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Evaluation of β-D-glucuronidase and particle-size distribution for microbiological water quality monitoring in Northern Vietnam

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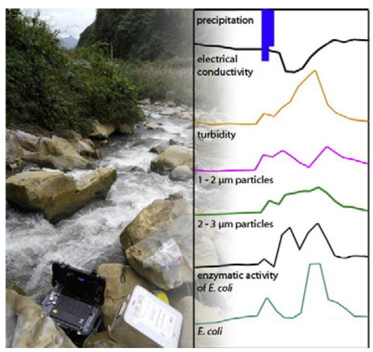
HIGHLIGHTS

- Enzymatic activity of *E. coli* was monitored at a karst spring in Northern Vietnam.
- We studied daily fluctuations of enzymatic activity and particle size distribution.
- A near real-time monitoring of fecal contamination patterns was possible.
- We found strong relations of particle concentrations and rising *E. coli* levels.
- Enzymatic activity is a promising, complementary monitoring parameter.

GRAPHICAL ABSTRACT



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ABSTRACT

In many karst regions in developing countries, the populations often suffer from poor microbial water quality and are frequently exposed to bacterial pathogens. The high variability of water quality requires rapid assays, but the conventional cultivation-based analysis of fecal indicator bacteria, such as *Escherichia coli* (*E. coli*), is very time-consuming. In this respect, the measurement of the enzymatic activity of *E. coli* could prove to be a valuable tool for water quality monitoring. A mobile automated prototype device was used for the investigation of β-D-glucuronidase (GLUC) activity at a remote karst spring, connected to a sinking surface stream, in Northern Vietnam. To assess the relationship between GLUC activity, discharge dynamics and contamination patterns, multiple hydrological, hydrochemical, physicochemical and microbiological parameters, including discharge, turbidity, particle-size distributions, and *E. coli*, were measured with high temporal resolution during ten days of on-site monitoring. A complex contamination pattern due to anthropogenic and agricultural activities led to high *E. coli* concentrations (270 to >24,200 MPN/100 ml) and a GLUC activity between 3.1 and 102.2 mMFU/100 ml. A strong daily fluctuation pattern of GLUC activity and particle concentrations within small size classes (<10 µm) could be observed, as demonstrated by autocorrelations. A Spearman's rank correlation analysis resulted in correlation coefficients of $r_s = 0.56$ for *E. coli* and GLUC activity and $r_s = 0.54$ for GLUC activity and the concentration of 2–3 µm particles. On an event scale, correlations were found to be higher ($r_s = 0.69$ and 0.87, respectively). GLUC activity and *E. coli* displayed a general contamination pattern, but with significant differences in detail, which may be explained by interferences of e. g. viable but non-culturable cells. Although further evaluations are recommended, GLUC activity is a promising, complementary parameter for on-site and near real-time water quality monitoring.

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1. Introduction

One of the main concerns in setting health-based targets for microbial safety of water is fecal contamination originating from humans or animals, which can be a source of pathogenic bacteria, viruses, protozoa, and helminths (WHO, 2008). In developing countries, where wastewater treatment is often insufficient or entirely absent, many individuals are exposed to pathogens leading to the widespread occurrence of waterborne diseases (Montgomery and Elimelech, 2007). Especially in karst areas, microbial contaminants can easily reach the groundwater surface due to rapid infiltration via sinking surface streams, swallow holes, or thin soils. Furthermore, the lack of granular texture, turbulent flow regimes in conduit systems, and short residence times, in addition to rapid hydraulic and hydrochemical responses to rainfall events, lead to a high vulnerability of groundwater (Ford and Williams, 1989; Goeppert and Goldscheider, 2008; Pronk et al., 2007; White, 1988).

The common hygienic-bacteriological evaluation of raw water is based on fecal indicator bacteria (FIB), such as total coliforms (TC), fecal coliforms (FC), *Escherichia coli* (*E. coli*), and intestinal enterococci (Ferguson et al., 2012; Servais et al., 2005). As microbial water quality often varies quickly over a wide range, and short-term peaks in pathogen concentrations may substantially increase the risk of disease outbreaks, there is a pressing need for rapid assays that enable near real-time quantification of FIB, particularly *E. coli* (Fiksdal and Tryland, 2008; WHO, 2008). However, standard culture-based techniques for the detection and enumeration of TC and FC require between 18 and 72 h, depending on the incubation time (Wildeboer et al., 2010), a time frame in which many individuals could be exposed to pathogens.

Enzyme assays are seen as a promising alternative to culture-based FIB assays (Heery et al., 2016), because they provide results in 1 h or less and are simple to perform (Fiksdal and Tryland, 2008; Noble and Weisberg, 2005). Roughly 97% of *E. coli* strains demonstrate β-D-glucuronidase (GLUC) activity, while the enzyme is absent in almost all other coliform bacteria (Kilian and Bülow, 1979; Wildeboer et al., 2010). Further, while a small number of non-coliform micro-organisms may exhibit activity of this enzyme, interference by these micro-organisms becomes negligible in heavily polluted systems (Caruso et al., 2002). Therefore, GLUC activity is thought to be a specific biomarker for *E. coli* detection in microbiological water quality control (Rompré et al., 2002; Togo et al., 2006; Wildeboer et al., 2010; Wutor et al., 2007). The assay is based on bacterial hydrolysis of the added substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) and fluorescence detection of the enzymatic reaction product 4-methylumbelliferone (MU) (Garcia-Armisen et al., 2005; Koschelnik et al., 2015).

In the last two decades, there has been an increasing interest in determining direct GLUC activity in order to evaluate microbial water quality (Farnleitner et al., 2002; Fiksdal et al., 1994; Fiksdal and Tryland, 2008; Servais et al., 2005). However, all measurements of GLUC activity in previously published studies were conducted in specialized laboratories. Recently, automated devices, such as the Coliminder® (VWM, Austria), have been developed to measure enzymatic activity in water by fluorescence photometry on-site (Heery et al., 2016; Koschelnik et al., 2015; Stadler et al., 2016). A first automated long-term monitoring of enzymatic activity was presented by Ryzinska-Paier et al. (2014), who tested an automated device (Coliguard, mbOnline, Austria). While most studies focused on the correlation between GLUC activity and *E. coli* or fecal coliforms (Fiksdal et al., 1994; Heery et al., 2016; Servais et al., 2005; Wildeboer et al., 2010), bulk parameters including turbidity, spectral absorption coefficient at 254 nm (SAC254), conductivity and water temperature were also considered. Pronounced correlations were obtained between GLUC activity with SAC254, GLUC activity and turbidity and GLUC activity and discharge, along with lower, but still significant, correlation levels for GLUC activity and *E. coli*.

Stadler et al. (2016) recently tested two ColiMinder instruments in a hydrological open air laboratory to evaluate the reliability of measurements and compared it to measurements of two BACTcontrol (formerly Coliguard) devices, based on similar technology (Ryzinska-Paier et al., 2014). They monitored GLUC activity dynamics in a stream for one year and assessed time series data by correlation. An essential outcome of the study was that GLUC activity was not a useful proxy for *E. coli,* but they concluded that further field experiments and detailed monitoring, with a specific focus on diurnal GLUC activity fluctuations, were required.

To date, on-line monitoring of enzymatic activity has been bound to well-established monitoring stations. However, in developing countries or in cases of natural disaster, required infrastructure is often insufficient or even absent. Therefore, in this study, a mobile prototype of ColiMinder was tested to investigate microbiological contamination patterns at an extremely remote karst spring in Northern Vietnam.

In the future, the water from the karst spring tested here, which is connected to a sinking surface stream, will be used as a water supply system for mountainous villages, underlining the importance of the investigation of water quality and the influences from the surface catchment area. Withal, suspended particles are important, since Abia et al. (2016) postulated that the risk of an infection due to *E. coli* increases approximately 10-fold if there is a river sediment disturbance, because bacteria tend to adhere to particles (Dussart-Baptista et al., 2003; Pronk et al., 2006; Schillinger and Gannon, 1985) and are more persistent within the aquatic environment than free-floating bacteria. Due to their attachment to a mobile solid phase, they can be transported and are influenced by processes of sedimentation and remobilization (Mahler et al., 2000). In karst systems, a rainfall event can lead to a pressure pulse and, consequently, to a primary turbidity signal, caused by the remobilization of sediments from the karst aquifer itself (autochthonous turbidity). A secondary turbidity signal can occur with the arrival of turbid storm-derived water from outside the karst aquifer, e. g. from the surface catchment area, that enters the swallow hole (allochthonous turbidity). This second turbidity signal is more likely associated with fecal contamination (Goldscheider et al., 2010). However, turbidity itself is a bulk parameter without any information about origin or nature. Detection of particle-size distribution (PSD) delivers more detailed information and is a valuable tool to specify the type of turbidity and to identify particle size classes that are related to microbial contamination, as shown in Pronk et al. (2007).

This study aims to determine the applicability of automated rapid on-site GLUC activity measurements by using a ColiMinder in an extremely remote, poor area, without public power supply, similar, for example, to the case of water quality monitoring during disaster management. Furthermore, PSD is evaluated as a complementary parameter to GLUC activity, to specify the type of contamination events and to assess the potential of this parameter combination for early warning systems.

The main research questions are: (i) are there daily fluctuations in GLUC activity and PSD caused by agricultural land use in the recharge area? (ii) how does GLUC activity react to hydrological events, such as rain fall, in comparison to PSD and other parameters? (iii) what is the relationship between GLUC activity and cultivation-based determinations of *E. coli,* as well as other water quality parameters (PSD, turbidity, electrical conductivity, water temperature)? and (iiii) what information can be obtained by using GLUC activity and culture-based methods and can they be used as complementary methods to indicate fecal contamination?

2. Materials and methods

2.1. Site selection and sampling campaign

The field site is located in Northern Vietnam, close to the Chinese border. The area was designated as a UNESCO Geopark in 2010, which has led to a strong increase in tourism and, consequently, in water demand. To meet this challenge, water from a remote karst spring will