum*, *Cosmarium*, *Closterium*, *Thorea* [41,42] antioxidants, lipids, structural polysaccharides
Sofia (incl. Sofia city)	***Anoxybacillus*, *Geobacillus*, *Bacillus* spp.** [11,14,17,20] thermostable xylanases, EPS, biofilm metabolites	*Mastigocladus*, *Calothrix*, *Symploca*, *Phormidium*, *Nostoc* [12,41] phycobiliproteins, EPS, antimicrobial compounds	*Cladophora*, *Spirogyra* [41,42] antioxidants, polysaccharides
Kyustendil	*Beggiatoa alba* biosurfactants, sulfur metabolism compounds	*Oscillatoria*, *Phormidium*, *Asterocapsa* sp. [12,41] pigments, EPS, thermophilic enzymes	—
Plovdiv	***Bacillus* spp., *Aeromonas sobria*** [11] hydrolytic enzymes, bioremediation metabolites	*Nostoc* [12,41] EPS, antioxidants	*Cladophora*, *Spirogyra*, *Chara*, *Cosmarium* [41,42] antioxidants, lipids, EPS
Pazardzhik*	***Geobacillus tepidamans*, *Bacillus* spp., *Staphylococcus*** [11,14,17,20] EPS, enzymes, antimicrobial compounds	*Leptolyngbya* [12,41] EPS	*Chlorella*, *Scenedesmus*, *Batrachospermum*, *Hildenbrandia*, *Closterium*, *Cosmarium* [41,42] lipids, antioxidants

Smolyan	—	*Spirulina subtilissima* [12,41] phycocyanin, antioxidants	—
Lovech	***Bacillus* spp.** [11] thermotolerant enzymes, biofilms	*Kampotonema*, *Microcoleus*, *Phormidium* [12,41] EPS, pigments	—
Montana	—	*Microcoleus* [12,41] EPS	*Cladophora*, *Spirogyra*, *Cosmarium*, *Closterium* [41,42] antioxidants, biosorption compounds
Pernik	—	*Lyngbya martensiana* [12,41] biosurfactants, lipopeptides	—
Varna	***Bacillus* spp.** [11] EPS, antimicrobial metabolites	*Lyngbya*, *Phormidium*, *Merismopedia* [12,41] biosurfactants, pigments	*Cladophora* [41,42] marine polysaccharides
Burgas	***Bacillus* spp., *Brevibacillus*** [11] antimicrobial compounds, EPS	*Lyngbya*, *Phormidium* [12,41] antimicrobial metabolites, biosurfactants	*Cladophora* [41,42] marine metabolites
Haskovo	***Bacillus* spp.** [11] hydrolytic enzymes, antimicrobial peptides, insecticidal proteins	—	—
Stara Zagora	***Bacillus* spp.** [11] amylases, proteases, EPS, antimicrobial compounds	—	—
Yambol	***Bacillus* spp.** [11] insecticidal toxins, hydrolytic enzymes	—	—
Sliven	***Bacillus* spp.** [11] enzymes, EPS, antimicrobial metabolites	—	—

*Legend: Pazardzhik administrative province is potential archaeal hotspot, rich in *Crenarchaeota* and *Euryarchaeota* [18].

7. Biotechnological Potential and Applications

Microbial communities from mineral and thermal waters represent a valuable reservoir of functional biodiversity with significant potential for applications in medicine, industrial biotechnology, and environmental technologies. Their adaptive evolution under extreme physicochemical conditions has led to the development of robust metabolic pathways, thermostable enzymes, and diverse bioactive compounds, highly relevant for biotechnological exploitation.

7.1. Applications in Medicine and Pharmaceuticals

Bacillus species constitute one of the most important microbial groups in medical and pharmaceutical biotechnology due to their ability to produce a wide range of bioactive peptides with antimicrobial activity against multidrug-resistant pathogens [182,183]. Strains isolated from Bulgarian mineral waters: *Bacillus cereus* 36/GI6, 53/R1, 54/GI3, *B. thuringiensis* 37/PBGK, 56/H3, and *B. subtilis* 47/YA1, have been identified as promising bacteriocin producers with potential medical applications [59]. *B. subtilis* and B. *licheniformis* are widely applied in probiotic formulations due to their immunomodulatory effects [184,185]. Thermophilic genera *Geobacillus*, *Anoxybacillus*, *Brevibacillus*, *Thermus*, and *Thermotoga* are important in pharmaceutical biotechnology due to their production of thermostable enzymes, which retain structural stability under extreme conditions and are widely applied in diagnostics and molecular medicine [186]. Thermophilic bacilli also produce exopolysaccharides with cytoprotective, antiviral, anti-inflammatory, and immunomodulatory properties and generally low cytotoxicity, which makes them attractive candidates for therapeutic applications [31]. *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* synthesise secondary metabolites with antibacterial and antitumour activity, while their rhamnolipids are considered promising agents for drug delivery systems and enhancement of hydrophobic drug solubility [187]. However, the pathogenic nature of certain *P. aeruginosa* strains restricts their direct biomedical use.

Archaea represent an emerging source of biocatalysts with high structural stability under extreme conditions. Their enzymes are important for the development of stable drug carriers, pharmaceutical synthesis, and enzyme-based therapies [188]. Acid-stable enzymes from *Sulfolobus* spp. enable carbohydrate biotransformations at low pH [189], while archaeal ether-linked lipid membranes can be applied in the design of stable liposomal systems for vaccines and anticancer agents [190].

Cyanobacteria are also significant producers of pharmacologically relevant compounds with antioxidant, antiviral, and immunomodulatory properties. Genera *Synechococcus*, *Nostoc, Oscillatoria*, *Phormidium*, and *Chroococcus* produce cyclic peptides, lipopeptides, alkaloids, and exopolysaccharides with antimicrobial and cytotoxic effects. EPS-forming genera (*Gloeocapsa*, *Aphanothece*, *Chroococcus*) are interesting for hydrogel-based drug delivery and wound-healing applications. *Chlorogloeopsis* spp. contribute carotenoids and phycobiliproteins with anti-inflammatory potential [65,191–195].

7.2. Application in Industrial Biotechnology

Bulgarian mineral waters represent a significant reservoir of microorganisms with high biocatalytic potential for industrial biotechnology. Members of the family *Bacillaceae* isolated from these environments exhibit strong enzymatic capabilities, although most studies remain focused on activity screening rather than process development [11]. Species *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*, *B. vallismortis* and *B. pumilus* exhibit an exceptional capacity for the synthesis of industrially relevant enzymes. *B. thuringiensis* is widely employed in agri-biotechnology for the production of biocontrol agents for plant protection [196,197]. Cultivation of *B. amyloliquefaciens* M, isolated from a spring in the Haskovo administrative province, has revealed its optimal potential for the biotechnological production of plant protection products [80]. Thermophilic microorganisms from hot mineral springs in southwestern Bulgaria constitute an important resource for enzyme production under extreme conditions [11,113]. The team of Dimitrov identified thermally stable xylanases in two bacterial strains [110]. *Geobacillus stearothermophilus* is able to produce stable proteases, α-amylases and DNA polymerases active at 50-80 °C. Species of *Anoxybacillus*, *Thermus*, *Thermotoga* and *Caldicellulosiruptor* contribute key hydrolases which are widely applied in biomass conversion and waste processing [198,199].

Cyanobacteria and microalgae from Bulgarian mineral waters are increasingly recognised as sustainable “cell factories” for the production of proteins, vitamins, antioxidants and pigments. Experimental cultivation began in the Rupite area, and industrial-scale implementation started in 2012 in the village of Banovo (Varna). Until recently, an industrial facility in the village of Varvara

(Southern Bulgaria) produced biomass from *Chlorella* and the cyanobacterium *Arthrospira platensis*. Its metabolomic profile indicates that this organism represents a valuable source of bioactive compounds suitable for incorporation into dietary supplements [200–202]. It remains unclear whether the strain used was isolated from native Bulgarian sources or cultivated in the mineral waters of Varvara. Nonetheless, these developments demonstrate that both the natural diversity of Bulgarian mineral waters and existing biotechnological infrastructure provide favourable conditions for the discovery and exploitation of novel high-performance strains, highlighting their future potential in industrial biotechnology [203].

7.3. Application in Ecology and Bioremediation

Microorganisms from mineral waters play an essential role in environmental restoration and biogeochemical stability. Species of *Bacillus* and *Pseudomonas* are particularly important due to their metabolic versatility and ability to degrade complex organic pollutants. The strain *B. amyloliquefaciens* M, isolated from a mineral spring in the Haskovo administrative province, suppresses competing microflora and contributes to the ecological restoration of soil fertility [77]. *B. megaterium* facilitates phosphate metabolism of mineral salts, thereby enhancing phosphorus bioavailability. The broad enzymatic profile of *B. vallismortis* supports efficient biodegradation of organic waste, while the high adaptability of *B. pumilus* enables its application in bioremediation processes under extreme conditions [204]. *B. thuringiensis* is highly effective against soil phytopathogens [196,197]. Anaerobic strains *Caldicellulosiruptor*, isolated from hot springs in Rupite, may also have potential for efficient degradation of plant waste biomass [17]. *Pseudomonas fluorescens* produces biosurfactants that enhance hydrocarbon bioavailability and support pollutant degradation [205]. *Stenotrophomonas maltophilia* and *Klebsiella oxytoca*, contribute to the removal of toxic aromatic hydrocarbons and heavy metals [206–208]. Methanogenic archaea (*Methanosarcinales*) are important for biogas production and wastewater treatment systems [209].

Cyanobacteria and algae contribute to carbon sequestration and nutrient cycling, while EPS-producing taxa *Gloeocapsa* and *Leptolyngbya* play important roles in heavy metal biosorption [210–214]. Furthermore, nitrogen-fixing cyanobacteria (*Nostoc*, *Calothrix*) improve soil fertility through atmospheric N_2 conversion and soil crust formation [215,216]. The presence of specific microbial taxa in mineral springs may serve as an indicator of water quality. Genera such as *Closterium*, *Cosmarium*, *Spirogyra*, *Mougeotia* and *Zygnema* are particularly sensitive to pollution. Their occurrence is associated with high water quality, low nutrient concentrations and the absence of toxic contaminants, which makes them reliable bioindicators in hydrobiological studies. Representatives of the genus *Chara* contribute to the maintenance of stable and clean aquatic ecosystems by stabilising sediments and limiting nutrient resuspension. Among red algae, *Batrachospermum moniliforme*, *Hildenbrandia rivularis* and *Thorea hispida* are characteristic of clean, oxygen-rich waters and are used as indicators of high ecological status [217,218].

7.4. Application in Cosmeceutical and Biomedical Biotechnology

In addition to industrial and pharmaceutical biotechnology, microbial communities from Bulgarian mineral waters also represent an important source of bioactive compounds with applications in cosmetics and wellness-related therapies. This extends the biotechnological significance of these ecosystems towards high-value cosmeceutical development.

Mineral springs in Bulgaria have long been associated with balneotherapy and cosmetic use. However, recent microbiological studies show their hidden potential as sources of skin-active metabolites. Lipopeptides surfactin and iturin, produced by *Bacillus subtilis*, are widely applied in probiotic and anti-ageing formulations due to their antimicrobial activity and ability to suppress acne-associated skin bacteria [183]. Similarly, cell extracts of *Bacillus amyloliquefaciens* contain proteolytic and antioxidant enzymes with regenerative and anti-inflammatory properties, while proteases from *Bacillus licheniformis* are considered suitable for enzymatic exfoliation in cosmetic formulations [219,220].

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Bacillus sp. strains isolated from Bulgarian mineral waters also demonstrate adhesion to human epithelial cells and modulation of immune responses. The highest activity, characterised by moderately strong adhesion, was observed in the strain *Bacillus subtilis* 0-2, isolated from a thermal spring in the Haskovo Mineral Baths, with approximately 10 adherent bacterial cells per 10 epithelial cells. The induction of transforming growth factor β (TGF-β) and suppression of pro-inflammatory cytokine IL-8 reveals their potential immunomodulatory relevance in topical applications [11]. Thermophilic glycosylases from *Geobacillus stearothermophilus* and *Thermus thermophilus*, expand cosmetic applications through their role in oxidative stress protection and DNA repair mechanisms in skin cells [186,221,222].

The diverse biotechnological applications derived from mineral spring microorganisms are directly linked to their evolutionary adaptation to extreme environmental conditions. These adaptations not only drive metabolic versatility but also determine the possible production lines of bioactive compounds, enzymes, and structural biomolecules with industrial relevance. As summarised in Figure 5, the biotechnological potential of microorganisms from mineral springs is intrinsically rooted in the ecological and taxonomic structure of these systems. Rather than representing isolated functional traits, enzymatic activities, bioactive metabolite production, and environmental processes emerge as interconnected outcomes of microbial adaptation to extreme physicochemical conditions.

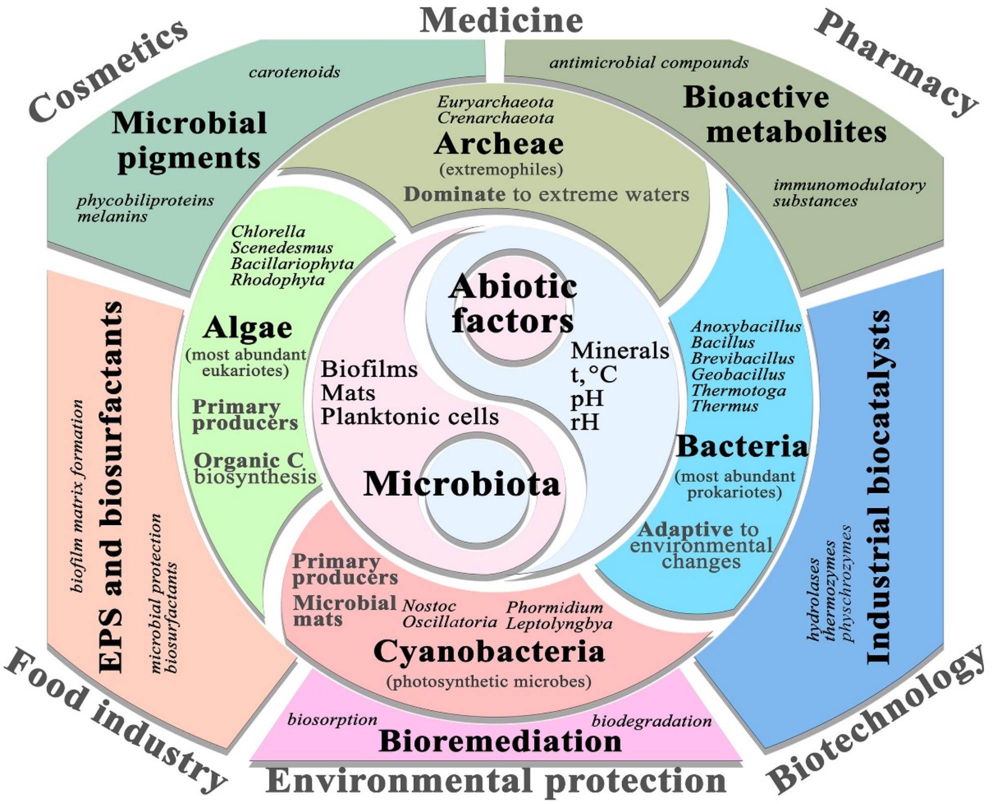

Figure 5. Functional microbial diversity and biotechnological potential of microorganisms from mineral springs.

8. Methodological Approaches in the Study of Microbial Communities Isolated from Mineral Waters

The study of microbial communities in mineral and thermal waters requires an integrated methodological framework, as culture-dependent approaches capture only a limited fraction of microbial diversity. A significant proportion of environmental microorganisms remains uncultivable under standard laboratory conditions, and form the so-called "microbial dark matter", which necessitates the use of molecular, omics-based, and imaging techniques to achieve a comprehensive ecological understanding [223–226].

Sampling represents a critical step that strongly influences downstream analyses. In mineral water ecosystems, sterile sampling techniques are required to prevent external contamination. Given the frequent presence of biofilms and microbial mats, it is essential to include not only water samples but also sediments and surface-associated communities. Preservation strategies to maintain the original microbial structure and prevent post-sampling community shifts include cooling, in situ filtration, and nucleic acid stabilisation [13,17,225]. Classical microbiological methods remain important for the isolation and physiological characterisation of cultivable strains. These include morphological analysis, Gram staining, growth profiling on selective media, and assessment of tolerance to pH, salinity, temperature, and mineral composition. Such approaches have enabled the isolation of numerous *Bacillaceae* strains with industrially relevant enzymatic activities from Bulgarian mineral waters [11,13,20]. MALDI-TOF mass spectrometry further enhances rapid identification and taxonomic validation of isolates based on protein fingerprinting and has been successfully applied in thermophilic systems from the Rupite region [25].

Modern molecular approaches provide deeper insights into microbial diversity and function. Amplicon sequencing of 16S and 18S rRNA genes enables taxonomic profiling and phylogenetic reconstruction without the need for cultivation [227]. These methods have revealed extensive diversity of thermophilic bacteria and archaea in South-Western Bulgarian geothermal systems [12,17,27,28,131]. Metagenomics allows the analysis of total community DNA and provides insights into functional gene pools and metabolic potential [228]. This is particularly valuable in extreme environments, where microbial communities exhibit diverse and specialized metabolic pathways. Complementary approaches metatranscriptomics and metaproteomics enable the assessment of gene expression and active metabolic processes and offer a functional perspective beyond genetic potential [229]. Spatial organisation and micro-scale interactions within biofilms and sediments are examined with fluorescence in situ hybridisation (FISH), which provides direct visualisation of microbial distribution and structure [230]. Finally, bioinformatic integration of multi-omics datasets enables taxonomic annotation, functional profiling, and ecological modelling. Network-based approaches and systems ecology tools facilitate the analysis of microbial interactions and prediction of community responses to environmental gradients such as temperature, mineralisation, and pH [231]. Together, these methodologies provide a robust framework for understanding microbial diversity and function in mineral water ecosystems.

9. Future Research Perspectives

Despite increasing interest in microbial diversity and functionality in mineral and thermal waters, many ecosystems in Bulgaria remain insufficiently studied. Existing data are often fragmented, spatially biased toward well-known geothermal zones, and limited by the predominance of culture-dependent methods. As a result, the full taxonomic and functional diversity of microbial communities, especially in cold and less accessible mineral springs, remains largely unresolved. One of the key challenges is the lack of systematic metagenomic and multi-omics surveys across different types of aquatic systems. Comparative studies between cold, mineral, and thermal waters are still scarce, although such approaches are essential to understand how temperature, mineralisation, and geochemical gradients shape microbial community structure and metabolic potential. Current evidence suggests that these environmental parameters are among the primary drivers of microbial selection and functional adaptation in extreme ecosystems [232–235]. Another important limitation is the insufficient integration of temporal dynamics into microbial studies. Seasonal variability, hydrological fluctuations, and anthropogenic impacts are rarely considered in a coordinated framework, despite their significant influence on microbial composition and activity. Long-term monitoring would therefore provide a more realistic understanding of ecosystem stability and resilience.

Future research should increasingly rely on integrated multi-omics approaches in combination with metagenomics, metatranscriptomics, metaproteomics, and metabolomics. Such strategies would enable a transition from descriptive microbiology toward functional ecosystem modelling. In addition, the integration of molecular data with physicochemical parameters would allow more precise identification of environmental drivers and shape microbial assemblages and their biogeochemical roles [236]. From an applied perspective, Bulgarian mineral waters represent an underexplored reservoir of novel enzymes, bioactive compounds, and microbial consortia with biotechnological potential. However, only a small proportion of the isolated and characterised microorganisms has reached patent protection or practical biotechnological use, which reveals a significant gap between scientific discovery and application []. Sustainable exploitation of these resources requires the establishment of local culture collections, standardised isolation protocols, and curated genomic databases. These infrastructures are essential to ensure reproducibility, biodiversity conservation, and efficient bioprospecting [238,239]. Future studies should focus on predictive ecological modelling and systems-level analysis of microbial interactions. Network-based approaches and ecological simulations could improve understanding of community stability, functional redundancy, and adaptation under environmental stress. From this perspective, Bulgarian mineral waters represent a valuable natural laboratory for the study of microbial evolution, ecosystem development, and biotechnological innovation under extreme conditions [240].

10. Conclusions

Bulgarian mineral waters represent unique limnological ecosystems that support diverse and highly specialised microbial communities shaped by the combined effects of geological conditions and stable physicochemical composition. Available studies are mainly concentrated in southern Bulgaria and demonstrate the presence of thermophilic and metabolically versatile microorganisms, with a strong predominance of the *Bacillaceae* family. *Bacillus* species produce a wide spectrum of bioactive metabolites, thermostable enzymes, antimicrobial peptides, and exopolysaccharides. *Geobacillus stearothermophilus*, *Anoxybacillus bogrovensis*, *Brevibacillus thermoruber*, and *Aeribacillus pallidus* are mainly studied for the production of thermostable hydrolases and exopolysaccharides with protective functions. In hot springs archaeal communities affiliated with *Crenarchaeota*, *Euryarchaeota*, and *Thaumarchaeota* participate in biomass transformation processes but remain poorly studied. Phototrophic communities are widely identified in southwestern Bulgaria. Dominant cyanobacteria include *Leptolyngbya*, *Oscillatoria*, *Chlorogloeopsis*, *Mastigocladus*, and *Nostoc*, which represent potential biomass producers. Microalgae *Navicula*, *Chlorella*, and *Cladophora* contribute to the synthesis of lipids, phenolic compounds, and antioxidant molecules. These microbial groups sustain major biogeochemical cycles and show a wide range of adaptations to thermal stress, oligotrophy, and high mineralisation. Despite significant progress, microbial diversity in Bulgarian mineral waters remains incompletely characterised, especially in cold systems, while archaeal ecology and microbial interactions require further investigation. Future research should prioritise integrated multi-omics approaches, long-term ecological monitoring, and cultivation-independent methods to enable systematic discovery of novel microbial resources and a deeper insight into ecosystem interactions.

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Abbreviations

The following abbreviations are used in this manuscript:

DNA	Desoxyribonucleic acid
EPS	Extracellular polymeric substances
FISH	Fluorescence In Situ Hybridisation
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight
TDS	Total dissolved solids

Appendix A. Low-Mineralised Waters with TDS ≤ 2 g/L

Appendix A.1. Pure Thermal Acratotherms

Table A1. Hyperthermal acratotherms.

Field (area*)	t, °C	TDS, mg/l	pH	Hardiness, °H
Drill R-1x (Va)	52	620	7.2	12.0
Chiflik (L)	51	305	7.6	2.0
Asparuhovo (Va)	50	611	7.8	11.0
Pancharevo (So-c)	48	495	7.1	16.7
Druzhba (Va)	46	592	7.2	13.0
Gorni Bogrov (So-c)	46	1,288	7.2	1.5
Starozagorski bani (SZ)	42	498	6.9	16.5

* Administrative areas in Bulgaria: Bl – Blagoevgrad; Bu – Burgas; D – Dobrich; G – Gabrovo; H – Haskovo; Ka – Kardzhali; Kyu – Kyustendil; L – Lovech; M – Montana; Pa – Pazardzhik; Pe – Pernik; Pl – Pleven; Pd – Plovdiv; Ra – Razgrad; Ru – Ruse; Sh –Shumen; Sl – Sliven; Sm – Smolyan; So – Sofia; So-c – Sofia city; SZ – Stara Zagora; T – Targovishte; Va – Varna; Vn – Vidin; Vr – Vratsa; VT – Veliko Tarnovo; Y – Yambol.

Table A2. Isothermal acratotherms.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H
Vyrshetz (M)	37	194	8.7	0.3
Devin – non Rn (Sm)	37	320	9.4	0.5
Topoli (Va)	37	643	7.2	16.5
Salmanovo (Sh)	34	1331	7.3	42.0
Tuzlata (D)	33	659	7.8	15.4
Voyvodinovo (Pd)	32	500	8.3	2.1
Dzhebel (Ka)	32	574	6.9	17.0
Topolyane (SZ)	32	1,381	7.7	4.0

Table A3. Hypothermal acratotherms.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H
Knyazhevo (So-c)	31	119	9.4	0.4
Spanchevtsi (M)	31	175	8.8	1.2
Golden beaches (Va)	31	560	7.5	15.0
Lozenets (So-c)	31	301	7.8	4.3
Dolni Rakovets (Pe)	31	462	7.4	12.0
Albena (D)	30	614	7.4	17.0
Kavarna (D)	30	555	7.4	12.2
Ivanyane (So-c)	28	266	9.2	0.5
Kostinbrod (So)	28	1,569	8.0	2.9
Belotintsi (M)	28	904	7.9	5.3

Slatina (M)	27	256	8.3	1.8
Targovishte (T)	27	592	8.0	2.0
Bozhurishte (So)	27	439	7.8	5.0
Razgrad (R)	26	607	7.3	17.2
Varbina (Sm)	25	239	7.6	5.7
Kranevo (D)	24	547	7.6	7.6
Provadiya – Ereka (Va)	24	710	7.4	10.3
Nevestino – drill 2 (Kyu)	24	668	7.2	16.2
Belovo (Pa)	24	551	7.0	16.0
Bansko (Bl)	22	202	7.5	6.2
Golden Panega (L)	22	587	7.3	20.0
Balchik (D)	21	622	7.5	8.0
Kirkovo (Ka)	21	291	7.5	8.0
Musomishta (Bl)	20	355	7.4	11.4
Tran (Pe)	20	417	7.3	12.0
Vlahovo (Sm)	20	288	7.4	9.7

Appendix A2. Siliceous, Fluoride and Siliceous-Fluoride Low-Mineralised Waters

Table A4. Siliceous mineral waters.

Field (area)	**t, °C**	**TDS, mg/l**	**pH**	**Hardiness, °H**	**H_2SiO_3 (mg/l)**
Aquarium (Va)	20	673	7.8	1.4	63
Zheleznitsa (So-c)	31	278	9.5	0.3	54
Kazichene (So-c)	60	357	8.6	0.4	53
Gorna banya (So-c)	42	149	9.7	0.3	52
Ravno pole (So)	52	730	7.8	3.7	52
Svetovrachene (So-c)	46	1,208	8.2	0.7	50
Elin Pelin (So)	43	868	7.5	3.0	48
Krichim (Pd)	28	788	7.3	23.0	48
Radomirtsi (Pl)	20	1,832	7.4	56.0	45

Table A5. Fluoride mineral waters.

Field (area*)	**t, °C**	**TDS, mg/l**	**pH**	**Hardiness, °H**	**F^- (mg/l)**
Yakoruda (Bl)	42	637	8.2	1.5	14.0
Gorna Breznitsa (Bl)	37	558	8.6	0.5	11.3
Poibrene (Pa)	42	856	9.4	2.6	10.6
Kromidovo (Bl)	47	610	8.9	0.4	9.6
Slivek (L)	12	1,289	8.8	0.8	8.4
Nevestino – drill 1 (Kyu)	29	777	9.5	0.3	7.6
Garmen (Bl)	9	197	9.1	0.5	5.6
Sadievo (Bu)	29	351	9.6	0.2	5.5
Gotsedelchevski bani (Bl)	43	222	9.1	0.4	5.3
Dobroslavtsi (So-c)	40	1,360	7.9	0.8	5.2
Tryavna (G)	14	1,052	7.5	9.0	5.2
Hotovo (Bl)	37	449	8.6	0.9	5.2
Dupnitsa (Kyu)	33	352	9.5	0.3	5.0
Momina salza – Hisarya (Pd)	41	234	9.3	0.5	5.0
Zheravna (Sl)	13	818	7.9	1.6	5.0
Trakiya (Pd)	28	843	7.8	2.0	5.0
Banichan (Bl)	19	308	7.5	1.3	4.8
Pchelinovo (SZ)	24	1,312	8.1	1.2	4.0
Topolitsa (Bu)	19	443	9.0	0.4	3.9

Dimitrovche (H)	46	249	9.2	0.6	3.9
Asenovgrad (Pd)	39	1,407	7.6	14.0	3.5
Bogatovo (G)	8	1,537	8.4	1.8	3.0
Preslav (Sh)	20	831	7.6	11.0	3.0
Dyulevo (Bu)	20	511	8.2	1.0	2.7
Aleksandrovo (Va)	18	973	9.2	0.8	2.6
Starosel (Pd)	20	766	8.3	2.9	2.5
Svoboda (So-c)	51	1,874	8.0	2.3	2.4
Dolno Botevo (H)	20	485	8.2	1.9	2.3
Tsarevtsi (Va)	42	1,687	7.8	10.3	2.1
Shipkovo – drill L-1 (L)	33	950	7.4	35.4	2.0
Ovcha kupel (So-c)	32	1,188	7.0	29.0	2.0
Burgas (Bu)	26	1,309	7.6	4.7	2.0
Vardun (T)	12	642	7.6	7.7	2.0
Bachevo (Bl)	26	222	8.7	1.3	1.8
Bolyarovo (Y)	18	1,591	7.3	37.0	1.8

Table A6. Siliceous-fluoride mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	F^- (mg/l)	H_2SiO_3 (mg/l)
Banya Korten (Sl)	41-57	502	7.9	1.8	24.0	83
Blagoevgrad – west (Bl)	60	678	9.1	0.3	13.0	72
Gradeshnitsa (Vr)	68	548	8.6	0.6	11.2	83
Simitli (Bl)	62	599	8.8	0.6	10.8	74
Draginovo – Velingrad (Pa)	94	648	8.2	1.2	10.4	74
Banya – Karlovo (Pd)	35-46	348	9.2	0.5	9.0	55
Banya (Pd)	41	604	9.9	0.3	9.0	94
Eleshnitsa (Bl)	56	302	9.7	0.2	8.7	58
Kamenitsa – Velingrad (Pa)	90	551	8.3	0.7	8.6	91
Dolno Osenovo (Bl)	55	560	9.0	0.7	8.5	81
Marikostinovo (Bl)	63	1,031	7.3	4.9	8.4	77
Aytos (Bu)	44	441	9.9	0.3	8.0	58
Stoletovo (Pd)	32	217	9.7	0.6	7.8	47
Straldzha (Y)	77	1,105	8.9	3.6	7.0	87
Varvara – Vetrendol (Pa)	90	773	8.3	1.8	6.5	102
Bedenski bani (Sm)	76	1,626	6.6	10.0	6.5	92
Kostandovo (Pa)	30	387	9.5	0.3	6.5	70
Levunovo (Bl)	85	911	7.6	1.9	6.5	110
Sandanski (Bl)	81	653	7.7	1.7	6.1	115
Posestra (Sm)	40	955	7.2	7.5	5.6	96
Ladzhene – Velingrad (Pa)	60	317	9.2	0.3	5.5	63
Belchinski bani (So)	40	312	9.5	0,3	5.0	50
Bani (Ladzha) (Pa)	41	913	7.1	4,8	5.0	69
Pesnopoy (Pd)	42	275	8.6	0.3	5.0	53
Erma reka (Sm)	76-90	1,078	7.6	7.7	4.3	160
Banya – Panagyurishte (Pa)	38-42	635	7.7	6.5	3.8	49
Pravdino (Y)	20	406	10.0	0.5	3.7	53
Panagyurishte (Pa)	48	578	7.6	6.4	3.5	60
Medovo (Bu)	29	545	9.7	0.1	3.3	88
Staro Zhelezhare (Pd)	30	299	8.3	1.2	3.2	54
Lakatska Gabrovnitsa (So)	28	975	7.9	9.2	3.1	50
Barziya (M)	32	271	9.4	0.7	2.5	80
Bankya (So-c)	37	280	9.6	0.4	1.7	48
Sofia – center (So-c)	46	302	9.4	0.2	1.6	46

Appendix A3. Radon Low-Mineralised Waters

Table A7. Pure radon mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	Radon, nCi/l
Rozino (Pd)	18	340	7.6	8.5	20-30
Rudartsi (Pe)	28	300	9.1	0.9	5,6
Granitovo (Y)	16	1,723	6.7	42.0	4,5

Table A8. Radon-fluoride mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	Radon, nCi/l)	Fluoride (mg/l)
Klisura (Pd)	20	328	7.8	8.5	26.0	1.6
Momina banya – Hisarya (Pd)	46	254	8.9	0.6	16.4	4.5
Eleshnitsa (Bl)	38	199	9.2	0.6	9.7	5.8
Simeonovgrad (H)	23	1,438	7.5	6.8	9.6	10.0
Hrabrino (Pd)	21	1,513	8.2	1.6	4.9	6.5
Devin – radone (Sm)	44	223	9.4	0.3	4.3	4.6

Table A9. Radon-siliceous mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	Rn, nCi/l)	H_2SiO_3 (mg/l)
Bratsigovo (Pa)	27	466	7.2	3.4	5.7	81

Table A10. Radon-fluoride-siliceous mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	Radon, nCi/l)	F^-, (mg/l)	H_2SiO_3 (mg/l)
Narechenski bani (Pd)	20-33	1,558	7.3	15.4	110.0	6.0	84
Momin prohod (So)	65	964	7.7	5.8	56.0	10.0	87
Dolna banya (So)	64	623	9.0	1.5	27.0	12.4	47
Strelcha (Pa)	36-56	288	8.8	0.8	22.0	3.8	50
Oshtava (Bl)	58	402	8.6	0.7	15.0	14.0	46
Kliment (Pd)	29	277	8.3	0.9	14.5	5.0	55
Pchelinski bani (So)	73	921	8.0	4.3	12.0	7.9	95
Rakitovo (Pa)	50	335	9.6	0.2	11.0	5.1	62
Pavel banya (SZ)	35-61	650	7.6	1.7	10.0	16.0	59
Chepino – Velingrad (Pa)	48	187	9.2	0.3	10.0	4.2	48
Ovoshtnik (SZ)	45	502	8.4	0.5	9.0	24.0	49
Drill 1 – Hisarya (Pd)	46	263	9.0	0.5	9.0	5.0	62
Dobrinishte (Bl)	43	309	9.0	0.3	7.2	6.3	67
Miromir (Pd)	37	323	8.8	0.6	7.0	3.9	57
Banya (Pd)	57	327	9.4	0.3	4.5	8.0	53
Haskovski bani (H)	56	1,571	6.9	24.8	4.4	4.1	76
Yagoda (SZ)	41	625	8.6	0.8	4.3	16.0	107
Krasnovo (Pd)	55	324	9.1	0.6	4.2	4.3	67

Appendix A4. Sulphide, Low-Mineralised Waters

Table A11. Pure sulphide mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	S^{2-}, mg/l
Krapets (D)	33	1,821	7.2	18	25
Rusalka (D)	32	1,225	7.5	14	21

Voneshta voda (VT)	13	1,275	6.7	30	11
Varbitsa (Sh)	20	1,378	7.0	22	9
Beronovo (Bu)	13	582	7.3	14	9

Table A12. Sulphide-fluoride mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	S^{2-}, mg/l	F^-, mg/l
Enchevtsi (G)	18	1,293	8.6	1.4	11.0	9.6
Plachkovtsi (G)	18	1,389	7.7	0.9	10.7	14.2
Dragoevo (Sh)	18	1,503	8.8	0.8	10.0	8.1

Table A13. Sulphide-fluoride-siliceous mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	S^{2-}, mg/l	F^-, mg/l	H_2SiO_3 (mg/l)
Sapareva banya (Kyu)	57-103	0.6	9.4	707	15.8	17.0	66
Kyustendil (Kyu)	73	0.2	8.9	678	9.0	7.6	80
Blagoevgrad (Bl)	56	1.4	8.1	678	9.0	13.0	66

Appendix A5. Carbonated, Low-Mineralised Waters

Table A14. Carbonated mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	H_2SiO_3 (mg/l)	CO_2, mg/l
Stefan Karadzhovo (Y)	21	1,473	6.4	40	26	515
Pravo bardo (Pe)	19	1,671	6.3	50	73	665

Appendix B. High-Mineralised Waters with TDS > 2 g/L

Appendix B.1. Hydrocarbonate Mineral Waters

Table A14. Hydrocarbonate-sodium mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	HCO_3^-, mg/l	Na^+, mg/l
Kurilo – Novi Iskar (So-c)	30	6,189	8.6	5.2	3,562	1,738
Al. Boykov – Novi Iskar (So-c)	40	3,110	7.7	3.7	2,001	814
Golyama fucha (Kyu)	22	2,210	7.7	2.3	1,538	582

Table A15. Hydrocarbonate-fluoride mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	HCO_3^-, mg/l	F^-, mg/l	Na^+, mg/l
Gnilyane – Novi Iskar (So-c)	43	3,254	7.8	2.5	1,904	3.5	870
Trebich (So-c)	51	3,822	7.1	11.0	1,831	3.8	1,023
Chepintsi (So-c)	52	2,890	8.0	1.9	1,347	2.2	8,149
Byalata voda (Pe)	26	2,522	6.9	10.4	1,294	3.8	651

Slivenski bani (Sl)	48	1,986	6.8	20.8	860	4.2	423

Table A16. Hydrocarbonate-fluoride-siliceous mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	HCO_3^-, mg/l	F^-, mg/l	Na^+, mg/l	H_2SiO_3 (mg/l)
General Todorov (Bl)	76	2,134	6.8	7.2	1,321	6.5	526	71

Appendix B.2. Sulphate Mineral Waters

Table A17. Pure sulphate mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	SO_4^{2-}, mg/l	Na^+, mg/l	Ca^{2+}, mg/l	Mg^{2+}, mg/l
Deventsi (Pl)	20	5,928	8.0	201	3,542	444	213	741
Dlagnevo (H)	20	3,073	6.6	121	1,279	253	618	150
General Inzovo (Y)	20	2,847	7.0	70	1,121	406	326	109

Table A18. Sulphate-siliceous mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	SO_4^{2-}, mg/l	H_2SiO_3, mg/l	Na^+, mg/l	Ca^{2+}, mg/l	Mg^{2+}, mg/l
Balgarene (L)	12	5,981	7.8	152	3,705	208	742	343	453

Table A19. Sulphate-fluoride mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	SO_4^{2-}, mg/l	F^-, mg/l	Na^+, mg/l	Ca^{2+}, mg/l	Mg^{2+}, mg/l
Harmanli (H)	20	4,414	7.6	13.7	1,866	10.0	1,366	388	172
Shipkovo (L)	19	2,476	7.2	9.8	1,576	1.9	31	489	120
Nova Varbovka (VT)	20	3,098	7.5	109	1,236	1.8	121	224	336
Polski Trambesh (VT)	46	2,476	7.8	70	1,119	3.2	870	301	120

Table A20. Sulphate-iron mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	SO_4^{2-}, mg/l	Fe^{2+}, mg/l	Na^+, mg/l	Mn^{2+}, mg/l	Ca^{2+}, mg/l	Mg^{2+}, mg/l
Breznik (Pe)	12	4,458-8,211	2.9-3.5	76-158	3,206-5,683	172-886	497-509	24-132	434-529	80-362

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Gorno uyno (Kyu)	20	2,241	2.6	23	1,514	413	53	4	99	41

Appendix B.3. Chloride (Chloride-Sodium) Mineral Waters

Table A21. Pure chloride-sodium mineral waters.

Field (area)	**t, °C**	**TDS, mg/l**	**pH**	**Hardiness, °H**	**Cl^-, mg/l**	**Na^+, mg/l**
Mirovo (So)	20	318,243	6.8	249	189,739	122,879
Dolni Dabnik (Pl)	60	121,065	6.2	1,086	734,482	39,139
Kozloduy (Vr)	37	74,994	7.6	424	43,349	25,959
Gomotartsi (Vn)	67	56,618	6.5	433	33,593	18,814
Slanchev bryag (Bu)	33	15,003	7.5	119	9,019	4,150
Varbitsa (Sh)	20	13,105	7.7	70	7,927	4,581
Dolno Ezerovo (Bu)	20	9533	9,7	70	5264	3147
Gradets (Sl)	32	4,361	7.3	28.6	2,328	1,421

Table A22. Iodine-bromine chloride-sodium mineral waters.

Field (area)	**t, °C**	**TDS, mg/l**	**pH**	**Hardiness, °H**	**Cl^-, mg/l**	**Na^+, mg/l**	**I^-, mg/l**	**Br^-, mg/l**
Staro Oryahovo (Va)	30	17,257	7.6	54	9,923	6,214	39	53
Shkorpilovtsi (Va)	30	14,483	7.4	29	8,137	5,628	27	70
Dolni Chiflik (Va)	29	12,180	7.6	16	6,552	4,417	21	35
Novo Oryahovo (Va)	62	9,841	7.6	17	4,908	3,443	15	7.5

Appendix B.4. Mixed Type Mineral Waters

Table A23. Sulphide-chloride (sulphate, iodine) mineral waters.

Field (area)	**t, °C**	**TDS, mg/l**	**pH**	**Hardiness, °H**	**H_2S, mg/l**	**SO_4^{2-}, mg/l**	**Cl^-, mg/l**	**Na^+, mg/l**	**Ca^{2+}, mg/l**	**I^-, mg/l**
Dalgodeltsi (M)	90	36,067	6.4	36,067	418	-	20,519	12,390	-	-
Slanotran (Vn)	40	57,544	6.3	57,544	50	-	33,741	18,867	-	-
Vidin (Vn)	46	57,346	6.8	57,346	44	-	33,172	18,518	-	-
Koshava (Vn)	30	53,828	6.8	53,828	18	-	31,524	17,630	-	-
Kaylaka (Pl)	64	18,583	7.2	18,583	15	-	9,519	5,696	-	-
Slavyanovo (Pl)	47	13,838	7.9	13,838	21	2,100	6,383	3,954	794	-
Shabla (D)	40	3,539	7.0	3,539	35	-	1,630	1,091	-	3.0
Zamfirovo (M)	44	2,643	8.6	2,643	30	-	1,182	922	-	-

Table A24. Sulphide-hydrocarbonate mineral waters.

Field (area)	**t, °C**	**TDS, mg/l**	**pH**	**Hardiness, °H**	**S^{2-}, mg/l**	**HCO_3^{2-}, mg/l**	**Na^+, mg/l**	**H_2SiO_3, mg/l**
Birimirtsi (So-c)	28	3,232	7.5	7.7	15	1,892	865	48

Table A25. Carbonated-hydrocarbonate mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	CO_2, mg/l	HCO_3^{2-}, mg/l	F^-, mg/l	SO_4^{2-}, mg/l	Na^+, mg/l	Ca^{2+}, mg/l	H_2SiO_3, mg/l
Mihalkovo (Sm)	26	3,406	6.1	52	1,150	1,977	3.2	-	607	297	91
Kurilo 2 – Novi Iskar (So-c)	30	6,190	6.5	5.2	658	3,562	-	-	1,738	-	-
Iliyantsi (So-c)	45	4,537	6.6	18.0	505	1,975	2.8	1,054	1,191	-	-
Mramor (So-c)	45	3,371	6.8	15.6	469	2,415	-	-	2,415	-	66

Table A26. Hydrocarbonate-sulphate-arsenic mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	HCO_3^{2-}, mg/l	As^{3+}, mg/l	SO_4^{2-}, mg/l	Na^+, mg/l	H_2SiO_3, mg/l	F^-, mg/l
Merichleri (H)	44.5	6,328	6.9	8	2,075	0.7	1,585	1,894	51	5.5

Table A27. Sulphate-chloride mineral waters.

Field (area)	t, °C	TDS, mg/l	pH	Hardiness, °H	Cl^-, mg/l	SO_4^{2-}, mg/l	Na^+, mg/l	Ca^{2+}, mg/l	Mg^{2+}, mg/l	H_2SiO_3, mg/l	F^-, mg/l
Krushuna (L)	56	11,404	7.9	151	2,053	5,040	3,103	706	229	-	4.0
Boyanovo (Y)	12	11,271	7.3	273	3,499	3,797	2,004	885	647	-	-
Marash (Sh)	67	8,036	7.4	102	3,678	1,153	2,207	560	103	-	-
Ovcha Mogila (VT)	45	4,557	7.9	76	1,585	1,177	1,064	344	123	-	2.3
Obedinenie (VT)	43	4,439	7.9	94	1,333	1,434	862	392	169	-	3.5
Resen (VT)	55	4,523	7.8	98	1,249	1,608	842	447	154	49	2.0
Svishtov (VT)	48	2,923	7.2	53	954	733	625	231	100	75	2.0
Bobov dol (Kyu)	65	2,085	7.6	13	427	797	586	-	-	-	7.6
Hayredin (Vr)	30	2,844	7.9	5,8	776	426	914	-	-	-	2.3
Pomorie (Bu)	30	2,275	8.3	54	729	170	357	-	225	-	7.0

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