The spectral absorption coefficient at 254 nm as a real-time early warning proxy for detecting faecal pollution events at alpine karst water resources

H. Stadler, E. Klock, P. Skritek, R. L. Mach, W. Zerobin and A. H. Farnleitner

ABSTRACT

Because spring water quality from alpine karst aquifers can change very rapidly during event situations, water abstraction management has to be performed in near real-time. Four summer events (2005–2008) at alpine karst springs were investigated in detail in order to evaluate the spectral absorption coefficient at 254 nm (SAC254) as a real-time early warning proxy for faecal pollution. For the investigation Low-Earth-Orbit (LEO) Satellite-based data communication between portable hydrometeorological measuring stations and an automated microbiological sampling device was used. The method for event triggered microbial sampling and analyzing was already established and described in a previous paper. Data analysis including on-line event characterisation (i.e. precipitation, discharge, turbidity, SAC254) and comprehensive E. coli determination (n > 800) indicated that SAC254 is a useful early warning proxy. Irrespective of the studied event situations SAC254 always increased 3 to 6 hours earlier than the onset of faecal pollution, featuring different correlation phases. Furthermore, it seems also possible to use SAC254 as a real-time proxy parameter for estimating the extent of faecal pollution after establishing specific spring and event-type calibrations that take into consideration the variability of the occurrence and the transferability of faecal material. It should be highlighted that diffuse faecal pollution from wildlife and livestock sources was responsible for spring water contamination at the investigated catchments. In this respect, the SAC254 can also provide useful information to support microbial source tracking efforts where different situations of infiltration have to be investigated.

Key words | diffuse faecal pollution, drinking water resources, early warning proxy parameter, microbial source tracking, satellite based automated event sampling, spectral absorption coefficient at 254 nm

INTRODUCTION

Groundwater from alpine/mountainous karst aquifers plays a significant role for public water supply in many European countries; for example the city of Vienna gets more than 90% of its water from alpine karst springs (Ford & Williams 1996). Alpine karst spring water resources are considered to have good raw water quality. It was demonstrated that alpine spring water issuing from aquifer areas with prevailing matrix-flow conditions reveal extremely low biological activities and thus shows excellent bio-stability. This characteristic of the water is a result of an ongoing self-purification process within the mountain (Wilhartitz et al. 2009). Unfortunately, strong precipitation events in alpine catchments can lead to transient contamination of spring water due to temporarily activated conduits (Drew & Hötzl 1999;
Doerfliger et al. 1999). During such situations, surface associated microorganisms and nutrients are transferred to the spring water and rapidly bypass zones with sufficient self-purification capacities. Although contamination with surface associated microbes/nutrients is a general nuisance for spring water quality (because it gives the possibility for re-growth activities and decreases bio-stability), pollution from intestinal pathogens from human and animal faecal excreta is considered the most critical and immediate health hazard for drinking water supply.

Sustainable management of microbial hazards in alpine karst water supplies is most suitably based on an integrated system including the following components: i) protection and minimisation measures against faecal pollution sources in the catchment area, ii) spring management using spring water only when criteria for the raw water quality are below the thresholds, and finally, iii) sufficient final water treatment to ensure drinking water quality that meets health risk based (i.e. infection or diseases related) microbial water quality targets (Fewtrell & Bartram 2001; WHO 2004). Efficient implementation and maintenance of such a multiple barrier approach demands sound information on several system levels (Farnleitner et al. 2008). For routine in-field monitoring, Escherichia coli turned out to be the most efficient standard faecal indicator bacterium available for the sensitive detection of event based faecal pollution in alpine spring water (Farnleitner et al. 2010a, b). E. coli is present in high average concentrations in human, livestock and wildlife faecal sources in mountainous areas (log 7.0–log 8.4 CFU per g faecal material). Although potentially found in soil from areas where livestock is held during summer, it does not occur ubiquitously in alpine mountain soil habitats (Texier et al. 2008). According to the average length of high water events (around days to weeks) and the observed microbial die-off under ambient spring water conditions (around 0.10 to 0.15 day$^{-1}$), E. coli qualifies as an indicator for recent faecal pollution from surface runoff at alpine karst spring habitats (Stadler et al. 2008). Furthermore, it could also be demonstrated that a correlation exists between E. coli and other indicators with different persistence and morphology (i.e. E. coli, enterococci, aerobic spore formers, Clostridium perfringens spores, aerobic spore formers, total cell numbers, and virus like particles) during event situations. E. coli was thus suggested as a very suitable indicator for the potential surface input of faecal-associated pathogens irrespective of their persistence (Farnleitner et al. 2007). Most importantly, E. coli can reliably be detected in spring water with commercially available detection kits (detection level: 1 CFU of E. coli per 100 ml sample). These kits enable analyses directly in the field without the need of specialised microbiological laboratories (Stadler et al. 2008).

Cultivation-based detection technologies (including standard and commercially available kits) require more than an 8-hour working day for the enumeration of E. coli. Even if alternative rapid monitoring tools are used, such as enzymatic direct detection (Fiksdal & Tryland 2008), sensitive quantification of E. coli (i.e. <1,000 cells or CFU per litre) still needs more than one hour. Efficient spring water abstraction management—i.e. using spring water for drinking water production only when raw water quality criteria are below the set thresholds (c.f. multi barrier concept, paragraph above)—often requires reaction times of ≤1 hour. For example, during thunderstorm events, E. coli concentrations in spring water can increase dramatically within a very short (<1 hour) time period (Stadler et al. 2008). Therefore although essential for many purposes (i.e. system and risk assessment, internal performance monitoring, surveillance, etc.), direct microbial detection seems inappropriate for supporting near real-time spring water abstraction management.

The term “proxy parameter” or “proxy data” is used in this paper according to the report of the Intergovernmental Panel on Climate Change 2007 (IPCC 2007). As the current definitions of “surrogate parameter” and “proxy parameter” are not clearly confined, we use the term “proxy parameter” in this paper for parameters, which can be used instead of the intrinsic parameters, which are not available in real-time, but only after laboratory analyses. Whereas we define the term “surrogate parameter” in the case, where the intended parameter is not available at all or can not be measured in any way. Since the resulting delay can be very crucial in quality surveillance systems, the potential proxy candidates have to show faecal pollution events in real-time or near real-time by in-situ measurements with a robust relation to the parameter they are used for.

At the investigated karst springs, a basic monitoring system is permanently established, where discharge, electrical conductivity, water temperature, SAC254,
and turbidity are measured continuously with a time increment of 10 minutes.

The aim of this study was: i) to evaluate whether the SAC254 of the spring water, measured by in-situ submergible measuring devices, can be used as a real-time early warning proxy for faecal pollution events due to surface runoff, and ii) to further investigate its suitability for the estimation of E. coli concentrations in the spring water. Preliminary investigations already indicated the potential usefulness of the SAC254 as an early warning proxy (Stadler et al. 2008). In contrast to the limited data set there, this study analyses in detail four late summer events at three different alpine karst springs from 2005 to 2008. Due to our limited capacities, the complexity of assembling and the costs of the analyses, we could only investigate four events during this period. To enable appropriate resolution, the investigation was based on portable hydrometeorological measuring stations coupled with LEO-satellite based data transfer and automated microbiological sampling (Stadler et al. 2008).

**MATERIALS AND METHODS**

**Investigated alpine karst springs and during summer flood events**

The three studied alpine karst springs (LKAS2, LKAS6, LKAS8) are located in the Northern Calcareous Alps and drain into Triassic limestone aquifers. From the year 2005 to 2008, four events at three different springs were investigated. The mean discharges, based on the respective catchment size and different mean altitudes, ranged from 250 to 5100 litres per second. As with typical karst springs from limestone aquifers, the discharge coefficient—based on daily mean values—exceeded the ratio of 1:11 at all springs (Table 1). All monitored events were caused by precipitation of up to 157 mm within 48 hours, where the amount of rainfall within the first day ranged between 28 mm and 53 mm (Table 1). These boundary conditions significantly effected the reactions both in changing of the discharge and the quality parameters. Table 1 shows a summary of the characteristic hydrological data of the springs. Please note that representative graphs of the observed E. coli dynamics from the investigated events 2005/2006 at the LKAS2 were previously presented to show the applicability of LEO-based event monitoring and automated microbiological sampling procedures (Stadler et al. 2008).

**Sources of faecal contamination**

The catchment areas of the springs show different land use: pasture and forest areas are dominant, but tourist activities of different scales also exist. Since the areas reach altitudes of up to 2,200 m above sea level, also alpine grasslands, krummholz areas, and wasteland also appear. Recent efforts concerning faecal hazard characterisation and microbial source tracking at the springs indicated ruminant animals as the main faecal sources in all investigated catchments (Reischer et al. 2008, Farnleitner et al. 2010a,b). For example, as much as 99.9% of the daily deposited intestinal E. coli populations in the LKAS6 environment could be allocated to wildlife (42.4%) or livestock ruminants (57.6%). In contrast to ruminant animal sources, the human faecal pollution sources were negligible (Farnleitner et al. 2010a,b).

**Automated LEO-satellite based flood analysis**

Since the system for automated flood analysis was already described in detail (Stadler et al. 2008), just a brief description is presented here. The system enables fully automated event sampling and near real-time availability of data. By means of networking via Low Earth Orbiting (LEO) satellites, data from the precipitation station (PS) in the catchment area are brought together with data of the spring sampling station (SSS) without the need of terrestrial infrastructure for communication and power supply. Therefore, a completely automated event sampling procedure is possible. Furthermore, the whole course of input and output parameters, like precipitation (input system), discharge (output system) and the status of the sampling system, is transmitted via LEO-Satellites to a Central Monitoring Station (CMS) which can be linked with a web-server to have unlimited near real-time data access (Heiner 2005; Stadler et al. 2009). When an event occurs, an automatically generated notice is transmitted to the local service team of the sampling station via internet, Global System for Mobile Communication (GSM), General Packet Radio Service (GPRS) or LEO-Satellites (Stadler et al. 2008).
Table 1 | Basic data of the investigated events. Note that “general data” are determined from the total observation period of the springs and the amount of precipitation is the event related amount

<table>
<thead>
<tr>
<th>General data</th>
<th>LKAS2</th>
<th>LKAS2</th>
<th>LKAS8</th>
<th>LKAS8</th>
</tr>
</thead>
<tbody>
<tr>
<td>MQ [l s^{-1}]</td>
<td>5,100</td>
<td>5,100</td>
<td>589</td>
<td>254</td>
</tr>
<tr>
<td>NQT [l s^{-1}]</td>
<td>443</td>
<td>443</td>
<td>170</td>
<td>87</td>
</tr>
<tr>
<td>HQT [l s^{-1}]</td>
<td>34,273</td>
<td>34,273</td>
<td>1,950</td>
<td>1,379</td>
</tr>
<tr>
<td>HQT/NQT</td>
<td>1:77:4</td>
<td>1:77:4</td>
<td>1:11:5</td>
<td>1:15:9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Event related data</th>
<th>LKAS2 event 2005</th>
<th>LKAS2 event 2006</th>
<th>LKAS8 event 2007</th>
<th>LKAS6 event 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitation [mm] 1st day</td>
<td>43.0</td>
<td>28.3</td>
<td>53.0</td>
<td>52.7</td>
</tr>
<tr>
<td>Precipitation [mm] 2nd day</td>
<td>45.3</td>
<td>128.4</td>
<td>0.2</td>
<td>18.9</td>
</tr>
<tr>
<td>Precipitation [mm] total</td>
<td>88.3</td>
<td>156.7</td>
<td>53.2</td>
<td>71.6</td>
</tr>
<tr>
<td>Reaction time [hh:mm]</td>
<td>02:45</td>
<td>08:15</td>
<td>03:10</td>
<td>05:19</td>
</tr>
<tr>
<td>Time of precipitation trigger</td>
<td>14.08.2005 18:10</td>
<td>03.08.2006 13:25</td>
<td>09.08.2770 18:45</td>
<td>15.08.2008 13:43</td>
</tr>
<tr>
<td>Time of reference sample</td>
<td>14.08.2005 18:23</td>
<td>03.08.2006 14:40</td>
<td>09.08.2007 18:51</td>
<td>27.08.2008 15:25</td>
</tr>
<tr>
<td>End of automated sampling</td>
<td>24.08.2005 07:15</td>
<td>16.08.2006 03:51</td>
<td>07.09.2007 04:50</td>
<td>27.08.2008 10:10</td>
</tr>
<tr>
<td>Q start [l s^{-1}]</td>
<td>4,536</td>
<td>4,553</td>
<td>352</td>
<td>214</td>
</tr>
<tr>
<td>Q max [l s^{-1}]</td>
<td>23,524</td>
<td>45,713</td>
<td>1,781</td>
<td>539</td>
</tr>
<tr>
<td>Δ Q [l s^{-1}]</td>
<td>18,988</td>
<td>41,160</td>
<td>1,429</td>
<td>325</td>
</tr>
<tr>
<td>SAC254 min [abs m^{-1}]</td>
<td>1.78</td>
<td>0.65</td>
<td>0.55</td>
<td>0.52</td>
</tr>
<tr>
<td>SAC254 max [abs m^{-1}]</td>
<td>10.02</td>
<td>6.31</td>
<td>2.48</td>
<td>8.18</td>
</tr>
<tr>
<td>Δ SAC254 [abs m^{-1}]</td>
<td>8.24</td>
<td>5.66</td>
<td>1.93</td>
<td>7.66</td>
</tr>
<tr>
<td>Lead time SAC254 to E. coli [hh:mm]</td>
<td>03:00</td>
<td>06:00</td>
<td>06:00</td>
<td>05:00</td>
</tr>
<tr>
<td>E. coli min [Colilert 100 ml^{-1}]</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>E. coli max [Colilert 100 ml^{-1}]</td>
<td>307.6</td>
<td>178.9</td>
<td>1,553.1</td>
<td>2,419.6</td>
</tr>
<tr>
<td>E. coli [Colilert 100 ml^{-1}]</td>
<td>302.6</td>
<td>176.9</td>
<td>1,553.1</td>
<td>2,417.6</td>
</tr>
<tr>
<td>Exponential coefficient E. coli versus time</td>
<td>11.85</td>
<td>3.74</td>
<td>3.39</td>
<td>38.89</td>
</tr>
<tr>
<td>Linear correlation SAC254 versus E. coli: slope</td>
<td>0.0097</td>
<td>0.0079</td>
<td>0.0006</td>
<td>0.0017</td>
</tr>
<tr>
<td>Linear correlation SAC254 versus E. coli: R²</td>
<td>0.95</td>
<td>0.78</td>
<td>0.78</td>
<td>0.99</td>
</tr>
<tr>
<td>Turbidity min [FNU]</td>
<td>0.23</td>
<td>0.21</td>
<td>0.07</td>
<td>0.09</td>
</tr>
<tr>
<td>Turbidity max [FNU]</td>
<td>2.90</td>
<td>12.06</td>
<td>3.77</td>
<td>2.55</td>
</tr>
<tr>
<td>Δ Turbidity [FNU]</td>
<td>2.67</td>
<td>11.85</td>
<td>3.70</td>
<td>2.46</td>
</tr>
<tr>
<td>Number of samples: automated</td>
<td>128</td>
<td>121</td>
<td>268</td>
<td>214</td>
</tr>
<tr>
<td>Number of samples: manual</td>
<td>25</td>
<td>28</td>
<td>44</td>
<td>29</td>
</tr>
</tbody>
</table>

Abbreviations: MQ—mean discharge, NQT—lowest daily mean discharge, HQT—highest daily mean discharge, HQT/NQT—discharge coefficient, SAC254—spectral absorption coefficient at 254 nm.

On-line measurements of hydrological and physicochemical parameters

Precipitation was measured with an ARG100 tipping bucket (Campbell Scientific Ltd., Loughborough, UK) with a resolution of 0.2 mm and recorded every 5 minutes with a GEALOG-Compact (Logotronic, Vienna, Austria). Discharge (pressure probe PDCR1830 from Druck, London), electrical conductivity and water temperature (WTW Tetracon 325 sensor from WTW, Weilheim, Germany) were measured with a GEALOG-S System (Logotronic, Vienna, Austria) with a time increment of 15 minutes. Turbidity and SAC254 were registered every 10 minutes with s:can sensors with integrated data logger (s:can, Vienna, Austria).
where SAC254 is measured without previous filtration, but with mathematical correction after a matrix specific calibration. Cross-sensitivities of such optical systems are known, but at low concentrations like in our case, they are unlikely of any significance. The ORBCOMM data communication was performed by PANASONIC Satellite modems KX-G7101 (Fukuoka, Japan).

Automated microbiological sampling and \textit{E. coli} field enumeration

The method of event triggered microbial sampling and analyzing was established in former investigations and described in detail (Stadler \textit{et al.} 2008).

Just briefly, for the automatic sampling procedures at the spring outlet, 24 sterile disposable plastic vessels (120 ml, IDEXX) were directly positioned in the automatic sampling device. Samples were recovered from the auto-sampler at least every 24 h and analysed immediately by Colilert-18 (IDEXX, Austria). Auto-samplers were placed directly at the spring outlets with dark and ambient spring temperature conditions (5°C). The time span between sampling by the auto-sampler and the time of analysis by Colilert-18 (i.e. storage time) was recorded for each collected sample. Individual storage times were used to estimate and to correct concentration losses by \textit{E. coli} inactivation kinetics as described previously (Stadler \textit{et al.} 2008). The performance of the \textit{E. coli} field enumeration by the Colilert-18 system compares favourably with the ISO 9308-1 standard reference method for the spring water habitat considered (Stadler \textit{et al.} 2008).

\section*{RESULTS}

Microbiological measurements and validation of results

In total, 731 microbiological samples from the four investigated summer events (2005–2008) were collected by the automated sampling procedures during the four investigated summer events 2005–2008 and analysed for \textit{E. coli} concentrations following the previously established method by Stadler \textit{et al.} 2008 (Table 1). The comprehensive data set allowed a detailed correlation analysis with SAC254 for differing event phases and springs.

To evaluate correctness of the auto-sampling procedures a small set of samples at every observed event from 2005 to 2008 was taken manually and analysed immediately with the Colilert-18 system. These results were than compared to the corresponding results from the auto-sampling. A maximum time difference of 30 minutes was accepted for auto-sampling vs. manually sampling. Using this time lag, 84 samples could be correlated. The correlation analysis (Figure 1) showed that both methods yielded comparable and reliable data sets. However, a perfect correlation was not expected because of the accepted time lag between manual and automatic sampling (because exact simultaneous sampling was not possible from a technical point of view).

As shown in Figure 2 all investigated events revealed an exponential rise of \textit{E. coli} during the first event-phase (i.e. rise of discharge). The slope and the exponential rise of \textit{E. coli} differed according to the respective hydrological conditions at the investigated springs (Figure 2, Table 1). The observation of the exponential increase of the \textit{E. coli} was only possible because of the high amount of time-based sampling during the first phase of the event. This frequent sampling was only attainable by using the automated LEO-Satellite based system.

\section*{SAC254 AS AN EARLY WARNING PROXY FOR EVENT BASED FAECAL POLLUTION DETECTION}

SAC254 as early warning proxy

SAC254 is qualified as an early warning proxy parameter for faecal pollution in karst aquifers because the SAC254
always rose earlier than the compared concentrations of E. coli in the spring water at the investigated events. Irrespective of the investigated spring or event type (i.e. LKAS2 or LKAS6 or LKAS8), the lead time—i.e. the time span between the significant increase of SAC254 and the significant increase of E. coli concentrations—ranged between three and six hours (Table 1). This lead time is most likely caused by differences in the transport behaviour of E. coli and fractions of dissolved organic carbon measured by SAC254. The observed lead time is a basic requirement for surveillance systems that allow water quality management within a given reaction time. It is important to note that there was no discernable relationship between the duration of the lead time and the extent of the subsequent exponential increase of E. coli. As shown in Table 1, E. coli concentrations in the spring water showed a remarkable increase during the exponential phase with values ranging from log 1.8 up to > log 3.2; however, this increase was unrelated to the observed lead time.

The lead time should be distinguished from the reaction time of the spring. The reaction time is a hydraulic indicator and shows the time lag between the start of the rainfall in the catchment area and the beginning of the rise of the discharge. This time is usually in the range 2.75 h and 8.25 h (Table 1).

For alpine karst aquifers, turbidity is not only surface related. In karst conduits and caves sediments are deposited and can be mobilized under increased transport velocities and shear forces due to the flowing water. This effect can cause a rapid increase of turbidity in the spring from increased hydraulic pressure in the aquifer (e.g. due to input of surface water). Therefore, a relationship between the surface dynamics and the early indication of E. coli is not expected; hence, it was not observed in our study (data not shown).

### SAC254 as a proxy parameter to estimate E. coli concentration during events

A synoptic comparison of E. coli concentrations and SAC254 as determined from seasonal spring monitoring commonly does not show any correlation between these two parameters. This situation is reflected in a data set for LKAS2 with 3 recovered samples per week for 2 years (Figure 3). During the winter and spring, a significant number of SAC254 values without detectable E. coli counts were observable (Figure 3). In general, seasonal analysis combining all data, irrespective of the given situation in the catchment and the aquifer, will mask possible relationship between E. coli and the SAC254 during event triggered periods.

In contrast to the data from seasonal monitoring, event triggered investigations with a high number of samples from the auto-sampling procedure resulted in an entirely different picture. According to the discharge behaviour of the investigated events, correlation analysis was segmented into different phases. Although the characteristics of the

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**Figure 2**  | Event-based exponential increase of E. coli concentrations during the first event-phase (i.e. the rise of discharge).

**Figure 3**  | SAC254 versus log E. coli as recovered from seasonal monitoring (LKAS, 2004–2005).
investigated event types showed remarkable variations (Table 1), as can be expected for karst springs, three distinct phases could be found throughout all analysed events. A detailed analysis of these phases is shown for event 2006 LKAS2 and event 2007 LKAS6 (Figures 4 and 5).

The following three phases could be distinguished:

- **Lead time phase** (as described above) is the time lag between the increase of SAC254 and the increase of *E. coli* concentrations. The observed lead time allows management measures to be initiated in a particular timeframe.

- **First increase phase**: during this time, SAC254 and *E. coli* are highly correlated. However, the correlation slope depends on several factors including the hydrogeological situation of the spring, the controlling hydrologic constraints, and the characterization of the triggering rainfall event. It is important to note that the availability of *E. coli* in the respective catchments is also a major controlling factor. The slope between *E. coli* and SAC254 thus varies with the respective situation of contamination in the considered area. For example, a series of significant rainfall and run-off events within a short period of time will increasingly clean the surface from accumulated faecal material and thus the slope of the correlation will change.

- **Decrease phase**: This phase shows also a good correlation between SAC254 and *E. coli* concentrations, but with a weaker relationship than during the increase phase. In Figures 4 and 5 a strong correlation between SAC254 and *E. coli* enumeration during the decreasing phase is also shown. This relationship offers the possibility to use the SAC254 proxy-parameter as guidance for starting the water intake again. However, this issue was not the aim of the investigation and is thus not further discussed.

During peak times of *E. coli* no correlation to SAC254 was shown. The correlation between the SAC254 and the *E. coli* concentrations showed correlation factors always higher than $r = 0.88$, with a corresponding $R^2 > 0.78$ (Table 1). The slope of the linear correlation varied between 0.0006 and 0.0097 (Table 1). This pronounced variation of slopes implies that a calibration of the proxy-parameter SAC254 must be carried out individually for every spring in order to make *E. coli* concentrations predictable. This calibration should be based on the hydrologic conditions of the spring such as soil moisture, amount, and type of precipitation, discharge before the event, and abundance of faecal pollution sources.

**DISCUSSION**

The spectral absorption coefficient at 254 nm (SAC254) seems to be a logic and promising candidate for an early warning proxy parameter for detecting faecal pollution events at alpine karst water aquifers. However, near
real-time spring water abstraction management using SAC254 or other available on-line parameters (e.g. turbidity) represents just one essential element for sustainable water supply from alpine karst water resources (cf. intro). For a system assessment, the whole line of natural and technical barriers has to be considered. In this respect, the extent of final water treatment requirements depends on the achievable raw water quality. Remarkably, abstraction management can reduce bacterial contamination levels by 2 to > 3 orders of magnitude (cf. Table 1 \( \Delta E. \ coli \)). When concentration levels exceed the thresholds, spring water is no longer used for drinking water production and is diverted from the water conduit until it complies again with the respective quality criteria.

Since the SAC254 measures detectable fractions of dissolved organic matter (DOC) that are leached from the surface and soil (e.g. humic substances), it is indicative of the surface related organic influence in the spring water habitat. It could be demonstrated that the onset of the increase of SAC254 always precedes the onset of the increase of surface related \( E. \ coli \) concentrations irrespective which spring and event type was investigated.

Because of this observation, application of SAC254 constitutes a conservative early warning signal for faecal pollution events in the investigated alpine catchment. In this respect, false positive indications (i.e. increase of SAC254, but no increase in \( E. \ coli \)) are far more likely to occur than false negative indications (i.e. no increase of SAC254, but an increase in \( E. \ coli \)). This occurs because: i) faecal pollution in the investigated catchments is associated with the occurrence and distribution of ruminant animals (Reischer et al. 2008; Farnleitner et al. 2010a,b); ii) ruminant animals are associated with the occurrence of vegetation; and finally, iii) vegetation is associated with the occurrence of soil and humic substances. The presence of soil and humic substances is thus a pre-requirement for the deposition of faecal material (but not vice versa). False positive indications occur when faecal contamination in the environment is limited and mainly organic substances are transferred to the spring water.

It should be highlighted that faecal influence from point pollution sources, such as sewage from alpine lodges without adequate soil contact, is unlikely be detectable by the presented SAC254 approach. As a result, all recovered results apply only for rural catchment areas where diffuse faecal pollution sources have had adequate soil contact and dissolved organic material could be flushed into the water.

During the investigation, it became obvious that the SAC254 may also be used as a real-time proxy to estimate the quantity of faecal pollution during events (c.f. partial correlations between SAC254 and the occurrence of \( E. \ coli \), Figures 4 and 5). An important prerequisite is the establishment of specific spring and event type calibrations that account for the variability on the occurrence and transferability of the faecal material in relation to the surface related organic material.

However, as mentioned above, only diffuse faecal pollution sources with adequate soil contact are supposed to be quantifiable by the described approach.

Point sources without soil contact—such as from human sewage—do not show the required association between SAC254 and \( E. \ coli \). The correlation between SAC254 and \( E. \ coli \) for the different phases of the investigated events gives strong evidence that diffuse animal sources were the dominating faecal pollution sources in LKAS2, LKAS6 and LKAS8. The outcome is in agreement to results recovered from microbial source tracking studies in the considered catchments, where ruminants were identified as the responsible pollution sources by source-specific genetic markers (Reischer et al. 2008; Farnleitner et al. 2010a,b). The SAC254 is thus a promising candidate for evaluating the relevance of faecal pollution from point sources compared to diffuse pollution sources with soil contact in rural areas. However, further studies are required to support the usefulness of SAC254 for source tracking and characterisation.

Although the described methodology was established for alpine karst environments, the use in other rural systems might be very interesting especially for systems showing rapid changes of the considered quality parameters. However, the necessity of adaptations should be taken in account. For example, evaluating the applicability in estuarine locations might need to replace or complement \( E. \ coli \) by enterococci.

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REFERENCES


