GROUNDWATER CONTAMINATION AND THE RELATIONSHIP BETWEEN WATER CHEMISTRY AND BIOTIC COMPONENTS IN A KARST SYSTEM (BIHOR MOUNTAINS, ROMANIA)

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Abstract. The physical and chemical characteristics, microbial contamination, and meiofauna of the Ocoale-Ghețar-Dobrești karst system (Bihor Mountains, Romania) were studied in order to assess the natural water quality by an interdisciplinary study. A total of 60 water samples were collected seasonally from 7 sites. Physico-chemical results showed a typical composition of karst waters, except for one site, where Ca^{2+} was absent, pH was very low, and the abundance and diversity of meiofauna were highest, demonstrating life support even for the most sensitive animals. No significant chemical pollution was found, but microbial contamination occurred in all samples, according to the national water quality standards of the analyzed springs. The Canonical Correlation Analysis and the Canonical Correspondence Analysis performed showed a strong connection between pH, nitrates and faecal pollution, indicating also a direct connection between microbial contaminants and dissolved oxygen.

Key words: karst aquifer, chemical pollution, organic pollution, groundwater bacterial contamination, meiofauna, water quality.

1. INTRODUCTION

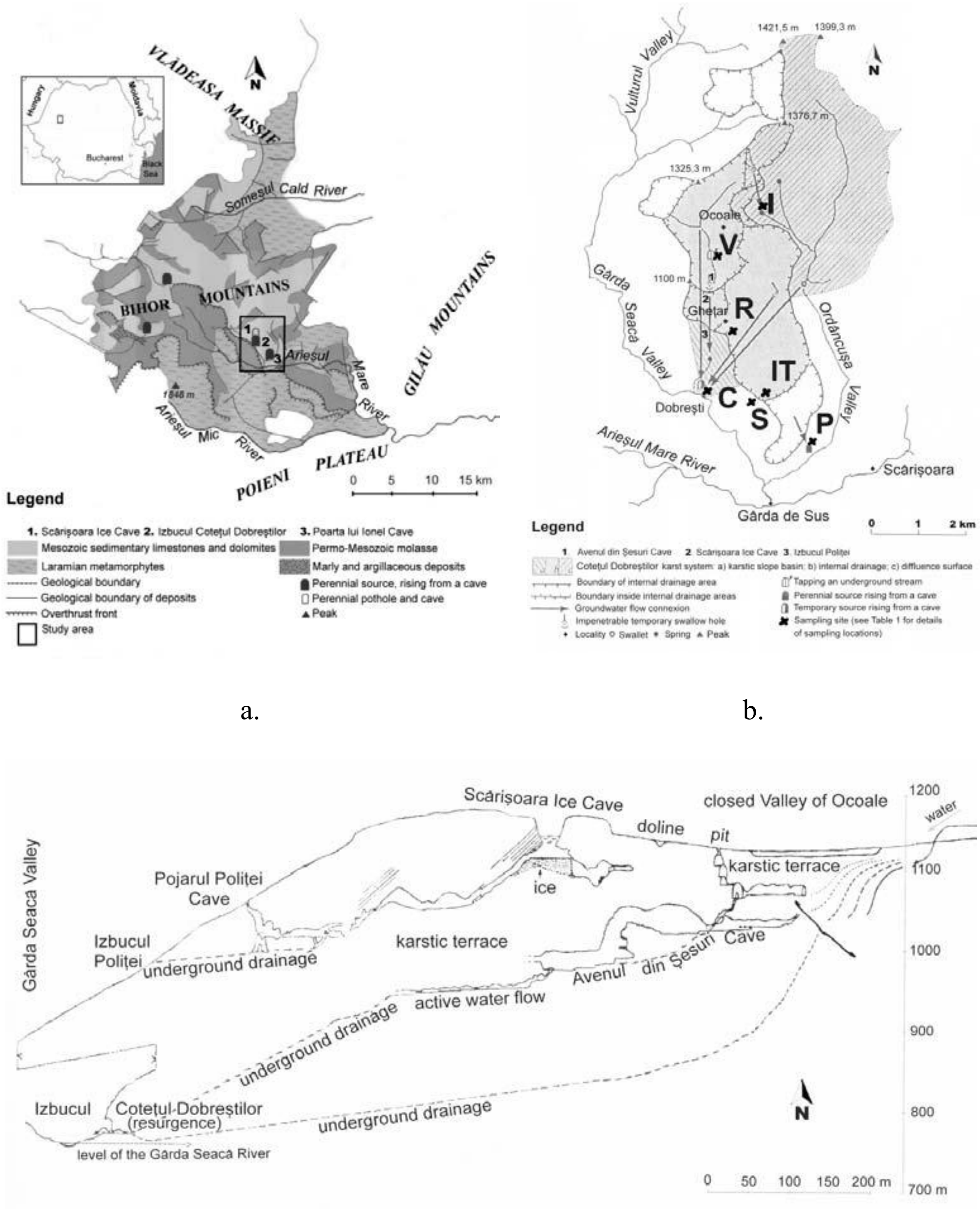
Groundwater contamination is an important global problem, due to its influence on water sources used for human needs. Groundwater ecosystems play an important role in providing good quality water (GRIEBLER *et al.*, 2010). In karst regions, the surface water is drained by natural percolation, by vertical drains, or via cave outlets. Because the natural filtration efficiency is lower compared to non-karstic terrains, aquifers are highly sensitive to pollution (POBST & LAVERN TAYLOR 2008). Pathogenic bacteria were found in high densities in karst waters (CURRENS 2002, KELLY *et al.*, 2009). Their presence in drinking water can cause serious diseases such as *E. coli*-induced gastroenteritis, cryptosporidiosis or dysentery epidemic (ZWIENER&FRIMMEL, 2004). Ammonia, nitrate, nitrite, chloride, and potassium, detected at all contamination levels in surface waters affect the aquatic life and underground biodiversity, being considered a serious problem (TAYLOR *et al.*, 2000). GRIEBLER *et al.* (2010) emphasized the lack of existing information regarding the direct relationships between fauna and microorganisms and the lack of biological/ecological standards in groundwater status assessment. They suggested that by investigating faunal and bacterial communities, important information on groundwater ecosystems and possible bio indicators can be obtained.

The aims of this study are (1) to evaluate the microbial and chemical contamination of water sources used by the Ocoale-Ghețar-Dobreşti inhabitants, (2) to estimate the drinking water quality in the Ocoale-Ghețar-Dobreşti karst system, according to the national legislation and (3) to understand biotic and abiotic interactions in the Ocoale-Ghețar-Dobreşti karst system groundwater.

1.1. BACKGROUND

Our study focused on groundwater quality in the karstic region of the Ocoale-Ghețar-Dobreşti plateau, located in the Apuseni Natural Park. This area is delimited to the East by Ordâncuşa Valley and to the West by Gîrda Seacă Valley (Fig.1.a, b and Table 1). The Bihor Mountains consist of Mesozoic sedimentary limestones and dolomites, combined with Permo-Mesozoic molasse, with double porosity (ORĂŞEANU, 2010). The Mesozoic rocks are characterised by a high effective infiltration; however, the Permo-Mesozoic molasse acts as a barrier for karst water reservoirs at large thickness; therefore the groundwater flow is mostly confined to the fracture and stratigrafic joints, (ORĂŞEANU 2010). This combination of stratigraphy and structure suggests a high permeability for groundwater flow. Most of the Ocoale-Ghețar-Dobreşti groundwater merge underground and outflow through two outlets: Izbucul Poliței (not in the study area) and Izbucul Cotețul Dobreştilor (C) (Fig. 1.c). The waters draining the East side of the plateau and outflow through two outlets, through Poarta lui Ionel Cave resurgence (P) (ORĂŞEANU *et al*., 1991).

The study area includes few villages, hamlets, and tourist facilities, located between 700-1 200m a.s.l. There is no intensive agriculture; therefore, the locals use mainly farmyard manure as fertiliser. The water resources for the local people are scarce. Due to this hydrological constraint, inhabitants have to use few wells and meteoric water supplies for drinking and domestic use. In order to connect the local households to a water supply system, a network, using the Iapa spring as water source, has recently been installed. In the last 20 years, the region has become a tourist destination because of the beautiful landscape and the famous Scărişoara Ice Cave. Because the area is not provided with a sewage network, the animal waste and the septic infiltrations from human settlements including rural pensions are the main sources of water contamination. The last two decades were marked by an intensive deforestation. Consequently, numerous wood processing points were installed (MARIN, 2002). Therefore, the sawdust resulting from the wood processing became a new pollution source in the area.



a. b.

c.

Fig. 1. a. Hidrogeological map of Bihor Mountains (modified from Orăşeanu 2010); b. Location of the study area with the sampling sites (modified from Orăşeanu 2010); c. Cross-section map of the Ocoale-Gheţar-Dobreşti karst system (modified from Şerban *et al.* 1957).

Table 1

Description of the sampling sites

Station	***Station abbrev.***	***Geographic Coordinates***	***Altitude (m a.s.l.)***	***Description***	***Utility***	***Potential contamination sources***	***Type of analysis***
Scaiu 2	S	N 46°28.203 E 22°49.714	998	Well	For human drinking	Farmyard manure and sewage; animal entry	Physico-chemical Microbiological Meiofauna
Rădăcini	R	N46°29.220 E22°49.537	1083	Well	For animal drinking	Farmyard manure and sewage; animal entry	Physico - chemical Microbiological Meiofauna
Iapa	I	N 46°30.610 E 22°49.521	1219	Spring, the catchment point of the piped distribution system	For human drinking	Farmyard manure and sewage	Physico - chemical Microbiological Meiofauna
Vuioagă	V	N 46°28.283 E 22°49.677	1171	Well	For animal drinking	Farmyard manure and sewage; sawdust; animal entry	Physico - chemical Meiofauna
Izbucul Cotețul Dobreştilor	C	N46°28.666 E22°48.529	710	Main drainage of the Ocoale-Ghețar-Dobreşti karst system	Not for drinking	Farmyard manure and sewage;	Physico - chemical Microbiological Meiofauna
Poarta lui Ionel	P	N46°27.934 E22°50.328	867	Cave resurgence	Occasionally human drinking	Farmyard manure and sewage	Physico - chemical Meiofauna
Iapa- tap	IT	N46°28.040 E22°49.898	1015	The end of the piped distribution system	For human drinking	-	Physico - chemical Microbiological Meiofauna

PLEŞA&BUZILĂ (2000) studied the stygobitic fauna of the Gârda Seacă valley, observing remarkable differences of faunal assemblages, linked to biotope structure. No studies regarding the groundwater contamination were performed in the Ocoale-Ghețar-Dobreşti karst system after the water network started to operate.

2. MATERIAL AND METHODS

2.1. SAMPLING PROCEDURE AND ANALYSIS

The study area includes 7 sampling sites (wells, drainage exits, and resurgences) of the Ocoale-Ghețar-Dobreşti karst system, located in the southern part of the Bihor

Mountains (Romania) (Fig. 1. a, b, c and Table 1). Wells S, R and V are low depth spring catchments protected by wood shelters. In order to determine physico-chemical parameters, microbial analyses and meiofauna counting, three water sample sets were collected from each sampling site in the autumn, winter, and spring of 2009-2010. A total of 60 samples were collected, because well R was completely frozen in winter, preventing us from collecting any data. Water physico-chemical characteristics, temperature (T ± 0.15 ^{o}C accuracy), pH (± 0.02 pH accuracy), electrical conductivity (EC ± 1μS/cm accuracy), total dissolved solids (TDS ± 1 mg/L accuracy) and dissolved oxygen (DO ± 1.5% of readings accuracy) were measured on the sampling sites, using a Portable Multiparameter HI 9828 (Hanna Instruments). Nitrates (NO_3^-), nitrites (NO_2^-), ammonia (NH_4^+), calcium (Ca_2^+), phosphorus (P) and iron (Fe) were measured in laboratory, with Photometer C99, HI 83099 (Hanna Instruments).

a. Microbiological analyses quantified the total aerobic bacterial count (TAC), the total coliforms (TC), and faecal coliforms (FC). The samples were collected in sterile recipients and transported to the laboratory within 12 hours. TAC was estimated using 1 ml of decimal dilutions (10^{-1}, 10^{-2}, and10^{-3}) of water samples in nutritive agar, incubated at 37^{o} C for 48 hours. For TAC, the number of colony forming units (CFU) was calculated using the formula: $\sum (n\times d)/\boldsymbol{N}$(where n = the number of CFU on a Petri plate, d = the dilution, N = number of Petri plates counted). For TC and FC the conventional method of multiple tubes was applied (Feng 2002). In the presumptive test, the water samples were inoculated in culture tubes containing lactose medium. To confirm the presence of TC, the bacterial colonies were then transferred on Levine's medium Petri plates and incubated at 37°C for 24 hours. FC presence was confirmed by gas production in brilliant green lactose bile medium (BGLB) incubated at 44.5°C for 24 hours. The most probable number (MPN/100 ml) was calculated for TC and FC. Water quality and contamination levels were estimated by comparing the results with the Romanian standards for drinking water quality (Law 458/2002, republished in 2011 (r.1), with adjustments and completions added by Law 311/2004, Law 124/2010 and Law182/2011).

b. Meiofauna was collected on all sampling sites by filtering 12 litters of water through a 65μm net. The invertebrates found were preserved in 96% alcohol, then sorted to taxonomic levels in laboratory, using a stereomicroscope (OPTIKA Microscopes Italy, model SZR).

c. Statistical analysis of data. In order to search for connections and patterns between data sets (physico-chemical, microbial, meiofauna), Canonical Correlation Analysis (CCorA) and Canonical Correspondence Analysis (CCA) were computed (XLSTAT, Version 2011 – Addinsoft SARL). CCorA was based on environmental chemical and physical properties of the water samples and the microbiological test results. CCA was computed with environmental physico-chemical parameters and meiofauna. To avoid results distortions, samples with no individuals or just one individual found were excluded from the statistical analysis.

3. RESULTS

3.1. WATER CHEMISTRY

Physico-chemical profile of the analysed water sources showed seasonal and spatial differences (Fig. 2). The highest amplitudes of water temperature were measured at Iapa-tap (6.5°C), which represent the terminal site of the water network (IT).

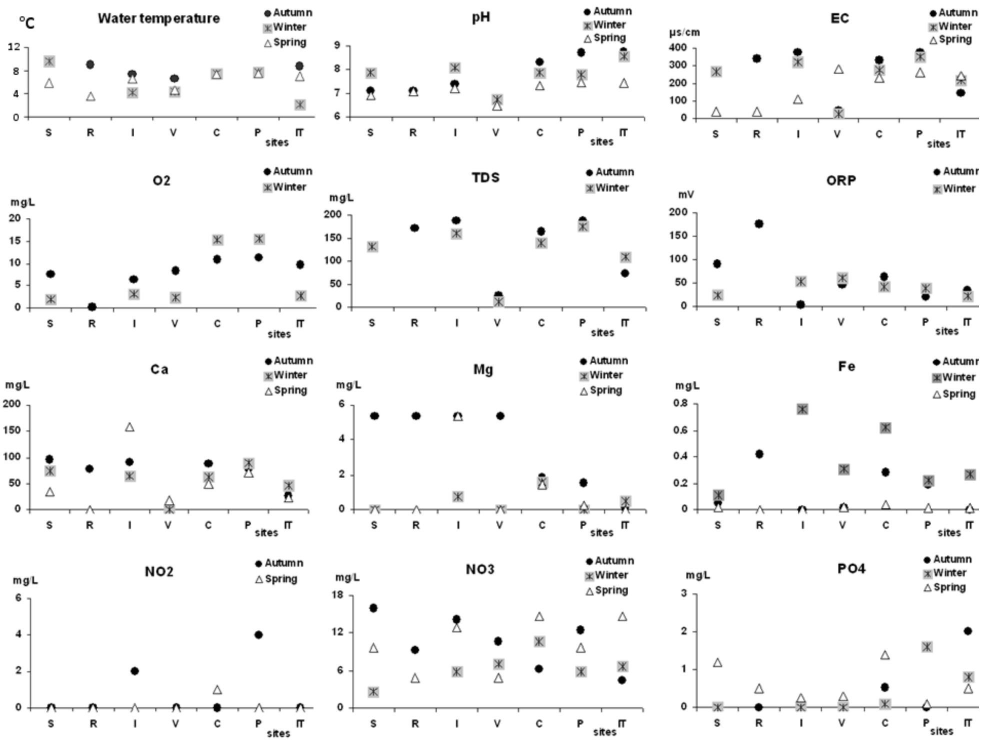


Fig. 2. Seasonal physico-chemical profiles of the studied sites from the Ocoale-Ghețar-Dobrești karst system.

The lowest temperatures differences (0.17°C and 0.18°C) were measured at the drainage exits of the study area (P and C). The pH revealed neutral to alkaline values in all samples, except for well V, where the pH was slightly acidic in all three seasons (pH 6.5-6.7). The highest pH values (pH 7.4 and pH 8.8) were measured at IT in autumn and winter. The highest electrical conductivity values (377µS/cm and 351µS/cm) were registered in the water rising from Poarta lui Ionel Cave (site P) in autumn and winter respectively. At well V, EC and TDS registered low values in autumn and winter, compared to the other samples. The highest values of DO were measured at the drainage points C and P in autumn and winter, while the minimal values were registered in wells, during autumn and winter. Nitrites were absent in most of the samples, except for I and

P in autumn, and C in spring. Measured values at these three sites exceeded those accepted by the law (maximum 0.5 mg/L). Nitrates and nitrites, however, registered the highest values in autumn. Calcium was present in high proportions (as expected in a karstic area) in all samples except for V, where calcium was absent in all seasons. Fe quantity exceeded the limits for drinking water quality, especially in winter, when the highest values were measured at sites V, C, I, and IT. According to the Romanian laws, concentrations over 0.2mg/L Fe will not be accepted in drinking water. In spring, it is the opposite case: all analyzed samples registered no Fe content.

3.2. MICROBIOLOGY

The microbiological analyses registered the highest values of TAC in the drainage points of the Ocoale-Gheţar-Dobreşti karst system (Fig. 3). The coliforms level was high on sites C and P as well. A seasonal difference in microbial content can be observed, the autumn samples revealing a high contamination, especially on coliforms. Microbial contaminants registered the lowest values in the cold season. The catchment of the water network (I) and its ending site (IT) were not contaminated with coliforms, in winter and spring. Moreover, one of the wells (S) and the ending site of the water network (IT) were not contaminated at all; neither coliforms, nor aerobic germs were found on those sites in winter.

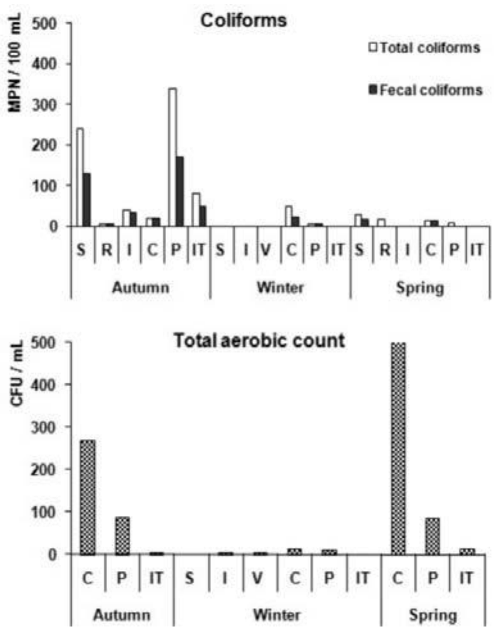


Fig. 3. The number of colony forming units of total aerobic count and the most probable number of total and faecal coliforms determined in water sources from the Ocoale-Gheţar-Dobreşti karst system.

3.3. MEIOFAUNA

A total of 112 individuals belonging to eleven taxa were counted in the analysed samples: Cyclopoida was the most abundant group (42%) followed by Acarina (19%) Harpacticoida (11%), and Plecoptera (9%) (Fig. 4). Half of the samples had no taxa and the majority of individuals collected over the three seasons (80 out of 112) were found on a single site (V). Site V was also marked by a high meiofauna diversity (7 taxa out of 11). Moreover, Acarina, Collembola, and Diptera were found only in this well.

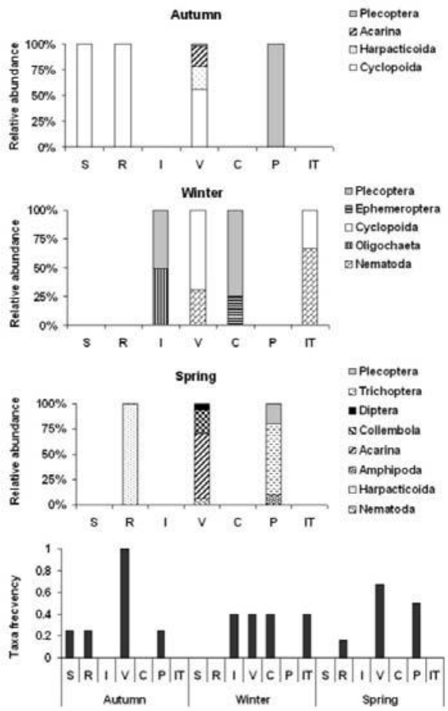


Fig. 4. The seasonal relative abundance of meiofauna and taxa frequency in the studied sites of the Ocoale-Gheţar-Dobreşti karst system.

4. DISCUSSION

4.1. THE PHYSICO-CHEMICAL PROFILE OF THE OCOALE-GHEŢAR-DOBREŞTI GROUNDWATER

Water physical features and its chemical profile, especially nutrients, may affect the presence and growth of aquatic biota. Water temperature is a major factor for mesophilic bacteria viability, growth, and abundance (BATTIN *et al.*, 2001), revealed by the lower concentrations of microorganisms and aquatic invertebrates in winter. Temperature at C and P showed the most constant values, because these two water sources are springs with continuous flow, emerging from underground, maintaining the average temperature of the karst system.

The pH profile showed high differences between the analysed water sources. The extreme values registered (pH 6.5 and pH 8.8) were out of the usual range (pH 7.0-8.5) maintained by carbonated system of karst areas (MARIN, 2002). The most acidic well (V) proves a high biodiversity and abundance of meiofauna. Its acidity is most probably due to the carbohydrates (cellulose and hemicellulose), lignin and phosphoric acid from fresh sawdust (STARBUCK, 1994) produced at the wood processing points located upstream.

Two of our sites (C and P) with the highest microbial content recorded also the highest dissolved oxygen content. This can be explained by local factors such as the contact with the surface or the flow velocity, which influences the O_2 concentration. The P resurgence and the C drainage will always register higher O_2 content compared to the wells, where water is stagnant and the process of mixing with the atmospheric oxygen is inefficient. Moreover, wells of this area are usually protected by wood shelters, keeping the light away and preventing the photosynthetic organisms from growing. At site S, measured physico-chemical characteristics showed little variations, except for dissolved oxygen which was in a low concentration in winter compared to the season before.

Natural waters of Gârda Seacă and the surrounding areas have a typical karst composition, with high concentrations of Ca^{2+} and Mg^{2+} and a reduced mineralisation (MARIN, 2002). The absence of Ca^{2+} in the samples collected from site V is correlated to the lowest values of EC and TDS and showed that this spring has no contact with the limestone, crossing a different rock layer that actually interferes with the predominant karst.

Nitrates increase in surface water and groundwater indicates the presence of farming fertilizer (KAZMI&KHAN, 2005; KARADAVUT *et al.*, 2011). Nitrate concentrations did not reach nor exceeded the maximum values accepted by the Romanian Water Quality Standards during our study. Additionally, nitrates are one of the water soluble pollutants (VESPER *et al.*, 2001) therefore, can rapidly be washed away from a karst aquifer system, due to the rock permeability. This explains the highest values of nitrates in wells (S, R, I, V) in autumn, followed by low concentrations in winter. An accumulation of nitrates was observed again in spring, as a result of the traditional fertilization.

An interesting dynamics was observed for Fe values. We measured high concentrations at S, R, C, and P in autumn. In winter we registered an increase on all sites, exceeding the law's maximum limits in I, V, C, and IT. The following season was marked by Fe absence or by its very low values. Iron and manganese normally appear in karst aquifers and cave deposits, being a part of the natural background (VESPER *et al.*, 2001). The factor controlling metal transport is their absorption onto various substrates that move towards the draining springs, when the particulates which they are absorbed on are carried out of the underground system by storm pulses (VESPER *et al*., 2001). The absence of this element in spring can be associated with the larger volume of water carried through the karst aquifer, transporting various substances or organisms which accumulated in the cold season. In winter a reduced volume of water is carried through the karst system.

4.2. THE MICROBIAL CONTAMINATION IN THE OCOALE-GHEȚAR-DOBREŞTI GROUNDWATER

Microbial contamination caused by human or animal waste is considered one of the most common health risk associated with drinking water (LALURAJ *et al.*, 2005). Microorganisms colonize groundwater environments by active or passive migration, via percolation from the surface, lateral migration from recharge areas, or by introduction into the sediments during deposition (GOUNOT, 1994). In karst areas, sinkholes and vertical shafts enable rapid and concentrated infiltration into the underground channels, draining toward the spring (HEINZ *et al*., 2009). In rural areas, organic pollution is mainly the result of sewage and waste impacts (VESPER *et al*., 2001). According to the Romanian drinking water quality standards, all water sources analysed in our study were not suitable for human and animal use, especially in warm seasons, when the highest contamination was observed. The influence of water temperature was proved by our seasonal results of the microbial contamination. The sterilising effect of lower temperatures was shown by the reduced number of viable colonies determined in the water samples collected in winter. Sites V and I showed no coliforms in the cold season. *E. coli,* termotolerant bacteria selected as indicator for faecal contamination (VESPER *et al*., 2001), prevailed from the total coliforms and proved a strong faecal contamination in the water aquifer of the Ocoale-Gheţar-Dobreşti area. In the Romanian legislation concerning drinking water quality, the presence of *E. coli* and coliforms are not accepted (Law 458/2002 (r.1)/2011). According to ORĂŞEANU ET AL. (1991), sites C and P represent the drainage points of the plateau. This explains the high values of TAC on these two sites. The absence of microbial contamination at I, in winter and spring, was followed by the same results of microbial contamination at IT, in the same seasons. Between I and IT, the water crosses a pipe, thus, we exclude a contamination source between the two sites. To avoid bacteria transportation along the drinking water network, a purification procedure at the catchment's place (site I in our study) could be a solution.

4.3. MEIOFAUNA OF THE OCOALE-GHEȚAR-DOBREŞTI GROUNDWATER

From all sampling sites monitored in autumn, winter and spring, V was particularly interesting and completely different from the others. It had the highest number of individuals in all seasons. It was the only acid spring from all seven, with the lowest conductivity measured in autumn and winter and Ca^{2+} completely absent. The fact that the majority of individuals (81% in autumn, 59% in winter and 61% in spring, from all fauna collected) were found on this site cannot be considered a coincidence. According to the meiofauna counted in our samples, most of the Nematoda were found at V in winter and spring (Fig. 4). Nematoda showed negative numerical relation to pollution and are more likely to be considered bioindicators, because they can be influenced more by soil conditions, source availability, disturbance, and interaction with other organisms (EKSCHMITT and KORTHALS, 2009). In terms of sensitivity to environmental variables, copepods are among the sensitive fauna (STOCH *et al*., 2011). In our samples Copepoda was the most abundant group (77% in autumn, and 45% in winter). The most obvious difference in rock composition between site V and the other sites is the absence of Ca^{+}. Because of all this characteristics, we can assume that site V supports life even for the most sensitive animals. Also external factors like vegetation, but especially hydrological exchanges with the surface, on a local scale (THULIN&HAHN, 2008) could influence meiofauna distribution. In our case, the sawdust pollution needs to be considered as well.

In fact, the forces controlling the distribution of organisms are more complex (GRIEBLER *et al*., 2010). To provide a more accurate image of the phenomena that influence meiofauna distribution, more factors like geology, hydrodynamics, weather, vegetation, or human activity impact need to be considered.

4.4. RELATIONSHIPS BETWEEN THE PHYSICO-CHEMICAL PROFILE AND THE BIOTIC COMPONENTS IN THE OCOALE-GHEȚAR-DOBREȘTI GROUNDWATER

Multiple factors play a detrimental role to groundwater communities. They are influenced by seasons, thus the hydrological exchanges with the surface contribute to changes in groundwater biotic assemblages. Oxygen supply and resource availability are factors that govern the faunal pattern (THULIN&HAHN, 2008). Our results revealed a direct connection between microbial contaminants and the water content in dissolved oxygen (Figs. 2 and 3). NOTENBOOM *et al*. (1994) emphasized that oxygen consumption occurs commonly in groundwater because of microbial activity and oxidation of organic matter. Copepod abundance are also affected by oxygen content (HSIEH&CHIU, 2002), being consistent with our observations. On site V the number of Copepods was much lower in winter compared to autumn, when the oxygen concentration had a lower value (Appendix 2).

The CcorA computed with physico-chemical and bacteriological results explained 100% of the variance (Fig. 5). This statistical analysis emphasized here which of the measured chemical factors is mostly affecting the FC and TC values, considering the biotic/abiotic variations in our samples. Mg, Ca and temperature were positioned closer to the F1 axis, in the negative section of the plot, whereas the Fe is in the positive sector of the plot, close to the F1 axis as well. Consequently, the

F1 axis (63% significance) is a gradient of decreasing temperature, Ca, Mg and increasing Fe. F2 (37% significance) is a gradient of decreasing pH, NO_3, EC and increasing NH_4 and PO4, with a strong correlation to TC and FC, positioned in the negative section of the plot as well, closer to the F2 axis. FC and TC are closer to pH and NO3 vectors. Therefore, the CCorA plot showed a strong connection between pH, nitrates, and microbial content, especially FC. The presence of NO_3 and NO_2^- in groundwater is often associated with animal and human waste, considered as indicators of remote and recent faecal pollution (KAZMI&KHAN, 2005). This was proved in our study by the high concentrations of these chemicals on the same sites where high values of FC were measured.

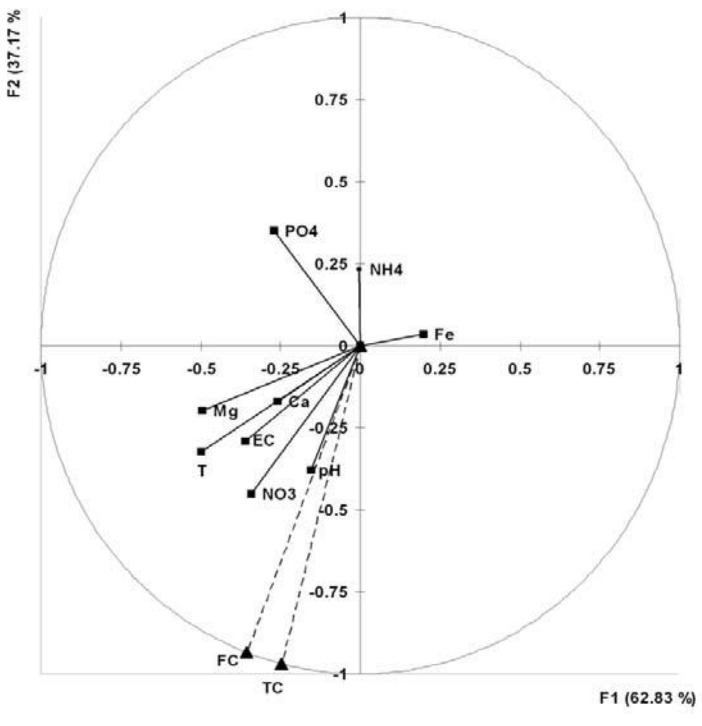


Fig. 5. Canonical Correlation Analysis based on total coliforms (TC), faecal coliforms (FC) and water physico-chemical characteristics.

The CCA calculated for meiofauna and the water physical and chemical characteristics explained 60% of the variance (Fig. 6).

The CCA analysis was computed in order to show the physico-chemical parameters mostly limiting the abundance of taxa collected. The pH, Ca, EC, Fe, NH_4 and temperature were positioned closer to the negative segment of F1 axis, whereas Mg is on its positive side. Consequently, the F1 axis, counting for 37% of the taxa-environment relationship, is a gradient of decreasing temperature, Ca, pH, EC, Fe and NH_4, and a gradient of increasing Mg. The second axis, counting for 23% of the relationship between meiofauna and environmental parameters, is correlated with increasing PO_4 and decreasing NO_3. F1 is a strong decreasing gradient of pH and Ca being related to Nematoda, Harpacticoida, Plecoptera, and Trichoptera, due to their position closer to this axis. F2 was strongly correlated to PO_4, being related with the rest of the taxa. Acarina and Collembola are positively related to F2, whereas Cyclopioda is negatively related to this axis.

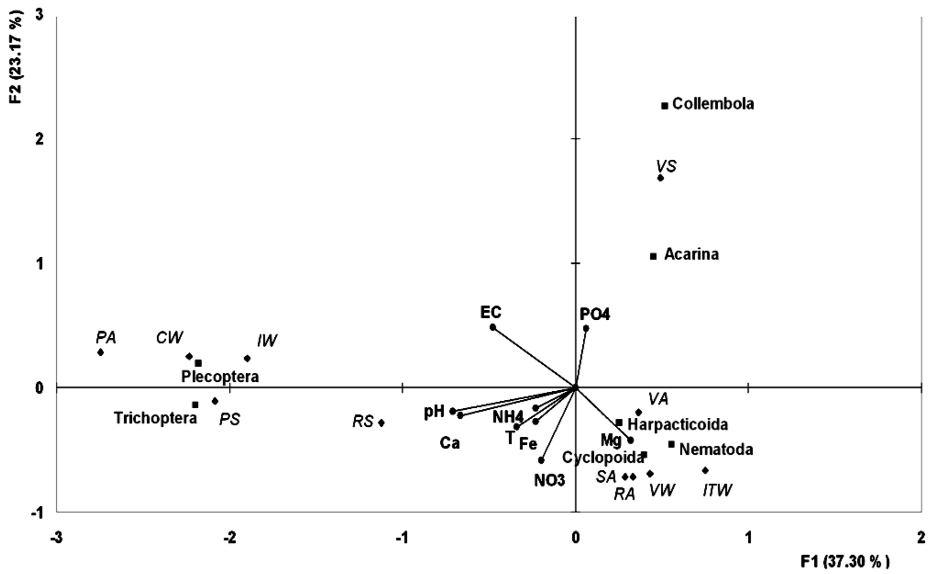


Fig. 6. Canonical Correspondence Analysis based on major taxa and water physico-chemical characteristics. *S, R, I, V, C, P, IT* – sites name, succeeded by *A*-Autumn, *W*-winter, *S*-spring.

Three taxonomic groups (Nematoda, Cyclopoida, and Harpacticoida) are positioned in the taxa-environment space, being related to increasing Mg. This indicates that Mg is affecting their abundance. The physico-chemical and microbial profile of the analysed water sources represents a condition of the poorly represented underground meiofauna in all three seasons. This was observed on site C and P that had a low number of groundwater invertebrates but, the highest microbial concentration. On site S, we counted Cyclopoida individuals only in autumn, together with a high number of coliforms. Furthermore, in winter and spring, the groundwater invertebrates were absent. At I and IT, which represent the beginning and ending sites of the water network, meiofauna was absent in autumn and spring but, poorly represented by Nematoda, Oligochaeta, Ciclopoyda, and Plecoptera in winter. This confirms that substratum has a critical impact over the meiofauna distribution and biodiversity, theory supported as well by the high biodiversity at well V.

5. CONCLUSIONS

(1) According to the national quality standards for drinking water, in the Ocoale-Ghețar-Dobreşti karst system no critical chemical pollution was found to affect the aquifer.

(2) The microbial contamination caused by human and animal waste is a serious problem; all water sources analysed were not suitable for human and animal use. This aspect needs more attention since these sources represent the water that locals use in their households and due to the high permeability of karst areas.

(3) From 7 water sources analysed, two were slightly affected by chemical and microbial contamination. Due to this fact, a drinking water network that respects the standards represents the best way to avoid the presence of coliforms and other bacterial communities in the water sources.

(4) This study can be an important contribution to understanding the relationships between microbiology and meiofauna with chemical and physical characteristics of a karst groundwater system, unaffected by intensive agriculture and modern society impact. Moreover, it can be a source of information for establishing the groundwater standards.

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