Natural attenuation potential of selected hydrokarst systems in the Carpathian Mountains (Romania)

Traian Brad, Alexandru Fekete, Mignon Severus Șandor and Cristina Purcașea

ABSTRACT

We examined the potential for natural attenuation of ten hydrokarst systems (HKS) in three mountain units in the Carpathian Mountains. We sampled in places where water enters below ground and where water emerges back to the surface in springs and is used as drinking water by the local communities. Water samples were used to assess the degree of chemical and microbiological pollution. Although the water in the ten HKS was rather clean, a general decrease in the concentration of most chemical compounds was observed along the flow path, regardless of the number of tributaries the underground stream receives. Dilution caused by tributaries could not account for the decrease in the concentration of most compounds. The contribution of other chemical immobilization processes, such as retention of pollution in the subsurface or sorption to sediment particles was suggested, in combination with the activity of microorganisms. The bacteria diversity is complex and decreases from upstream to downstream locations due to dilution with water provided by tributaries or retention of bacteria in the subsurface by adhesion to substrates. We suggest that karst can have a significant potential for natural attenuation by retaining the pollution underground, in combination with biodegradation performed by microorganisms.

Key words | bacteria community, groundwater, karst, natural attenuation, pollution

INTRODUCTION

Contamination of groundwater in karst is an apparent phenomenon wherever human activity exists. In karst terrains, contaminants originating from agricultural practices, cattle pasturing, or domestic activities are ultimately driven by the percolating water towards the subsurface with severe consequences for the adjacent aquifers and human and ecosystem health. Karst aquifers are, in general, oligotrophic with organisms that have adapted to food shortage. Additional input of organic matter into karst aquifers can cause disturbance, with proliferation of some microorganisms and death of those organisms vulnerable to increases of organic loads in their environment. Besides an extra input of carbon, the groundwater in a karst is often enriched with nutrients such as nitrogen, especially in the form of nitrates (Katz 2012) and phosphorus (Mellander et al. 2012). Karst groundwater is in many places polluted with heavy metals (Vesper 2012), pharmaceutical products (Metcalfe et al. 2011), waste waters originating from mining activities (Durand 2012), herbicides, insecticides and chlorinated compounds. Karst aquifers are often contaminated with parasite protozoans (Khaldi et al. 2011) and coliform microorganisms (Ribeiro et al. 2012). The hazard associated with the presence of this type of microorganism in groundwater is high, as resurgences (end points of the hydrokarst systems (HKS)) are frequently used as drinking water sources by the local communities.

Increasing awareness in recent decades of the poor quality and importance of groundwater recently led to the development of remedial measures. Conventional cleaning-up strategies based on physical removal of pollution followed
by controlled storage and treatment, are often hard or even impossible to apply due to high costs or limited effect. Natural attenuation of pollution is suggested as the cheapest means of remediation of polluted sites (Röling & Van Verseveld 2002). Pollution natural attenuation consists of a multitude of factors that collectively contribute to decreases in the amount of contaminants in the environment. Besides physical means for diminishing the pollution loads, such as dilution, sorption, precipitation or decantation (Christensen et al. 1994), the indigenous microorganisms transform the chemical compounds continuously to simpler and less- or non-hazardous molecules (Röling & Van Verseveld 2002). To verify whether biodegradation of pollutants occurs at the contaminated sites and to estimate whether this process is efficient enough to substitute conventional cleaning-up strategies, a detailed evaluation of the potential for in-situ biodegradation is essential. Besides a comprehensive characterization of the polluted site, this strategy requires information on the presence of degrading microorganisms in the polluted environment and how they transform and mineralize the pollutants in-situ, if they do this at significant rates, and how the process will evolve over time.

In karst areas, the local communities rely almost entirely on karst springs as drinking water sources, which are in many places subjected to upstream pollution. These water sources are often trapped and chemically treated to avoid the risks associated with pollution. The aim of this research was to characterize hydrochemically and microbiologically ten HKS located in the Western and Southern Carpathian Mountains (Romania), in order to estimate the potential of karst environments for natural attenuation of pollution.

**METHODS**

Ten HKS (the water underground course from upstream sinking point to downstream emergence) were selected in three mountain units of the Western (i.e. Pădurea Craiului and Bihor Mountains) and Southern (Șureanu Mountains) Carpathians, Romania (Figure 1, Table 1).

Water samples for chemical and microbiological analysis were collected for each investigated hydrokarst system in autumn 2011 and spring 2012 from upstream locations, after the water course passed areas with different degrees of anthropogenic impact (e.g., pastures, agricultural crops, domestic activities) and entered below ground in ponors. Samples were also collected from downstream locations after the water emerged back to the surface in springs, which are used as drinking water sources (Table 1). Due to drought-related absence of water in some upstream locations and to limited access in remote areas, parts of the investigated HKS were only sampled during either autumn 2011 or spring 2012. Samples were collected aseptically in three 2-litre sterile containers, transported to the laboratory in portable cool boxes and stored at 4 °C until further processing.

**Chemical analysis**

Physico-chemical parameters (i.e., temperature, pH and electrical conductivity) were measured in-situ using Hanna HI98128 pHep®5 and Hanna HI98311 DiST®5 (Hanna Instruments Inc., Woonsocket, RI, USA). The concentration of 30 dissolved metals and metalloids (Li, Be, B, Na, Mg, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Sn, Sb, Te, Ba, Ta, Ti, Pb, U) were analysed within 48 h of sampling by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on an Agilent 7500cx system (Agilent Technologies, Santa Clara, CA, USA). The concentration of phosphates, nitrites, nitrates and iodine was measured using a Hanna C200 Multiparameter Ion Specific Meter (Hanna Instruments, Woonsocket, RI, USA) according to the manufacturer’s instructions. The chemical oxygen demand (COD), a measure of the concentration of organic matter, was analysed volumetrically, by the Small Scale Tube Dichromate method (ISO 15705:2002).

**Microbiological analysis**

The density of coliform microorganisms was estimated using the Envirocheck Contact C (Merck KGaA, Darmstadt, Germany) according to the manufacturer’s instructions.

For each sample, a volume ranging from 1 to 2.51 of groundwater, was filtered through 0.22-μm-mesh filters (Sartorius AG, Göttingen, Germany) using a vacuum pump. The filters were aseptically cut into smaller pieces and DNA extraction was performed with the ZR Soil Microbe DNA MiniPrep™ extraction kit (Zymo Research Corporation, Irvine, CA, USA) according to the manufacturer’s instructions.
Bacteria 16S rRNA gene fragments were amplified with two sets of oligonucleotide primers. The first pair (Felske et al. 1998) consisted of forward primer 8f and the reverse primer 1512r, and was used to amplify almost the complete (approximately 1.5 kb) 16S rRNA gene fragments present in the initial DNA extracts. The obtained polymerase chain reaction (PCR) products were used later in amplified ribosomal DNA restriction analysis (ARDRA). The second pair of primers was formed of F357-GC and R518 (Muyzer et al. 1993), and the ~200 bp-long products were used in denaturing gradient gel electrophoresis (DGGE) analysis.

PCR-amplification was performed in a 25-μl volume containing 0.4 μM forward primer, 0.4 μM reverse primer, 0.4 mM of each deoxynucleoside triphosphate (dNTP), 2 mM MgCl₂, 1U Taq polymerase (Thermo Scientific, Waltham, MA, USA), PCR buffer and 1 μl of undiluted DNA template. Amplification was performed in an MJ RESEARCH PTC-200 Peltier Thermal Cycler (Bio-Rad, Hercules, CA, USA), using an initial denaturation of 95 °C for 2 min,
followed by 35 cycles of 95 °C for 30 s, 54 °C for 60 s, and 72 °C for 90 s, and a final elongation at 72 °C for 5 min.

ARDRA was performed in a 20-μl volume containing 5U of BshFI (HaeIII) restriction enzyme with 2 μl corresponding buffer, 7.5 μl water and 10 μl PCR product. After 30 min digestion at 37 °C, electrophoresis was performed for 1 h and 10 min at 100 V on 3% agarose gels.

DGGE was performed with a DGGE-4801-220 instrument (C.B.S. Scientific Company Inc., Del Mar, CA, USA). PCR products were loaded onto 0.75-mm-thick 8% (wt/vol) polyacrylamide (ratio of acrylamide to bisacrylamide, 37.5:1) gels. A 30 to 55% linear denaturing gradient was used, where 100% denaturant was defined as 7 M urea and 40% (vol/vol) formamide. Electrophoresis was performed in 1× TAE buffer (40 mM Tris-acetate, 1 mM Na-EDTA; pH 8.0) at 200 V and 60 °C for 4 h. The gels were stained in 1× TAE buffer containing 1 μg/ml ethidium bromide, and their images were analysed by band-assignment, which was performed manually.

RESULTS AND DISCUSSION

Hydrochemistry

Physico-chemical measurements and chemical analyses were performed with the aim of establishing whether there were hydrochemical differences between samples collected upstream (ponors) and downstream (springs) within each hydrokarst system (HKS). All chemical analyses revealed that the water in the analysed samples was relatively clean, with no particular increase of contaminant concentration. The water temperature values of the 20 sites varied between 2.5 and 13.8 °C and increased (paired t-test, $P < 0.01$) downstream by 25.65% (Figure 2(a)). The increase of water temperature downstream is an apparent phenomenon in karst areas due to altitude differences and especially to the contact with warmer calcareous rocks.

The pH decreased by 2.28% from upstream to downstream locations (paired t-test, $P < 0.05$) with values ranging between 7.4 and 8.8 (Figure 2(b)).

The concentration values of all measured chemical parameters were, in general, lower than the accepted limits in drinking water according to the European Union regulations (EU limits are provided in Figure 2 legends for each parameter). However, differences in hydrochemistry between upstream and downstream locations were apparent for concentrations of Na (25.02%), Al (15.06%), K (28.75%), Mn (73.73%), Fe (56.64%), Cu (37.83%), Zn (45.51%), As (35.25%), PO$_4$ (46.46%), NO$_2$ (20.12%) and NO$_3$ (16.5%) (Figures 2(c)–2(m), respectively), which decreased (% general decrease in parentheses) from ponor to spring (paired t-test, $P < 0.05$).

Table 1 | Description of the ten HKS prospected within the present study

<table>
<thead>
<tr>
<th>Mt. Hydrokarst system ponor – spring</th>
<th>Season</th>
<th>I</th>
<th>D</th>
<th>E</th>
<th>L (m)</th>
<th>T (h)</th>
<th>H (m)</th>
<th>UT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pădurea Craiului Mountains I Potriva Cave – Aștileu Cave</td>
<td>autumn</td>
<td>8</td>
<td>21</td>
<td>8</td>
<td>2620</td>
<td>10</td>
<td>107</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>spring</td>
<td>16</td>
<td>16</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II Gălaşeni Cave – Groapa Moțului Spring</td>
<td>autumn</td>
<td>2</td>
<td>28</td>
<td>7</td>
<td>1750</td>
<td>13</td>
<td>95</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>spring</td>
<td>7</td>
<td>25</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III Toaie Ponor – Dâmișenilor Spring</td>
<td>autumn</td>
<td>13</td>
<td>19</td>
<td>5</td>
<td>3550</td>
<td>90</td>
<td>255</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>spring</td>
<td>17</td>
<td>14</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV Runcșor Ponor – Bulbuci Spring</td>
<td>autumn</td>
<td>14</td>
<td>15</td>
<td>8</td>
<td>950</td>
<td>11</td>
<td>140</td>
<td>1</td>
</tr>
<tr>
<td>V Albioara Ponor – Izbucul Topliței de Roșia</td>
<td>autumn</td>
<td>5</td>
<td>25</td>
<td>7</td>
<td>2500</td>
<td>89</td>
<td>155</td>
<td>5</td>
</tr>
<tr>
<td>VI Cociului Cave- Water Cave at Bulz</td>
<td>spring</td>
<td>8</td>
<td>26</td>
<td>3</td>
<td>3100</td>
<td>29</td>
<td>270</td>
<td>4</td>
</tr>
<tr>
<td>Bihor Mountains VII Vuiaga Spring – Poliței Spring</td>
<td>autumn</td>
<td>16</td>
<td>19</td>
<td>2</td>
<td>1360</td>
<td>10</td>
<td>225</td>
<td>1</td>
</tr>
<tr>
<td>VIII Colba Mică Cave – Tăuze Spring</td>
<td>autumn</td>
<td>15</td>
<td>14</td>
<td>8</td>
<td>2650</td>
<td>322</td>
<td>110</td>
<td>1</td>
</tr>
<tr>
<td>Șureanu Mountains IX Ponorici Cave – Cioclovina cu Apă Cave</td>
<td>spring</td>
<td>5</td>
<td>27</td>
<td>5</td>
<td>1550</td>
<td>8</td>
<td>180</td>
<td>2</td>
</tr>
<tr>
<td>X Fântâna Socilor Ponor – Cocolbea Cave</td>
<td>spring</td>
<td>11</td>
<td>22</td>
<td>4</td>
<td>4600</td>
<td>126</td>
<td>460</td>
<td>0</td>
</tr>
</tbody>
</table>

(I) is the number of chemical elements with increasing concentration, (D) is the number of chemical elements with decreasing concentration, (E) is the number of chemical elements whose concentration was equal in upstream and downstream locations, (L) stands for the aerial distance between ponor and spring, (T) is the residence time, (H) is the altitude difference between ponor and spring and (UT) is the number of known underground tributaries (data collected from Orașeanu & Iurkiewicz (2010)).
Figure 2 | Physico-chemical measurements (i.e., temperature (Figure 2(a)), pH (Figure 2(b)), EU limit 6.5–9.5 and electrical conductivity – EC (Figure 2(o), EU limit 2500 μS/cm) and chemical analysis (measurements of concentration in ppm of Na (Figure 2(c), EU limit 200 mg/l), Al (Figure 2(d), EU limit 0.2 mg/l), K (Figure 2(e), EU limit no reference), Ca (Figure 2(n), EU limit 200 mg/l), Mn (Figure 2(f), EU limit 0.05 mg/l), Fe (Figure 2(g), EU limit 0.3 mg/l), Cu (Figure 2(h), EU limit 2 mg/l), Zn (Figure 2(i), EU limit 5 mg/l), As (Figure 2(j), EU limit 0.01 mg/l), PO₄ (Figure 2(k), EU limit no reference), NO₂ (Figure 2(l), EU limit 0.5 mg/l), NO₃ (Figure 2(m), EU limit 50 mg/l)) on water samples obtained from places located upstream (dark bars) and downstream (white bars) of the ten HKS prospected within this study in autumn 2011 (A) and spring 2012 (S). Each hydrokarst system is noted with roman numerals that relate to numbers in Figure 1 and Table 1. (continued)
Lower nitrite (NO$_2$) and nitrate (NO$_3$) concentration values were also obtained in downstream locations compared to upstream locations (Figures 2(l) and 2(m)). The nitrate concentration was in general 100$\times$ larger compared to the concentration of nitrites because of the obvious nitrification processes in the environment. The concentration of
nitrate was significantly related to the concentration of nitrite ($r = 0.53$, $P < 0.01$). The phosphates concentration ranged from 0.02 to 11.8 mg/l in samples collected upstream of the prospected HKS, and had values between 0.03 to 4.2 mg/l in downstream samples (Figure 2(k)).

Larger amounts of phosphates in upstream samples can be related to agricultural practices of adding cattle manure or chemical fertilizers to the soil. The concentration of phosphates appeared to decrease along the flow path (paired $t$-test, $P = 0.07$, average concentration 2.45 mg/l upstream, and 1.31 mg/l downstream). The amount of organic matter (analysed as COD-K$_2$Cr$_2$O$_7$) in the samples collected from the investigated HKS was, in general, below the accepted limits in drinking water (<10 mg O$_2$/l), with no significant differences between upstream (range 2–9 mg O$_2$/l) and downstream locations (range 2–12 mg O$_2$/l, paired $t$-test, $P > 0.05$).

On the contrary, the concentration of calcium and the electrical conductivity values (Figures 2(n) and 2(o)) increased at the HKS resurgences (paired $t$-test, $P < 0.01$) by 37.83% and 28.77%, respectively. A strong relationship was noticed between calcium concentration and electrical conductivity values ($r = 0.83$, $P < 0.01$). The increase in the concentration of calcium is mostly due to the dissolution of CaCO$_3$ while water passes through karst (Ford & Williams 2007).

In general, a larger number of chemical elements (average elements number 20.85 ± 5.08) whose concentration decreased from upstream to downstream locations, was obtained in comparison with the number of elements
(average elements number 10.45 ± 4.99) whose concentration increased while water passed through the subsurface (t-test, \( P < 0.01 \)), regardless of the number of tributaries that each karst system receives (Table 1).

Dilution with groundwater provided by the tributaries that the karst systems receive underground cannot thus explain entirely the decrease in the concentration of the various chemical compounds from upstream to downstream locations. When dilution is solely responsible for the decrease in the concentration of the various chemical compounds, such reduction is then expected for all measured chemical elements, especially when the HKS are recharged by cleaner waters. Likewise, when an HKS has no underground tributary, no major changes are expected in water chemistry from the place where the water enters below ground and where it appears back to the surface downstream, except the dissolution processes that occur as water passes rocks with variable geology. In our study, a reduction from ponors to springs was obtained in the concentration of several chemical parameters when more tributaries feed two of the prospected HKS (i.e., HKS V and VI), while the concentration of other chemical parameters in these two systems increased from upstream to downstream locations. For HKS with no tributaries (i.e., HKS II and X), changes in hydrochemistry have also occurred along the flow path. The concentration of several parameters decreased, while the concentration of other parameters increased as the water flowed through the subsurface karst environment. Other types of immobilization of chemical compounds underground, such as sorption to sediment particles, decantation of solid particles containing pollutants, contaminant condensation and dispersion, may thus also contribute to the reduction of the concentration of these chemical compounds downstream.

**Bacteria community characterization**

The density of coliform microorganisms in water samples obtained from upstream and downstream locations in the ten HKS investigated in the Carpathian Mountains (Romania) was estimated using culture-dependent techniques. Coliform microbes were only detected in samples collected in autumn 2011, when their density in upstream locations was comparable to that obtained in samples collected downstream of the HKS (i.e., 10^2–10^4 colony forming units (CFU)), and were no longer detected in spring 2012. The presence of coliform bacteria in samples collected in autumn can relate to cattle pasturing and the various household activities with waste deposition in the rural areas located upstream of the karst systems in our study. The activity of coliform bacteria appears to decrease during the winter as no coliforms were detected in the following spring.

ARDRA and DGGE fingerprinting were used to evaluate the diversity of bacterial communities present in water samples collected from the upstream and downstream locations of the ten prospected HKS. The bacterial community profiles, as revealed in ARDRA (Figure 3(a)) and DGGE (Figure 3(b)) analyses were complex and presented large variations between upstream and downstream locations. In most of the cases, only a reduced number of 16S-rRNA gene fragments from the upstream community profiles were recovered in downstream profiles. However, additional DNA bands were present in the downstream ARDRA and DGGE profiles as compared to those obtained from upstream samples for nearly all investigated HKS, suggesting the occurrence of different bacterial species at the two end-points of the HKS. The appearance of new microorganisms downstream of the HKS occurs mostly during autumn, when the amount of water flowing through karst is, in general, lower than in spring. More common bacterial species are expected at higher water levels in spring due to larger debits and intense mixing.

Overall, a lower bacterial diversity was obtained (paired t-test, \( P = 0.02 \)) in samples collected downstream of the HKS (average number of bands 3.92 ± 2.02) as compared to the bacterial diversity from upstream locations (average number of bands 7.54 ± 4.91), as revealed by the number of 16S-rRNA fragments from DGGE profiles (Figure 3(b)). Accordingly, ARDRA profiles suggest a change of the diverse bacterial community in water samples during the passage through karst with a higher number of 16S-rRNA gene fragments upstream, and a general reduced number of digested DNA fragments downstream, except for HKS IV-autumn, VI-autumn, VIII-autumn and I-spring (Figure 3(a)). The bacterial diversity (number of bands in DGGE profiles) was not related (Pearson correlation, \( P > 0.05 \)) to the length of the hydrokarst system or to the water residence time in the subsurface.
The decrease in the number of DNA fragments could correspond to a lower diversity of microorganisms in downstream locations. This phenomenon could be partly due to the dilution with groundwater provided by the HKS tributaries. As microorganisms usually attach to substrates, and have higher metabolic activity in sediments than in corresponding groundwater (Albrechtsen & Wind-\textit{ing} 1992), the decrease in bacterial diversity from upstream to downstream locations could also be due to microorganism retention underground by adhesion to surfaces and substrates.

The caves are sometimes considered as the below-ground conduits of surface rivers, with the same hydrochemistry, and suggestions are made in order to consider karst springs with the same suspicions as surface streams (White 2012). This study showed that, in most cases, the concentration of most chemical parameters analysed decreased along the subsurface water course, and the diversity of bacteria was reduced downstream of the karst systems.

In karst environments located in mountain areas characterized by high hydraulic gradients, the groundwater retention time is short. This implies faster rates of contaminant being washed out compared to rates of biodegradation (Einsiedl \textit{et al.} 2009). In these settings, contaminant biodegradation has, thus, smaller or non-existent chances to occur. Therefore, research on natural attenuation in karst environments was apparently avoided because of this relatively incorrect perception that contaminants are quickly washed out of the aquifers, with little residence time in the subsurface (Painter \textit{et al.} 2011). On the contrary, in phreatic aquifers developed in unconsolidated sediments on relatively flat terrains, the groundwater flow can be very slow (Van Breukelen \textit{et al.} 2005), and consequently the time of water retention underground is long. In this type of aquifer, biodegradation of contaminants is more likely to occur as degrading microorganisms present in these environments can actively transform the polluting compounds in situ and are not washed away from the system very fast.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Band assignment-based analysis of ARDRA (a) and DGGE (b) community profiles of bacteria obtained from upstream (dark bands) and downstream (white bands) samples collected from the ten HKS prospected within the present study. The HKS are noted with roman numerals that relate to the numbers in Table 1.}
\end{figure}
A few studies, however, have used chemical tracers to reveal the potential that karst aquifers have to attenuate pollution. As an example, caffeine was biodegraded faster while transiting a karst aquifer in southwest Germany, compared to the conservative reference tracer uranium (Hillebrand et al. 2002). Another tracer was resazurin which, in combination with sodium chloride as conservative tracer, was used in a study where the metabolically active transient storage was quantified in a surface stream in comparison with an adjacent hyporheic environment (Haggerty et al. 2009). Sometimes, karst aquifers may store considerable volumes of water, thus increasing their time of water retention. In these settings, the migration of contaminants is slower and their mass can reduce with the aid of degrading microorganisms under proper redox and other geochemical conditions, i.e., availability of limiting nutrients.

The decrease of various compounds’ concentration along the main karst drainage pathway is often linked with dilution caused by inputs of clean groundwater entering the HKS (White 2012), but also with the filtration capacity of limestone (Kresic et al. 2007). Hydrocarbons and chlorinated compounds are mostly insoluble in water and thus easily trapped in sediments or in the epikarst before reaching the underground water course (White 2012). Likewise, polycyclic aromatic hydrocarbons (PAHs) are retained in the upper part of the karst and become transportable only at major floods due to solid particles mobilization (Schwarz et al. 2011). Heavy metals with different degrees of toxicity, such as chromium, cadmium, lead, or zinc, can precipitate underground, they can be adsorbed to clay particles and other sediments, or they can be incorporated into iron or manganese oxides (White 2012).

CONCLUSIONS

In spite of the general opinion that contaminants are expelled very fast out of karst aquifers, and the generally accepted hypothesis on the absence of natural attenuation-related processes in karst, our results suggest that karst can have significant potential for natural attenuation by retaining the mass of pollution below ground. We observed a general decrease in the concentration of most chemical elements and a reduction of microbial diversity from above sinking points to the springs located downstream. This information can be useful in the management of karst waters, with the aim of improving drinking water quality. However, further investigation of the physical retaining of contaminants in karst and of the metabolism of naturally-occurring microorganisms in relation to contaminant biodegradation processes, is needed in order to understand the mechanism of natural attenuation in karst.

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