ANTHROPOGENIC IMPACT ON THE KARST AQUIFER QUALITY: PRELIMINARY RESULTS FROM DAMIȘ – PONORAȘ KARST AREA, ROMANIA

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Abstract. Karst aquifers are crucial water resources but are highly vulnerable due to their geological fragility and anthropogenic pressures. This study assesses water quality in the Damiș-Ponoraș Karst Area (DPKA), where the expansion of the drinking water network has occurred without proper sewerage systems. The investigation focused on the Dămișenilor Karst System (DKS) and included, for comparison, the Ponoraș Karst System and three other springs from the Brătcuței Valley that also collect discharge from the DPKA resurgences. In total, twelve water sources were sampled seasonally from 2023 to 2025, with analysis of physicochemical parameters, microbial content, and aquatic invertebrate assemblages. The geological structure of the DPKA shapes distinct physical and chemical properties among springs, ponors, and resurgences, as well as within its drainage systems. While all waters exhibit typical karst characteristics, including high alkalinity, conductivity, dissolved solids, and hardness, DKS sites show specific variations: springs had lower pH, calcium hardness and conductivity, ponors showed higher turbidity and lower redox potential, suggesting pollutant susceptibility, while the increased magnesium hardness at resurgence indicates flow through dolomitic strata. Measured concentrations of nitrates, nitrites, ammonia, and phosphates were low and complied with drinking water quality standards, but widespread microbial contamination with total mesophilic bacteria, *Klebsiella oxytoca, Pseudomonas aeruginosa,* and *Escherichia coli* was present. In the DKS resurgence, anthropogenic pressures, mainly bacterial contamination from domestic wastewater, are linked to reduced invertebrate richness and abundance, indicating declining ecological quality. A concurrent, gradual decrease in microbial and invertebrate abundance along the karst hydrological continuum, from springs to ponors and resurgence points, suggests the influence of poorly understood subsurface processes. These results highlight that ongoing human impacts may threaten aquifer water quality and ecological stability, underscoring the need for integrated monitoring using abiotic and biotic indicators to support timely management actions.

Key words: karst drainage systems, springs, ponors, resurgences, water physical-chemical profile, microbial content, aquatic invertebrates, anthropic impact.

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1. INTRODUCTION

Karst is a complex environment with a diversity of landforms and a special hydrology that develops mainly underground. In spite of the high heterogeneity of each karst unit, karst hydrology follows few general rules: the conduit network structure and groundwater flow conditions (BAKALOWICZ, 2005). Karst aquifers, developed in carbonate rocks, contain precious groundwater resources that can be exploited as a water supply (BAKALOWICZ, 2005). However, this resource, which accounts for approximately 25% (PARISE and GUNN, 2007) – 30% (DANIELOPOL *et al.*, 2003) of the global potable water supply, is highly susceptible to anthropogenic influences and environmental variations. The pronounced vulnerability of karst aquifers arises from the direct hydraulic connectivity between surface and subsurface systems, the rapid groundwater movement through conduit networks, and their limited natural attenuation capacity (PARISE *et al.*, 2015). The limited availability of surface water, caused by the rapid infiltration of precipitation into the subsurface, makes the karst aquifer an indispensable resource for local communities, while simultaneously rendering them highly vulnerable to degradation and contamination. Due to the influence of numerous natural factors, such as geological characteristics (rock types, soil mineralogy), hydrological conditions (residence time, flow paths), and climatic variables, as well as anthropogenic activities, the chemical equilibria within karst aquifers are highly dynamic. Consequently, physical-chemical analyses are essential for determining water suitability for various purposes (BOYD, 2020). Furthermore, since physical-chemical alterations directly affect the biological components of aquatic ecosystems, integrating ecological criteria into groundwater quality assessment and long-term monitoring is recommended, in alignment with methodologies traditionally employed for surface water evaluations. (CAIRNS, 1993; DANIELOPOL and GRIEBLER, 2008; STEIN *et al.*, 2009). By integration of abiotic and biotic indicators (microbial and water invertebrates), and monitoring their temporal trends, it is possible to detect early warning signals of system degradation and identify the underlying causes of existing impacts (CAIRNS, 1993). Because the restoration of karst aquifers to their original pristine condition is highly challenging, understanding the specific vulnerabilities of each karst system is crucial for the sustainable management of karst water resources.

In Romania, karst aquifers constitute a critical source of potable water for numerous rural communities, primarily through springs, wells, and other natural outlets in mountainous and plateau karst regions (MOLDOVAN *et al.*, 2022). Although many karst springs demonstrate favorable chemical characteristics (EPURE and BORDA, 2014), low heavy metal concentrations and moderate mineralization (HOAGHIA, 2021; MOLDOVAN *et al.*, 2022), elevated nitrogen compound levels persist as a recurring concern, particularly in agriculturally intensive or rural areas (BORDA *et al.*, 2019, 2022; MOLDOVAN *et al.*, 2020).

More concerning, karst aquifers exhibit a high susceptibility to microbiological contamination originating from surface sources, particularly due to manure-derived fertilizer runoff, inadequately treated sewage, and animal fecal matter (EPURE and BORDA 2014; BORDA *et al.*, 2019, 2022, 2024). Surface-derived microorganisms can infiltrate rapidly, with limited natural attenuation. Indicator organisms such as *E. coli* and coliforms are commonly detected, and pathogens may also occur, particularly following high-recharge events.

The purpose of this study is to address an integrative ecological assessment of the aquifer quality in the Damiș Karst System (DKS), consequent to the extension of the drinking water network in the absence of a proper sewerage system for Damiș hamlet. The DKS represents an appropriate model for study, because it is a well-documented geomorphological (RUSU, 1973, 1978, 1981, 1988) and hydrogeological (ORĂȘEANU, 1991, 2010) karst system, has a high level of karstification, and is relatively small and clearly defined. These features allow the efficient evaluation of the impact of additional water inputs within the depression, where, in the absence of sewerage infrastructure, water is recirculated via natural drainage pathways. The research constitutes a long-term investigation, and the present work discusses the findings from the first two years of monitoring. The physical-chemical water profile, microbial content, and aquatic invertebrate dynamics were analyzed to address the following research questions: 1) What is the geochemical signature characterizing the drainage systems within Damiș-Ponoraș Depression karst area? 2) What are the dominant pollution patterns in the Damiș Karst System? 3) How do human activities influence the water quality and ecological integrity of the karst aquifers in this region?

2. MATERIALS AND METHODS

2.1. STUDY AREA

The research was held in the Damiş karst depression from Pădurea Craiului Mountains, Romania. It is part of the Damiş-Ponoraş karst area that is located in the northeastern sector of the Pădurea Craiului Mountains, south of the village of Bratca. The depression is bounded by the Mişid Valley to the west and the Brătcuţa Valley (BV) to the east, encompassing two karstic catchment basins: Damiş and Ponoraş (RUSU, 1988). Both surface and subterranean drainage networks define two distinct karst systems, Damiş Karst System (DKS) and Ponoraş Karst System (PKS). Within the Damiş system, surface waters are absorbed through the Toaia sinkhole (the sample site S2), which functions as an intermittent hydrographic conduit, or through dolines that collect and infiltrate precipitation. These waters re-emerge at the Izbucul Dămişenilor resurgence (the sample site S6), located on the left slope of the Brătcuţa Valley (LSBV). The Ponoraş karst system, whose principal collector in area is the Ponoraş Cave, discharges its waters through the Izbucul Brătcanilor resurgence (the sample site S9), situated approximately 2 km downstream from the Dămişenilor resurgence. The two karst systems are morphologically separated by a ridge oriented northeast–southwest, marked by the elevations of Glimeia and Ouaşul Hill. There are also existing faults with the same orientation that separate the two underground hydrogeological systems. The surface watershed dividing these systems corresponds to the subsurface hydrological boundary, indicating a close correlation between surface topography and subterranean drainage organization (Fig. 1).

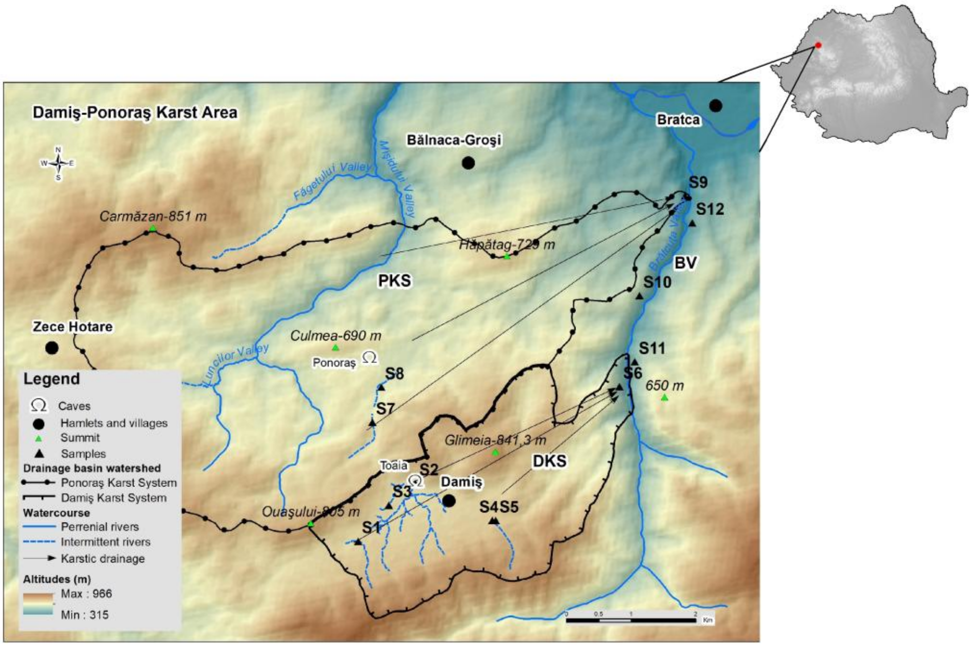

Figure 1. Study area showing the delineation of the Damiș Karst Systems (DKS), Ponoraș Karst System (PKS), and Brătcuței Valley (BV) zones, along with the locations of the sampling sites (S1–S12) .

2.1.1 Geological background

The existence of the Damiș karst depression is a consequence of the geological structure and the succession of strata that develop in the Damiș-Ponoraș karst area from Pădurea Craiului Mountains (Fig. 2). In the southern part of the area, the oldest formations are the metamorphic rocks of the Someș Series, represented by mica schists containing tourmaline, garnet, and biotite. The sedimentary rocks begin in the Permian with conglomerates composed of metamorphic rock fragments, bound by a reddish sandy-clayey matrix, with thicknesses of approximately 100 meters. These are followed, unconformably, by Early Triassic detrital sediments, represented by quartzitic conglomerates, yellowish sandstones, siltstones, or reddish-violet to greenish clay schists. Next is a thick package of black platy limestones with dolomite intercalations, which can total over 300 meters in thickness. The upper part of the Triassic is represented by massive, yellowish-white or pink marbled limestones, with grey dolomites at the base, which can also reach up to 300 meters thick. At the end of the Triassic a general emergence is produced, along with a paleo relief on which limestone breccias with a red clayey binder sometimes accumulate. The Early Jurassic (the Lias) begins transgressively with red detrital deposits at the base, followed by white or grey deposits, which in the upper part show limestone or marl intercalations. These dominate the Middle Jurassic (the Dogger), which is characterized by a reduced thickness due to sediment condensation and several unconformities. The Late Jurassic (the Malm) is represented exclusively by stratified limestones, that is grey to dark-grey with siliceous inclusions in the lower part, and white, massive, reef-like in the upper part. At the end of the Jurassic, another emergence takes place, during which a low-altitude karst plateau relief develops, with characteristic paleo dolines (Pop and Mârza, 1977; Cociuba, 1999). The Cretaceous begins with bauxite deposits accumulated in these paleo dolines, followed by a few meters of bituminous black limestones containing characeae, ostracods, and gastropods from freshwater and brackish environments. These are overlain by several hundred meters of marine limestones of Barremian age, containing pachyodonts, foraminifera, and algae.

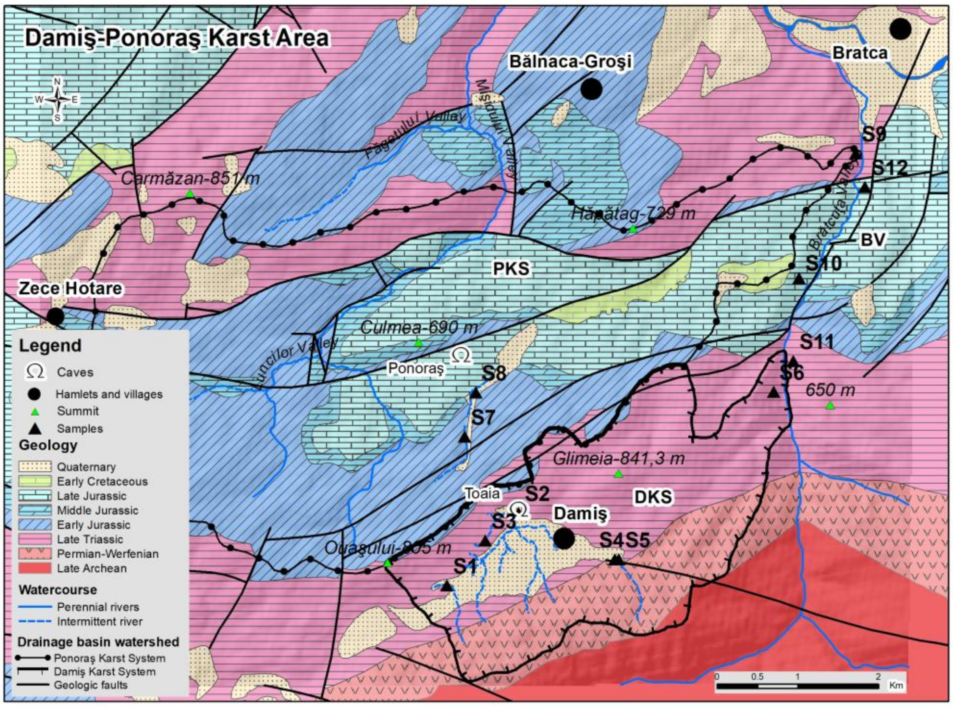

Figure 2. The geology map of the Damiş-Ponoraş Karst Depressions (after Patrulius *et al*., 1973, modified and simplified map section).

It is worth noting that the entire area of DKS develops on Triassic formations. In the south, Permian and Early Triassic detrital formations occur, which are generally impermeable and feature a network of short watercourses flowing northward toward the Damiş Depression. Along this path, the waters encounter Middle and Late Triassic limestone, which allows their infiltration underground, re-emerging at the surface at the Izbucul Damişenilor Resurgence in the Brătcuța Valley, a tributary of the Crişul Repede River. By following the morphology of the relief westward, it becomes evident that the watercourses in the Damiş area initially flowed westward, acting as tributaries, via the Roșia Valley, of the Crişul Negru River (Rusu, 1973). However, the presence of Mesozoic limestone levels in the Damiş area enabled the waters to infiltrate underground and be captured by the Crişul Repede, due to its lower base level of erosion and its geographical proximity. This sequence of events ultimately led to the formation of the DKS.

2.1.2 Hydrogeological background

From a karstological point of view, the stratigraphic column described above can be grouped into generally impermeable non-karstifiable detrital levels and karstifiable limestone levels. The Permian and the base of the Triassic form the basal impermeable layer, followed by a Triassic carbonate sequence. The sequence of detrital calcareous-marly sediments from the Early and Middle Jurassic forms the impermeable layer that separates the underlying Triassic limestone level from the overlying Jurassic-Cretaceous limestone level. In addition to the presence of thick limestone stacks, hundreds of meters thick and separated by generally impermeable detrital sediments, the block tectonics, characteristic of continental-type units, plays a major role in the evolution of karst in the Pădurea Craiului region. Also important is the presence of open fracturing, generated during the most recent major tectonic phases that occurred in the Carpathian area at the beginning and during the early Quaternary, when the Apuseni Mountains were uplifted by approximately 1000 meters.

The DPKA is located within the antithetic faults area (IANOVICI *et al*., 1976), characterized by strata dipping towards the NNW and tectonic blocks that deepen towards the SSE, separated by major faults-oriented SW-NE with significant vertical displacements. These large-scale faults can separate certain karst systems, as Damiş Depression, or can bring different limestone levels into hydrogeological contact, so that at the scale of the Pădurea Craiului region all limestone formations form a single, unified aquifer. However, at the local level, almost all springs are associated with fault zones or impermeable layers. In our case, the drainage from the Izbucul Damişenilor Resurgence developed in Triassic limestones is separated from others by large-scale fault lines located to the north of the Damiş Depression.

2.2. SAMPLING AND ANALYSIS

Based on the extensive hydrogeological researches undertaken by RUSU (1973, 1978, 1981, 1988) and ORĂȘEANU (1991, 2010) in Pădurea Craiului Mountains, including the DPKA, we selected 12 sampling sites, with the well-known hydrogeological connections between them (table 1, fig. 1).

Table 1

Sampling sites description. Abbreviations used: DKS = Dămișenilor Karst System, PKS = Ponoraș Karst System, LSBV = Left Slope of Brătcuței Valley, RSBV = Right Slope of Brătcuței Valley.

Sites	Toponym	Water type	Karst system	Geology and site description
S1	Peșteruța	Ponor	DKS	Sinkhole of a stream that flows over Triassic sandstones and enters underground in Triassic dolomites through a sinkhole
S2	Toaia	Ponor	DKS	It represents the main contributor to the DKS, collecting the four main watercourses from the Damiș karst depression, which flow over Quaternary gravels and Liassic sandstones.
S3	Izvorul Rece	Spring	DKS	This spring originates from the Triassic dolomites and is one of the contributors to S2. It is designed for watering animals.
S4	Pârâul Munău	Ponor	DKS	Surface water that flows on the Triassic sandstones and goes underground in Triassic limestone.
S5	Izvorul cu Vălău	Spring	DKS	It is supplied by a marshy area over Triassic sandstones. The flow is channeled through an artificial drain, arranged for livestock watering purposes.
S6	Izbucul Dămișenilor	Karst Resurgence	DKS	This is the resurgence of the DKS, located on the left slope of the Brătcuța Valley. It has three neighboring emergence points, situated at very similar elevations. We sampled the lowest elevation point. It is tapped for the water supply of the village of Damiș.
S7	Valea Huții	Ponor	PKS	Surface water flows over Lower Jurassic sandstones and disappears into a marshy area at the contact with Jurassic limestones.
S8	Valea Ponoraș	Ponor	PKS	Surface water flowing over Lower Jurassic sandstones, sinking into Upper Jurassic limestones via a chain of dolines and detritic quaternary deposits.
S9	Izbucul Brătcanilor	Karst Resurgence	PKS	This is the PKS resurgence, situated in the lower sector of the Brătcuța Valley, within Triassic limestones. It is captured for the potable water supply of Bratca village. The natural stream was sampled.

Sites	**Toponym**	**Water type**	**Karst system**	**Geology and site description**
S10	Izvorul pietrificat	Spring	LSBV	A left-side tributary of the Brătcuța Valley. This karst spring, independent of the DKS and PKS systems, is situated between the two major hydrological systems. Over its short surface course before discharging into the Brătcuta Valley, it deposits travertine.
S11	Izvorul de la Sălcii	Spring	RSBV	A right tributary of the Brătcuța Valley emerges among slope breccias of the Triassic limestones.
S12	Izvorul M	Spring	RSBV	A right tributary of the Brătcuta Valley, which gathers the flow of two springs emerging from slope deposits of Triassic limestones. It also features a travertine deposit.

From DKS we sampled 2 springs, 3 ponors (swallow holes) and the Izbucul Dămișenilor resurgence, from the nearby PKS we sampled 2 ponors and Izbucul Brătcanilor resurgence, as well as 3 springs from different karstic systems located in the Brătcuței Valley. The 12 water sources were sampled for a timespan of two years (2023–2025), with a seasonal frequency (summer, autumn, winter and spring). A total of eight sampling campaigns were usually performed, with exceptions for sites where water was frozen or absent due to extreme meteorological events (Sites S1, S4, S7, and S10 were sampled seven times, while site S8 was sampled six times).

The physical-chemical features of the water were measured in situ with a portable multiparameter (HI 9829, Hanna Instruments) for the following parameters: water temperature (WT, ±0.15ºC accuracy), pH (±0.02 pH accuracy), turbidity (TUR, ±0.3 FNU), oxido-reduction potential (ORP, ±0.1 mV accuracy), electrical conductivity (EC ±1µS/cm accuracy), and total dissolved salts (TDS ±1 mg/L accuracy). The concentrations of chemical pollutants in the 12 studied water sources were determined in laboratory using a photo-colorimeter for waste and drinking water analysis, COD ISO 15705 (HI83399, Hanna Instruments). We determined the concentration of Nitrate (NO_3 ±0.5 mg/L accuracy), Nitrite Low Range (NO_2 ±0.02 mg/L accuracy), Ammonia Medium Range (NH_4 ±0.5 mg/L accuracy), Phosphate Low Range (PO_4 ±0.04 mg/L accuracy), Dissolved Oxygen (DO ±0.05 mg/L accuracy), Calcium Hardness and Magnesium Hardness (CaH, MgH, ±0.11 mg/L accuracy).

The microbial content of the water sources was determined by using the TC Compact Dry and EC Compact Dry selective cultivation media (R-Biopharm, Germany), which were activated in situ with 1 ml of sampling water, transferred from water source on the media with a sterile pipette. The activated media were directly transported to the laboratory and incubated at 36ºC for 48 hours before readings. The microbial content, expressed as colony forming units (UFC/mL), was established for the total count of aerobic mesophilic bacteria (TC), *Escherichia coli* (*E.c.*) and coliforms (*Klebsiella oxytoca – K.o.* and *Pseudomonas aeruginosa – P.a*).

The aquatic invertebrates were collected by filtering 10 L of water through a 50 μm sieve from each sampling site. The filtrate was preserved in 70% ethanol and water invertebrates were sorted in the laboratory under a stereoscope (OPTIKA Microscopes Italy) at the major taxon levels. For the water invertebrates we established the total abundance on site, calculated as the total number of water invertebrates found in a water site from all samples (TAWS). The total abundance on the water type (TAWT) was calculated as an average value of all TAWS from the same water type in every karst system. A major taxa richness (MTR) was calculated as the sum of all major taxa found in each water type from every karst system. We considered major taxa (MT) as clustering different representatives of a particular higher taxon (genera, families, orders, phylum) based on water invertebrate morphological divergences. The total richness of invertebrate taxa on water sites (TRWS) was calculated as the number of MT found in all samples from a water site. The total richness of invertebrate taxa on water type (TRWT) was calculated as the average of all TRWS from the same water type in every karst system.

To have an overall picture on the investigated waters we analysed together the same water types in every karst unit and depicted separately for DKS, PKS, LSBV, and RSBV. The average of individual values, seasonally obtained over the two years of monitoring (4 seasons × 2 years), were plotted in our graphical representations as average values. When we had more water samples of the same type we plotted the means of these averages, as follows: for DKS springs we plotted the means of 2 springs, for DKS ponors we use the mean of the 3 ponors, for PKS ponors we plotted the mean of the 2 ponors, and for RSBV springs we plotted the mean of the 2 springs. DKS-Resurgence, PKS-Resurgence, and LSBV-spring were plotted as individual averages. Charts and statistical analyses were performed using Excel Microsoft Office 2019.

The two-samples unequal variance Student's t Test was performed to examine for significant differences between the karst water features from different water types (springs, ponors, and resurgence) in different karst systems (PKS, LSBV, and RSBV). The differences were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1. PHYSICO-CHEMICAL PROFILE OF WATER

All physical-chemical parameters of the waters from DKS show differences both between the water types and karst units (Fig. 3).

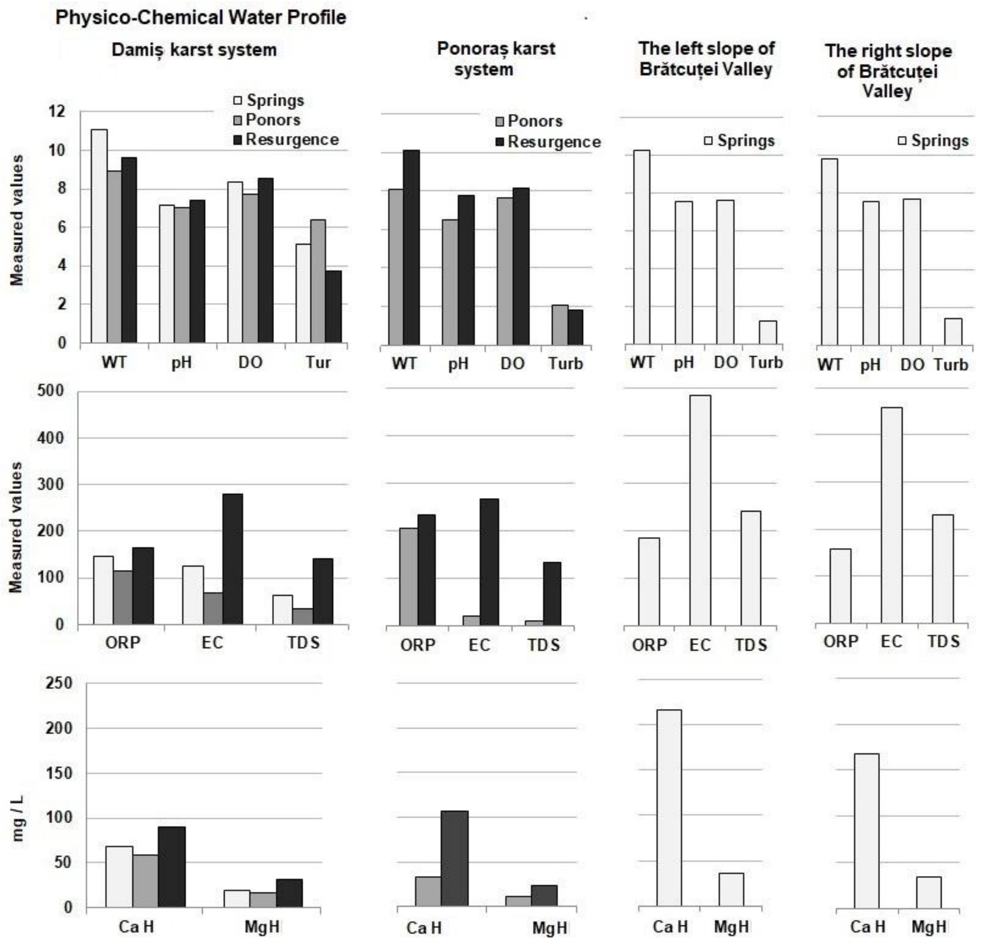

Figure 3. The averages of physical-chemical parameters in sampled water types. For parameters abbreviations see the Materials and methods section.

Although not all differences reached the level of statistical significance, we recorded significant differences (Tab. 2) for pH between DKS springs (7.12 pH) and LSBV (7.57, $p<0.05$), respectively RSBV (7.55 pH, $p<0.05$) springs; between DKS resurgence (7.38 pH) and PKS resurgence (7.77, $p<0.01$); as well as inside of PKS between ponors (6.5 pH) and resurgence (7.77 pH, $p<0.001$). The Oxidation-Reduction Potential registered a significant difference in ponors between DKS (113.99 mV) and PKS (204.69 mV, $p<0.01$). The turbidity had a high variation between the water types and karst systems and reached the statistical level for significance only for ponors of DKS (6.36 FNU) and ponors of PKS (2.06 FNU, $p<0.01$). The Electrical Conductivity and the Total Dissolved Solids, which are directly related to EC, had the most statistically significant variation. Therefore both parameters showed significant differences in DKS between springs (125.19 µS/cm, respectively 62.63 mg/L, $p<0.01$), ponors (67.93 µS/cm, 34.22 mg/L, $p<0.001$), and their resurgence (279.13 µS/cm, 139.75 mg/L); in PKS between ponors (21µS/cm, 10.42 mg/L) and resurgence (267.56 µS/cm, 134 mg/L, $p<0.001$); in ponors between DKS (67.93 µS/cm, 34.22 mg/L) and PKS (21µS/cm, 10.42 mg/L, $p<0.01$); as well as in DKS springs (125.19 µS/cm, respectively 62.63 mg/L) versus LSVB (485.43 µS/cm, 242.71 mg/L, $p<0.001$) and RSVB (458.01 µS/cm, 229.13 mg/L, $p<0.001$). The Calcium Hardness shows significant differences between DKS springs (67.93 mg/L) and LSBV (215.57 mg/L, $p<0.05$), respectively RSBV (167.88 mg/L, $p<0.01$); and also in PKS between ponors (32.84 mg/L) and resurgence (106.88 mg/L, $p<0.01$); while the Magnesium Hardness exceeded the significance threshold only in DKS between ponors (18.93 mg/L) and resurgence (31.14 mg/L, $p<0.05$).

Table 2

Results of t-Test analyses indicating statistically significant differences among water types sampled from Dămișenilor Karst System (DKS), Ponoraș Karst System (PKS), Left Slope of Brătcuței Valley (LSBV), and Right slope of Brătcuței Valley (RSBV). Significance levels: * = results statistically significant ($p < 0.05$), ** = results highly significant ($p < 0.01$), *** = results very highly significant ($p < 0.001$). Pon = ponors, Sp = springs, Res = Resurgence. For other abbreviations see 2.2 Materials and methods section.

Comparisons	**pH**	**ORP**	**EC**	**TDS**	**TUR**	**PO4**	**CaH**	**MgH**	***K.o***	**HAR**	**DIP**	**MTR**
DKS Pon / PKS Pon		**	**	**	**	*			**			
DKS Pon / DKS Sp			**	**								
DKS Pon / DKS Res			***	***				*		*	*	*
DKS Sp / LSBV Sp	*		***	***			*					
DKS Sp / RSBV Sp	*		***	***			**					
DKS Res / PKS Res	**										*	**
PKS Pon / PKS Res	***		***	***			**					
LSBV Sp / RSBV Sp												

3.2. CHEMICAL POLLUTANTS AND MICROBIAL CONTAMINATION

The nitrates, nitrites, ammonia and phosphates concentrations are low and do not exceed the legal limits for drinking water (Directive 2020/2184/EC), but the obtained values show a different dynamic in the two drainage systems. In DKS, pollutant concentrations exhibit a heterogeneous pattern. Nitrates and nitrites are elevated in springs and ponors but decrease in the resurgence, while ammonia shows the opposite trend, reaching its highest concentration in the resurgence. Phosphate concentrations in the resurgence are intermediate, falling between the values observed in springs and ponors. By contrast, in PKS, pollutant behavior is more uniform, with the resurgence consistently displaying the highest concentrations of all measured pollutants. A statistically significant difference was proved only by phosphates from ponors, between DKS (0.04 mg/L) and PKS (0.01 mg/L, $p<0.05$).

According to our findings, DKS has the most contaminated waters with the total count of mesophilic bacteria, coliforms and *E. coli* (Fig. 4b.). Among coliforms, *Klebsiella oxytoca* was the most abundant bacteria, while *Pseudomonas aeruginosa* and *E. coli* were more reduced. Regarding the water type, the greatest contamination was recorded in DKS springs followed by ponors and resurgences. The statistically significant differences were raised by *Klebsiella oxytoca* in ponors, between DKS (68,05 CFU/mL) and PKS (20.2 CFU/mL, $p<0.01$), same as phosphorus (Tab. 2).

3.3. WATER INVERTEBRATES

Overall, a total of 21 major taxa were counted in all samples collected from the 12 water sources. Similar with the microbial content, the biggest abundance of invertebrates in DKS was recorded in springs, followed by ponors and resurgence (Fig. 4c.). The same dynamic was also recorded by PKS between ponors and their resurgence, while springs from the other karst systems showed a lower abundance than DKS.

The total richness of major taxa follows the same pattern in DKS, but showed an opposite dynamic in PKS, where the resurgence has the highest diversity (Fig. 4 d.). Statistical analysis revealed significant differences between ponors and their resurgence in DKS in MTR (2.95 MT/sample vs. 1.25 MT/sample, $p<0.05$), dipterans (8.41 ind./sample vs. 0.63 ind./sample, $p<0.05$), and harpacticoids (0.59 ind./sample vs. 0 ind./sample, $p<0.05$). The DKS resurgence also significantly differed from PKS resurgence, as MTR (1,25 MT/sample vs. 4 MT/sample, $p<0.001$) and dipterans (0.63 ind./sample vs. 3 ind./sample, $p<0.05$) (Tab. 2). Analysis of the taxonomic composition of aquatic invertebrates in the investigated karst systems revealed 15 taxonomic groups in DKS, dominated by dipterans, copepods, amphipods, and stoneflies (Fig. 5). It is followed by PKS and RSBV both with 14 taxa, in which dipterans, amphipods and nematodes, respectively amphipods and dipterans were the main groups. In contrast, LSBV counted only 7 taxa with dipterans and springtails being the most-well represented.

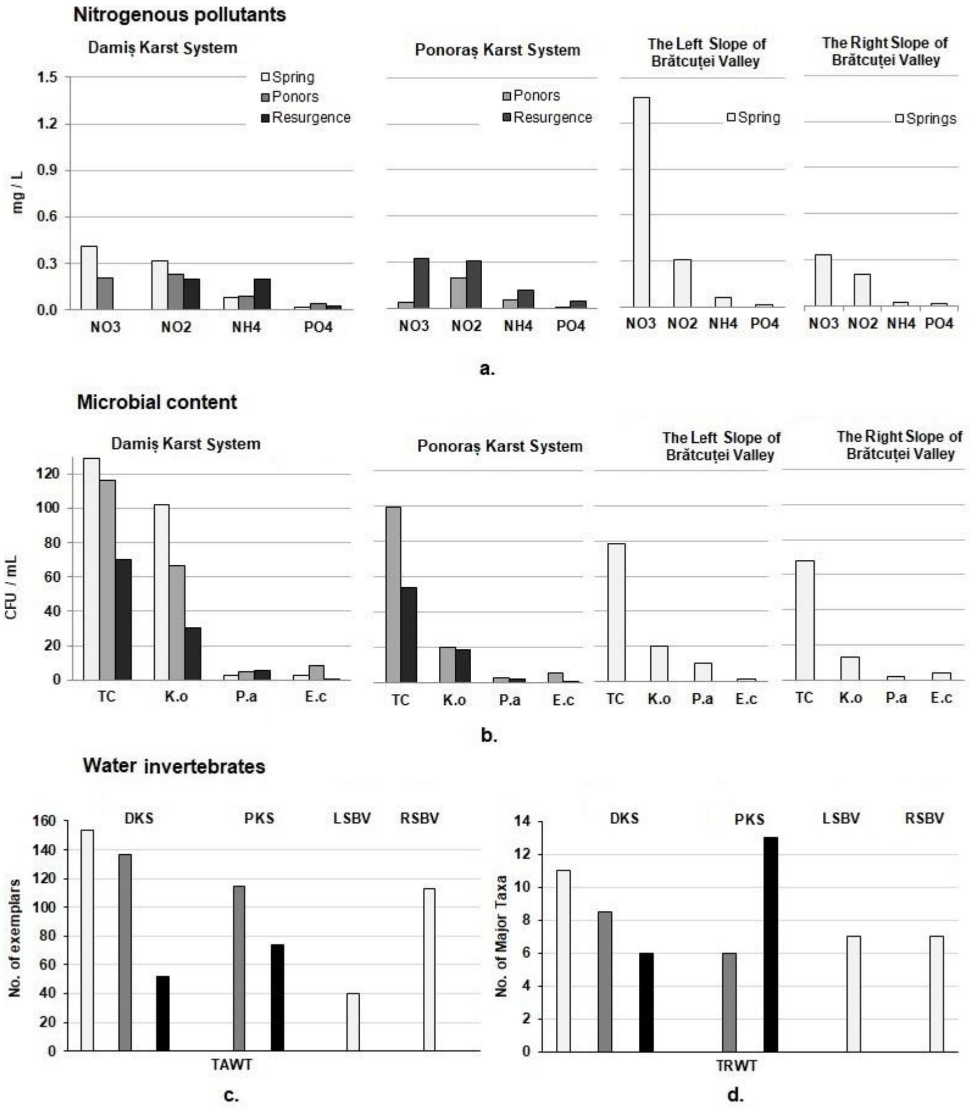

Figure 4. a) The nitrogenous pollutants. b) The microbial CFU averages on sampled water types. c) The total abundance in water types (TAWT) of water invertebrates. d) The total richness of invertebrate taxa on water types (TRWT) of water invertebrates. Abbreviations used: TC = Total Count, E.c = *Escherichia coli,* K.o = *Klebsiella oxytoca,* P.a = *Pseudomonas aeruginosa,* CFU / mL = the number of colony forming units from 1 mL of sampled water types

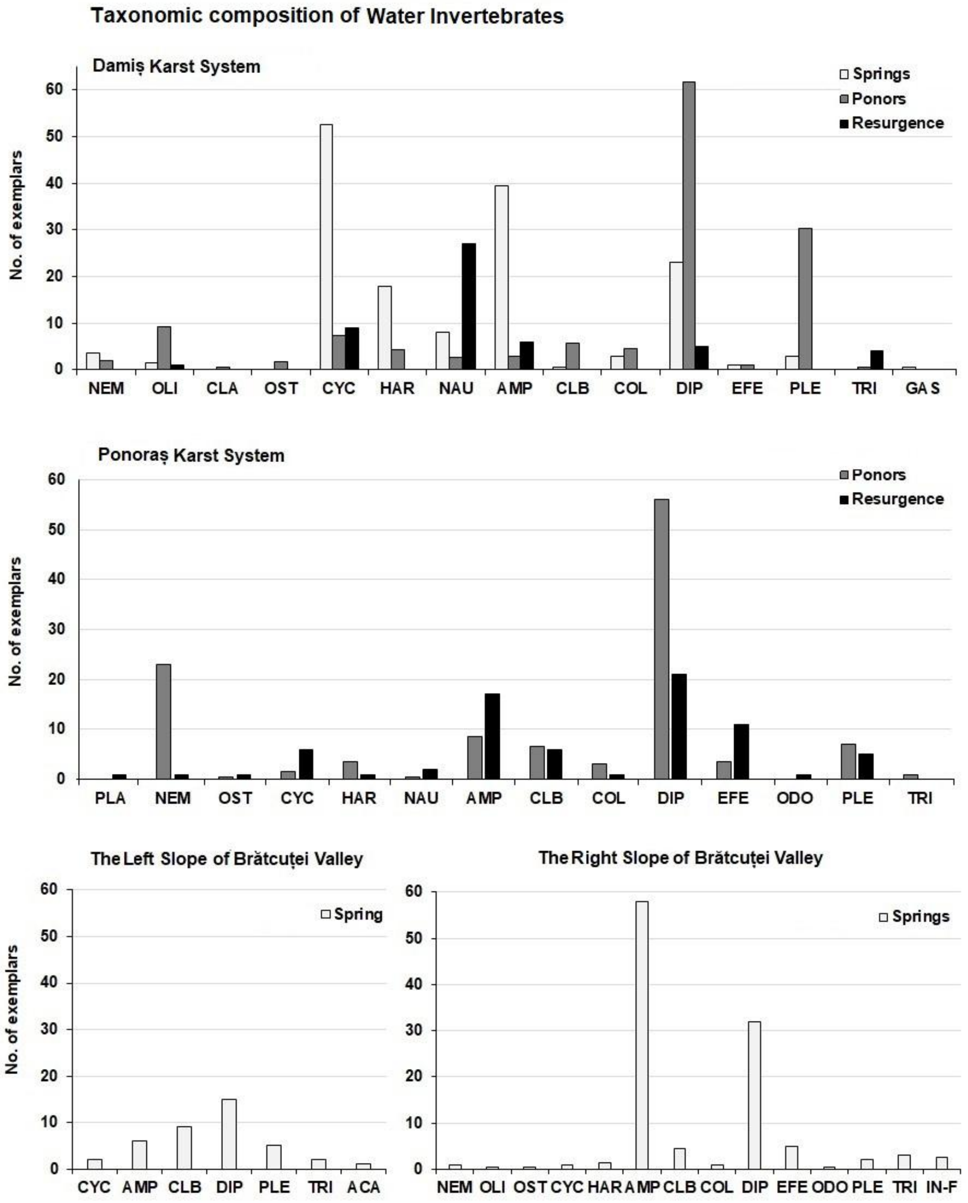

Figure 5. The taxonomic composition of aquatic invertebrates plotted as the average of the water sites grouped on water types and drainage systems. Taxa abbreviations ACA = Acari: Hydrachnidia, AMP = Amphipoda, ARA = Araneae, CLA=Cladocera, CLB = Collembola, COL= Coleoptera, CYC = Cyclopoida, DIP = Diptera, EFE = Ephemeroptera, GAS = Gastropoda, HAR= Harpacticoida, HET = Heteroptera, IN-F = insect fragments, NAU = copepods nauplii, NEM = Nematoda, ODO = Odonata, OLI = Oligochaeta, OST = Ostracoda, PLA = planarians (Tricladida), PLE = Plecoptera, TRI = Trichoptera.

4. DISCUSSION

4.1. GEOLOGICAL SIGNATURE OF DPKA ON THE PHYSICAL-CHEMICAL FEATURES OF KARSTIC WATERS

The geochemical evaluation of representative springs and streams provides insights into the functioning of karst aquifers (LECOMTE, 2016). In karst systems, water movement from recharge zones to discharge areas involves continuous dissolution and precipitation processes within carbonate rocks (NARANY, 2019; HERMS, 2021). As demonstrated by LECOMTE (2016), surface waters are influenced by lithological characteristics and seasonal dynamics. These interconnected factors collectively shape the geochemical signature of the water, reflecting the intensity of water–rock interactions and the duration of subsurface residence (DEPRETIS *et al.*, 2014).

Considering the geological framework of the DPKA area and the water–rock interactions, this study provides a comprehensive characterization of the physicochemical properties of the waters forming the local drainage systems. The analysis is based on statistically significant results, thereby reducing the likelihood that the observed patterns occurred by chance. Although the findings are preliminary and derived from a two-year survey, several statistically validated trends are already evident. Therefore, the geochemical signature of Damiș-Ponoraș karst area on the sampled waters consist in the prevalence of water alkalinity in all sites, together with higher values of the electric conductivity, total dissolved salts, calcium and magnesium hardness and turbidity, explained by the limestone nature of the land. Moreover, the DKS displays geochemical characteristics that slightly differ from those observed in the PKS and BV. For example, pH in DKS springs has lower values than the BV springs, mainly because of S5 spring from DKS which feeds in a marshy land from sandstone, while the springs from BV originated exclusively from limestones. Also, the distinctly lower pH measured at the DKS resurgence relative to the PKS resurgence implies that the DKS groundwater system interacts primarily with Early Triassic sandstones, while the PKS groundwater route is mainly associated with limestone lithologies of the Late Jurassic, Early Cretaceous and Late Triassic. The different geological structures of DPKA drainage systems also imprint different characters in terms of EC, TDS, Ca and Mg hardnesses. Thus, EC and TDS show major differences between all water types, but the highest differences are displayed between the ponors in sandstones with a lower salt content and the resurgence with underground circulation, passing through limestone and soluble dolomites. Also, the springs from DKS have a significantly lower CaH than the BV's springs that come from limestone and behave similarly to resurgents, in sharp contrast with S5 spring from DKS. The higher values of MgH in DKS resurgence show us that the waters drained meet underground dolomitic levels of the Triassic to a greater extent than the waters from ponors, which run predominantly in Triassic sandstones and quaternary gravels. Another feature of DKS is the lower ORP that makes it more vulnerable to pollutants, because this parameter plays an essential role in the decomposition processes of pollutants. The presence of an inverse proportion pattern between ORP and turbidity, observed in all types of water and statistically reinforced by ponors, suggests that the evolution of these parameters could be the result of the swamp portion on DKS streams due to the clays from the waterproof substrate. Understanding the geochemical signature of karst waters has become a powerful tool for tracing contaminant transport, and assessing the vulnerabilities of the local karst system.

4.2. POLLUTION PATTERNS AND MICROBIAL CONTAMINATION IN DPKA

The geological structure, drainage surface, water volumes and time spent underground by water before returning to the surface are once again highlighted by the different dynamics of pollutants in Damiș-Ponoraș drainage systems. Based on the Directive on the Quality of Water Intended for Human Consumption (Directive 2020/2184/EC), all measured nitrogen and phosphorus compound concentrations in the DPKD systems were within permissible limits, indicating the absence of chemical pollution. Because of the high variability of individual pollutant values due to unpredictable pollution events, the statistical significance of these results was generally not evident, as well, excepting for the phosphates. Our results, however, clearly show the trend of pollutant variation in the drainage systems of DPKA, as well as on the water types. Pollutant variation in DKS relative to PKS shows higher values, which are associated with the level of population density in the focused study area. Thus, DKS overlaps with the Damiș hamlet and has an index of discharge of 83 l/s (ORĂȘEANU, 2010), from a smaller area of drainage, which makes it more exposed to pollution. VB is undergoing anthropization, while the PKS drainage area is much larger, having an index of discharge of 305 l/s (ORĂȘEANU, 2010) and does not include human settlements in the sampled locations. Due to its larger drainage basin, our study evidenced a concentration of chemical pollutants in the PKS resurgence, while in the DKS resurgence this tendency is not so clear.

Microbiota represent a fundamental component of karst aquifers, playing a key role in sustaining the functioning of aquatic ecosystems. Primarily, microorganisms are responsible for the major turnover of energy and matter, mineral formation processes, redox reactions, and biodegradation, while also constituting a substantial reservoir of biomass (DANIELOPOL *et al.*, 2003). But at the same time a lot of pathogenic microorganisms are transmitted through contaminated water and impact public health, and socio-economic structures, especially in low- and middle-income countries (DONG *et al.*, 2023). The animal wastes and improper sewage are the main sources of waterborne pathogens frequently detected in aquifers (EPURE and BORDA, 2014; BORDA *et al*., 2019, 2022, 2024; ZHANG *et al*., 2022). Because microbial activity is strongly influenced by the chemical composition and environmental conditions of the habitat, microbiological parameters are essential indicators for assessing water quality and sanitary safety in karst aquifers. A notable level of microbial contamination was detected in all monitored waters, revealing a consistent trend of decreasing contamination from springs to ponors and resurgences. This pattern reflects the mesophilic character of the analyzed bacterial communities and their limited capacity to persist under environmental stress conditions. The significant differences in *Klebsiella oxytoca* levels in ponors between DKS and PKS confirm that DKS is more vulnerable to coliform pollution. The detection of coliforms and *E. coli* points to anthropogenic influence, implying fecal contamination affecting both surface water and groundwater in DPKA systems. Degradation of these karstic systems deteriorates the raw quality of source waters, thereby increasing the magnitude and complexity of treatment processes required to achieve potable water standards for human consumption. Under the Drinking Water Directive (DIRECTIVE 2020/2184/EC) and national legislation (LEGEA 458/8.07.2002), waters intended for human consumption must exhibit 0 CFU of total heterotrophic bacteria (TC), coliforms, and *Escherichia coli*. Runoff originating from point and diffuse sources can mobilize pathogenic microorganisms, which infiltrate through surface soils and the unsaturated zone to reach the groundwater (BAGORDO *et al*., 2024). Although these mesophilic microorganisms are capable of surviving sufficiently long to reach and contaminate groundwater, their retention efficiency in saturated zones is typically lower than in unsaturated conditions (BALKHAIR, 2017), a pattern consistently observed in our current investigation and previous studies (EPURE and BORDA, 2014; BORDA *et al*., 2019, 2022, 2024). The pronounced temporal and spatial variability of microbial loads emphasize the need for systematic monitoring and protection of recharge areas to ensure the safe and sustainable use of these essential water resources.

4.3. WATER INVERTEBRATE ASSEMBLAGES

Despite substantial knowledge of the hydrogeological linkages between surface and groundwater in karst systems, and of the physicochemical characteristics of these waters, relatively little is known about the distribution patterns of invertebrate assemblages from recharge zones across the various groundwater habitats (DI CICCO *et al*., 2021). In our study, the total abundance of aquatic invertebrates displayed a spatial distribution closely aligned with microbial content across the distinct water types within the Damiș-Ponoraș drainage systems. From the perspective of major taxa richness, the DKS and PKS systems exhibited contrasting dynamics, emphasizing the ecological vulnerability of DKS and the structural complexity of PKS. The pronounced decline in richness of major taxa and abundance from surface sites to the underground further indicates anthropogenic disturbance within the DKS karst system. Even though the springs and ponors from DKS were characterized by higher diversity and abundance of aquatic invertebrates, the resurgence reveal the sensitivity of groundwater to organic pollution. A positive correlation between the microbial content and water invertebrates has also been reported in groundwaters of the Runcuri Karst Plateau, a neighboring karst system (BORDA *et al.*, 2022). STEIN *et al.* (2010) showed that microbial diversity and subterranean faunal composition mirror anthropogenic pressures, especially from agriculture, underscoring the value of aquatic fauna and microbial communities as indicators of groundwater quality.

Across all taxa surveyed, Diptera and Harpacticoida demonstrated a consistent spatial distribution, reflecting the general pattern observed in the DPKA invertebrate assemblages. Similarly, DI CICCO *et al.* (2021) reported distinct responses of surface-water copepod species and obligate groundwater taxa to the hydrogeological characteristics of the karst unit. Their study revealed a shift from Cyclopoida dominance in surface waters to Harpacticoida prevalence in cave groundwater, reflecting contrasting infiltration dynamics, whereby rapid endorheic flows transport surface species underground, while diffuse epikarstic pathways sustain populations of obligate groundwater dwellers.

4.4. ANTHROPOGENIC IMPACT IN DPKA

Human activities contribute to contaminant accumulation in karst aquifers, disrupting ecosystem structure and function and increasing risks to human health (DANIELOPOL *et al.*, 2003). The DKS represents the outcome of a complex interplay between geological-structural and hydrogeological factors that have shaped the area, particularly during the Quaternary period. The presence of extensive outcrops of non-karstifiable, impermeable rocks on the hills in the southern sector of the area has led to the formation of temporary surface watercourses. After relatively short surface flow paths, these streams descend into the depression, where they disappear through ponors, contributing to the recharge of an aquifer hosted in Quaternary gravels, with thicknesses ranging from 4 to 6 meters, which cover the depression floor. In the northern part of the depression, the land surface is predominantly composed of Triassic limestones, through which precipitation rapidly infiltrates via fissures and dolines. Once percolated into the subsurface, the water has developed a network of subterranean conduits that facilitate groundwater circulation, eventually re-emerging at the surface through the Izbucul Damișenilor resurgence.

Due to favorable environmental conditions and the availability of water resources, the area has been continuously inhabited, with dwellings dispersed across the hills of the hamlet of Damiș, which is documented as a human settlement as early as 1264 (MIRCESCU *et al.*, 2008). The demographic evolution of Damiș fluctuated, with the population varying from 1,338 inhabitants (1966 census) to 680 inhabitants (2011 census). During the demographic peak, the local economy was primarily based on livestock farming and subsistence agriculture (MIRCESCU *et al.*, 2008). Currently, these activities have undergone a pronounced decline, a process substantiated by our results, which demonstrate negligible levels of chemical contamination within the study area. In recent years, a new anthropogenic factor has emerged with the implementation and progressive expansion of a potable water distribution network in the village of Damiș. The system is supplied by the Izbucul Damișenilor spring, which is properly captured and treated for human consumption. However, in the absence of a sewerage system, part of the distributed water re-enters in the karst environment, transporting a variety of pollutants, including nutrients, detergents, and microorganisms, thereby contributing to the natural contaminant load. Our research demonstrates that the primary anthropogenic impact in the DKS area is the significant microbial contamination, including fecal and pathogenic bacteria. This situation presents a significant public health risk to the population of Damiș hamlet and places additional demand on the water purification and treatment systems supplied by the Izbucul Damișeni resurgence, thereby increasing the operational costs required to maintain potable water quality. Stable isotope analyses (δ^2H and δ^{18}O) indicate that karst areas in Romania receive recharge from mixed sources: a rapid component occurring through ponors, swallow holes, and conduits, and a slower, diffuse infiltration through the soil and karst cover (FEHER, 2024). The microbial contamination identified in this study affects both the ponors recharge path, but is mostly associated with diffuse infiltration through soil and quaternary gravel aquifer, ultimately affecting the karst aquifer of the Damiș Depression. Additionally, in DKS the distributed water increases the overall volume and rate of recharge of the groundwater system with a polluted amount of water, adding extra stress to the water treatment processes.

5. CONCLUSIONS

The geological structure and lithology of the DPKA plays a determining role in shaping the hydrogeochemical diversity observed among its springs, ponors, and resurgences, as well as across its drainage systems. Although all analyzed waters share the characteristic signature of karst environments, marked by elevated alkalinity, electrical conductivity, total dissolved solids, and calcium–magnesium hardness, distinct spatial differences were evident within the DKS. The hydrochemical patterns of DKS indicate spatial differentiation along the karst system: springs had lower pH, calcium hardness and conductivity, ponors showed higher turbidity and reduced redox potential, suggesting pollutant susceptibility, and resurgence waters exhibited increased magnesium hardness linked to flow through dolomitic formations.

The evidence points that anthropogenic pressures within the DKS are predominantly biological in nature, arising from bacterial contamination, including pathogenic species, linked to domestic wastewater discharges. These impacts have been also reflected in the aquatic invertebrate communities. Our findings demonstrate a progressive reduction in microbial load and invertebrate abundance and major taxa richness along the karst hydrological continuum, from springs to ponors and resurgence points. This trend suggests that biological abundance and diversity in karst waters diminishes along to the subsurface transport, likely driven by complex and still poorly understood ecological and physicochemical mechanisms that warrant further long-term investigation.

In the long term, persistent anthropogenic influences are expected to progressively impair the water quality of the DKS karst aquifer, affecting its ecological integrity and functional resilience. A comprehensive assessment framework integrating abiotic and biotic indicators, supported by continuous seasonal monitoring is therefore essential for early detection of ecological deterioration and for guiding effective management actions to prevent or mitigate ecosystem destabilization.

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