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# Environmental isotope $^{18}O$ in coastal karst spring waters as a possible predictor of marine microbial pollution

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*We tested the validity of the traditional hypothesis of a causal effect between rainfall occurrence, groundwater discharging into the sea, and marine microbial contamination. For groundwater characterization, we used the $^{18}O$ isotope. This stable isotope, together with $^{2}H$, proved to be a useful physical, naturally occurring tracer primarily due to its abundance variations at different stages of water cycle. Test locations include Bakar Bay and selected adjacent Rijeka city beaches (Croatia). To test for statistical associations, we used the Panel Data Pairwise Granger Causality test. At examined locations, we found statistically significant relationships between the amount of rainfall and the abundance of $^{18}O$ isotope in groundwater, as well as relationships between the abundance of $^{18}O$ isotope in groundwater and faecal bacteria concentrations. Accordingly, $^{18}O$ isotope, when used as an indicator for the functioning of karstic groundwater systems, may also be used as predictor of faecal contamination of bathing waters in associated karst littoral areas. We believe this physical method could be a valuable addition to present methods of predicting microbiological contamination and economic allocation of stock and flow pollutants in scarce common pool resources such as fresh water basins, springs and beaches.*

**Key words:** common property resources: natural resources, microbial contamination, nuclear physics: stable isotopes, spectroscopic techniques

## INTRODUCTION

Human health hazards due to recreational exposures in faecal contaminated marine waters include eye, ear, and skin infections as well as gastrointestinal and respiratory illnesses (GRIFFIN *et al.*, 2001). *Escherichia coli* and enterococci are widely used as indicators of microbial quality of recreational waters throughout the world. *E. coli* is present in high concentrations in faeces, while enterococci are not as numerous in faeces as *E. coli*, but are salt-resistant and therefore good indicators of faecal contamination of marine waters (HARTZ *et al.*, 2008). According to the revised European Union Bathing Water Directive (BWD 2006/7/EC) both, *E. coli* and enterococci concentrations should be determined for seawater quality classification.

Croatian coastal bathing waters are regularly controlled for microbiological quality during the bathing season (mid May – end September). Kvarner, an area in the Northern part of the Croatian Adriatic, is a popular tourist destination for recreational bathing. Although the bathing water in this area is excellent or of at least good quality, there are some reports about its occasional worsening (VUKIĆ LUŠIĆ *et al.*, 2017). Globally, there have been reports about the worsening of microbiological quality of marine waters after heavy rainfalls (HARAMOTO *et al.*, 2006; DWIGHT *et al.*, 2011). In Kvarner, the most probable reason of such worsening is microbial contamination originating from catchment areas of coastal and submarine springs, faults in sewage systems, as well as leakages of private septic tanks (VUKIĆ LUŠIĆ *et al.*, 2017).

The Kvarner coastal area is part of the Dinaric littoral karst (BONACCI, 2015). The dominant groundwater flow from the Dinaric karst is towards the Adriatic Sea, where the main karst groundwater outlets are coastal and submarine springs. Due to complex and heterogeneous groundwater flow systems in karst, non-conventional methods need to supplement traditional hydrological and hydrogeological ones. One of such non-conventional methods is the analysis of hydrogen and oxygen stable isotopes ($^{2}H$ and $^{18}O$) in water (LECOMTE *et al.*, 2016; MANDIĆ *et al.*, 2008; OZYURT *et al.*, 2014; PALMER, 2010; ROLLER-LUTZ *et al.*, 2013). $^{2}H$ and $^{18}O$ environmental isotopes are widely used for karst aquifer characterization: analysis of recharge and discharge processes, study of reservoir water mixing, estimation of water transit times, etc. (DÓŠA *et al.*, 2011; LONG & PUTNAM, 2004; MALOSZEWSKI *et al.*, 2002; MANCE *et al.*, 2014).

Although Kvarner is known for its numerous karst springs, there are no regular discharge measurements on any of them. During the period from April 2010 to March 2012, selected springs in Kvarner were sampled on a weekly basis for the analysis of $^{2}H$ and $^{18}O$ content (MANCE, 2014).

Our goal was to test whether these environmental isotopes could serve as predictors of marine coastal water microbial contamination. In other words, since it was not possible to establish a direct precipitation → groundwater discharge → seawater microbial contamination connection, we tested for the precipitation → groundwater stable isotopes → seawater microbial contamination statistical association instead.

Shore-seawater and adjacent open-sea water samples were not collected, so isotope-based freshwater admixture determination was not possible. Nevertheless, admixture locations are known from literature (BENAC *et al.*, 2003) and approximately correspond to our measurement locations.

## MATERIAL AND METHODS

### Study area and data

Geographically, the study area belongs to the Kvarner Bay (Fig. 1A). That is a semi-enclosed bay in the Adriatic Sea located between the Vinodol – Velebit coastline in the east and the Istrian peninsula to the west, with many islands within. Geologically, cretaceous carbonate sedimentary rocks (limestones, dolomites and carbonate breccia) prevail, but Paleogene limestones and Paleogene flysch are also present (JURAČIĆ *et al.*, 2009).

Specifically, the study area includes two micro-locations: Bakar Bay and the Pećine dis-


*Fig. 1. Satellite images A) Kvarner Bay with city of Rijeka; B) sampling locations in Bakar Bay and Pećine (source: Google Earth). 1 – beach of the Hotel Jadran (HJ); 2 – Sablićevo (SB); 3 – Glavanovo west (GLW); 4 –Glavanovo (GL); 5 – Ružićevo (RZ); 6 – Grčevo (GR); 7 – Uvala Dobra (UDB); 8 – Bakarac (BKC); ZV – Zvir; MB – Martinšćica well; KUK – Kukuljanovo; PER – Perilo; DB – Dobra; DBC – Dobrica*

trict of the city of Rijeka (Fig. 1B). Bakar Bay is 4.6 km long, 1.1 km wide and 40 m deep. Approximately 1,500 people live in the Bakar Bay area, with some 6,000 more in its approximate hinterland. Pećine is Rijeka city district with many popular beaches. There are some 2,500 inhabitants in Pećine, and some 30,000 more in the surroundings.

Marine water samples were collected at two beaches in Bakar Bay: Bakarac (BKC) and Uvala Dobra (UDB), and on six Pećine district beaches: Grčevo (GR), Sablićevo (SB), Ružićevo (RZ), Glavanovo (GL), Glavanovo west (GLW) and the beach of the Hotel Jadran (HJ). Samples were collected biweekly during the bathing seasons 2010 and 2011. The sampling is part of the national bathing water quality-monitoring programme. Altogether 160 samples were analysed for *E. coli* and enterococci.

Three springs: Dobra (DB), Dobrica (DBC), and Perilo (PER) were chosen as representative of springs that discharge in the Bakar Bay. Zvir (ZV) and one of the Martinšćica wells (MB) are representative groundwater outlets for Pećine. Sampled groundwater outlets belong to two karst systems for which we may expect (MANCE, 2014):

a) to be best described by a dual porosity model that includes a fissure-porous matrix and karstic channels;
b) groundwater base flow to originate from the matrix and groundwater quick flow to originate from widened channels – conduits;
c) to have precipitation as main water input;
d) recharge dominated by winter precipitation; and
e) higher $^{2}H$ and $^{18}O$ groundwater contents in periods of heavy rain in comparison to periods of scarce rain and drought.

For the purpose of stable isotope analysis, spring water samples were collected on a weekly basis from 2010 to 2012 (MANCE, 2014), but only samples that coincided with the collecting of the samples for microbiological analysis were included in this study ($N$ = 100).

The Croatian Meteorological and Hydrological Service provided data on daily precipitation amounts from meteorological station Kukuljanovo (KUK, Fig. 1B).

### Measurements and analysis

Stable isotope composition is expressed as stable isotope abundance deviation of the sample relative to the standard: δ (‰) = ($R_{sample}$ / $R_{standard}$) − 1 (COPLEN, 2011; BRAND & COPLEN, 2012). In case of water, δ values of interest are $\delta^2$H and $\delta^{18}$O. For hydrogen, R ratio represents $^2$H/$^1$H and for oxygen the R ratio is $^{18}$O/$^{16}$O. Delta values are expressed relative to VSMOW2 (Vienna Standard Mean Ocean Water) standard.

Non-marine natural waters are less abundant in heavier isotopes compared to the VSMOW, and therefore, fresh water δ values are commonly found to be negative. $\delta^2$H and $\delta^{18}$O values of natural waters that are not under influence of evaporation, are linearly correlated (CRAIG, 1961). There is strong and statistically significant linear correlation between $\delta^2$H and $\delta^{18}$O values of groundwater discharging at the analyzed springs (MANCE, 2014) and therefore, in this paper, as an environmental tracer we used only $^{18}$O. Groundwater base flows have a common property of quite constant d contents, while isotopic shifts towards less negative δ values are usually an indication of newly infiltrated precipitation (MANCE *et al.*, 2017).

Stable isotope measurements were performed on a Delta plusXP (Thermo Finnigan) isotope ratio mass spectrometer (IRMS). Peripheral units of the IRMS were HDO eq48/24 (IsoCal) equilibration unit and a Dual Inlet system (Thermo Finnigan). Prior to the measurement, all samples were equilibrated, as it is common for the water equilibration technique (HORITA & KENDALL, 2004). USGS Laboratory Information Management System (LIMS) was used for normalization and analyses of the measurement data. The measurement precision for $\delta^{18}$O was better than 0.1 ‰.

Traditional culture-based methods were performed using the membrane filtration technique and reference methods. *E. coli* was analyzed by means of Rapid test EN ISO 9308-1:2000 (ISO, 2014) using TSA/TBA media, which enables identification of *E. coli* within 24 hours. The Rapid test was based on membrane cultivation under selective conditions on double-layer Petri dish consisting of Tryptone Soy Agar (Tryptic digest of casein, soy peptone and sodium chloride, Biolife) and of Tryptone Bile agar (Tryptic digest of casein and bile salts, Biolife). The membrane was placed on a freshly-prepared double-layer plate and incubated first at (36 ± 2) °C for 4 hours (resuscitation period) followed by an additional incubation at (44 ± 0.4) °C for 20 hours. Indole test was used for further *E. coli* confirmation. Following incubation, the membrane was placed on an adsorbent pad saturated with indole reagent and irradiated with an ultraviolet lamp for 20 min. All red colonies on the membrane filter were counted as *E. coli*.

Intestinal enterococci were analyzed according to the EN ISO 7899-2 (ISO, 2016), placing the filter membrane on a selective Slanetz- Bartley medium (Sodium Azide, Triphenyl Tetrazolium Chloride - TTC). The plate was incubated at (36 ± 2) °C for (44 ± 4) hours. The membrane was carefully examined and all red or brown colonies were counted as presumptive enterococci. The membrane with typical colonies was transferred to a Petri dish with Bile Esculin Azide Agar (Esculin, Ferric Ammonium Citrate) which was incubated at (44 ± 0.5) °C for 2 hours. After incubation, typical colonies characterized by the brown-black halo were verified as intestinal enterococci.

### Statistical analysis and methodology

We analyzed the spatial differences for $\delta^{18}$O, *E. coli* and enterococci with the Kruskal-Wallis test supplemented by the Nemenyi post-hoc test. The analysis was carried out in the PMCMR package of the R statistical software (R Development Core Team, 2018). In order to find out the level of correlation between these variables we calculated the Spearman's rank correlation coefficient. The results were accepted as statistically significant at the level of $P < 0.05$.

Correlation is just a measure of statistical association. Statistical correlation analysis of cross-sectional data measures occurrences at the same point in time. Causal conjectures between dependent and independent variables need to be tested by testing the lagged past values of the independent since the cause precedes the effect, and not vice-versa. To test for a statistical association between indicators, factors, and determinants conjectured to have a causal relationship between them, we used the Panel Data Pairwise Granger causality test provided with the statistics software EViews 9 (IHS Markit Ltd, London, UK).

In statistics, panel data include observations with both cross-section identifiers (e.g. marine water sampling locations) and within-cross-section identifiers (e.g. sampling dates). Panel data are thus multi-dimensional data involving measurements over time containing observations of multiple phenomena obtained over multiple periods for the same locations. Panel data analysis is common in biostatistics (MANDEL, 2010), epidemiology (WANG *et al.*, 2015), ecology (OU *et al.,* 2013), econometrics (MADDALA, 2001) and social sciences (MANCE & PEČARIĆ, 2016).

Granger causality test shows how much of the current dependent variable is explained by its own past values and the lagged values of the independent variable. A dependent variable is said to be Granger-caused by an independent variable, if the independent variable helps in its prediction. Granger causality test is a useful method to falsify conjectures of causality where the dependent variable is temporally successive to the independent. In its simplest form, Granger causality test between variables $X$ and $Y$ is computed by running bivariate regressions (EVIEWS, 2018):

$$Y_{i,t} = \alpha_{0,i} + \sum_{j=1}^{n} \alpha_{j,i} Y_{i,t-j} + \sum_{k=1}^{n} \beta_{k,i} X_{i,t-k} + \varepsilon_{1i,t} \quad (1)$$

$$X_{i,t} = \alpha_{0,i} + \sum_{j=1}^{n} \alpha_{j,i} X_{i,t-j} + \sum_{k=1}^{n} \beta_{k,i} Y_{i,t-k} + \varepsilon_{2i,t} \quad (2)$$

where $i$ denotes cross-sectional dimension, $t$ denotes time period dimension of the panel and is the idiosyncratic error term. The null hypothesis ($H_0$) reads: "the independent variable does not Granger cause the dependent variable". The Granger causality test measures the statistical significance of the a and b coefficients and $H_0$ is rejected if those coefficients are statistically significantly different from zero at the 5 % significance level.

We tested both non-differenced and differenced data for Granger causality. By analyzing non-differenced data, we conjecture associations between factor states, and by analyzing differenced data, we conjecture associations between factor changes. Additionally, by manipulating time lags, we may get some insight into the amount of time that is required for the pollution to develop, or to disappear.

## RESULTS AND DISCUSSION

The results of the Kruskal-Wallis ($H$ = 20.04, $P$ < 0.001) and the Nemenyi post-hoc tests ($P$ < 0.001) indicate ZV and MB having on average lower $\delta^{18}O$ values than DB and DBC (Fig. 2). Observed differences are persistent over longer periods of time and can be explained by higher mean elevations of the springs' recharge areas in the Rječina River catchment (ZV and MB)

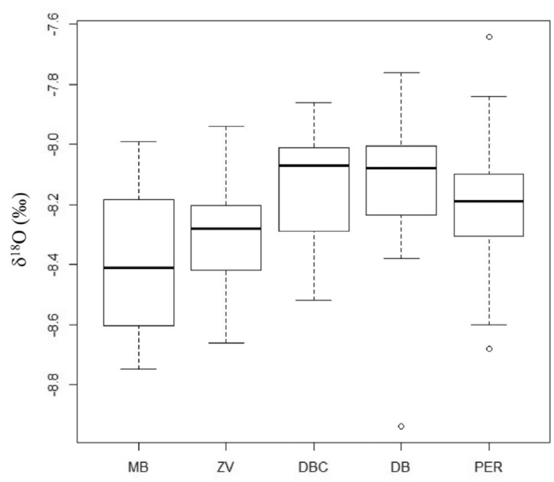


*Fig. 2. Spring and well water: $\delta^{18}O$ for Martinšćica well (MB), Zvir (ZV), Dobrica (DBC), Dobra (DB) and Perilo (PER). The bottom and top of the box are LQ and UQ, respectively, the line in the box represents the median. The ends of the whiskers represent LQ – 1.5 IQR and UQ + 1.5 IQR. Circles designate data that were recognised as outliers. LQ – lower quartile; UQ – upper quartile; IQR – interquartile range*

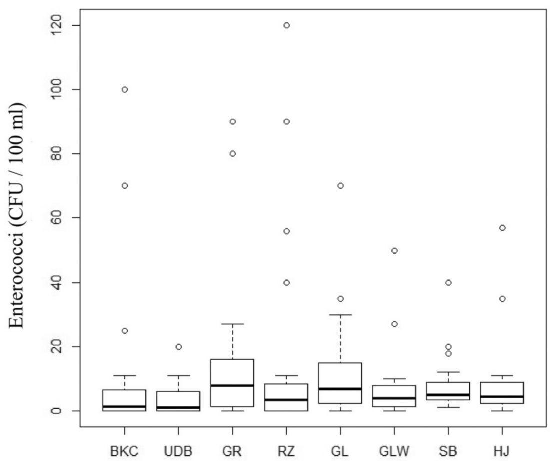


*Fig. 3. Marine water Enterococci counts for Bakarac (BKC), Uvala Dobra (UDB), Grčevo (GR), Ružićevo (RZ), Glavanovo (GL), Glavanovo west (GLW), Sablićevo (SB) and beach of the Hotel Jadran (HJ). The bottom and top of the box are LQ and UQ, respectively, the line in the box represents the median. The ends of the whiskers represent LQ – 1.5 IQR and UQ + 1.5 IQR. Circles designate data that were recognised as outliers. LQ – lower quartile; UQ – upper quartile; IQR – interquartile range*

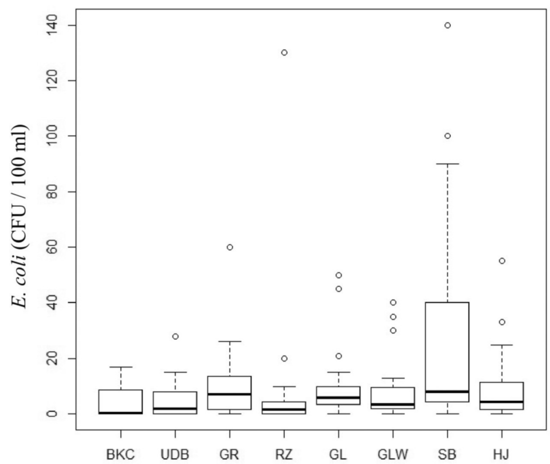


*Fig. 4. Marine water Eschericia coli counts for Bakarac (BKC), Uvala Dobra (UDB), Grčevo (GR), Ružićevo (RZ), Glavanovo (GL), Glavanovo west (GLW), Sablićevo (SB) and beach of the Hotel Jadran (HJ).. The bottom and top of the box are LQ and UQ, respectively, the line in the box represents the median. The ends of the whiskers represent LQ – 1.5 IQR and UQ + 1.5 IQR. Circles designate data that were recognised as outliers. LQ – lower quartile; UQ – upper quartile; IQR – interquartile range*

compared to the springs belonging to the Bakar Bay catchment (MANCE, 2014; ROLLER-LUTZ *et al.*, 2013).

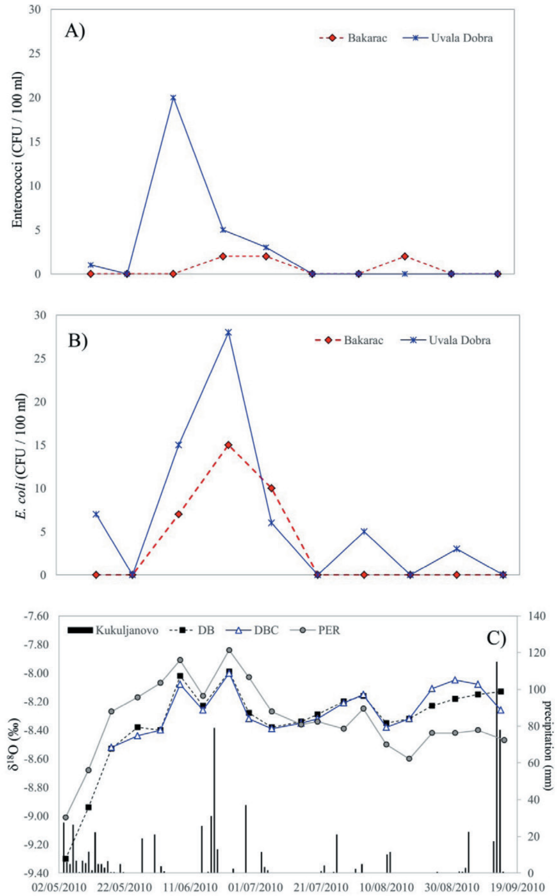


*Fig. 5. Bakar Bay, bathing season 2010: A) enterococci; B) E. coli; C) daily precipitation amount at station Kukuljanovo and spring water $\delta^{18}O$ time series. BKC – Bakarac; UDB – Uvala Dobra; DB – Dobra; DBC – Dobrica; PER – Perilo*

In case of enterococci (Fig. 3), the Kruskal-Wallis test showed no statistically significant differences among sampling locations ($H$ = 13.77, $P$ = 0.06).

Although location SB has the highest median and maximal value for *E. coli* in comparison to all examined marine locations (Fig. 4), the Nemenyi test indicated significantly lower *E. coli* counts only at locations BKC and RZ ($P$ < 0.001).

The summer 2010 $\delta^{18}O$ time series of Bakar springs beginns with the most negative values recorded since the beginning of the sampling (Fig. 5C). Since we can expect significantly lower δ values for snow than those for rain, the most negative $\delta^{18}O$ values of the time series are most likely the consequence of water infiltration

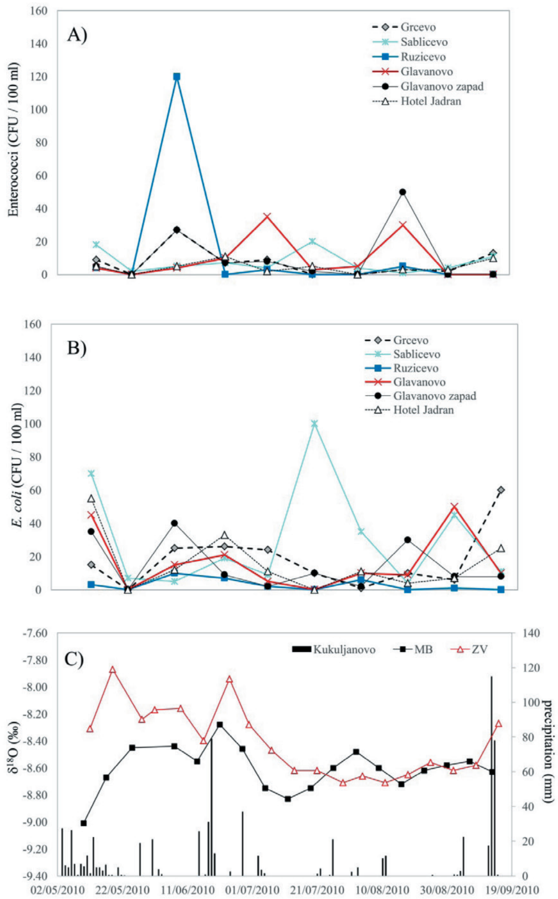


*Fig. 6. Pećine, bathing season 2010: A) enterococci; B) E. coli; C) daily precipitation amount at station Kukuljanovo and spring and well water δ$^{18}$O time series. GR – Grčevo; RZ – Ružićevo; GL – Glavanovo; GLW – Glavanovo west; SB – Sablićevo; HJ – beach at Hotel Jadran; MB – Martinšćica well; ZV – Zvir*

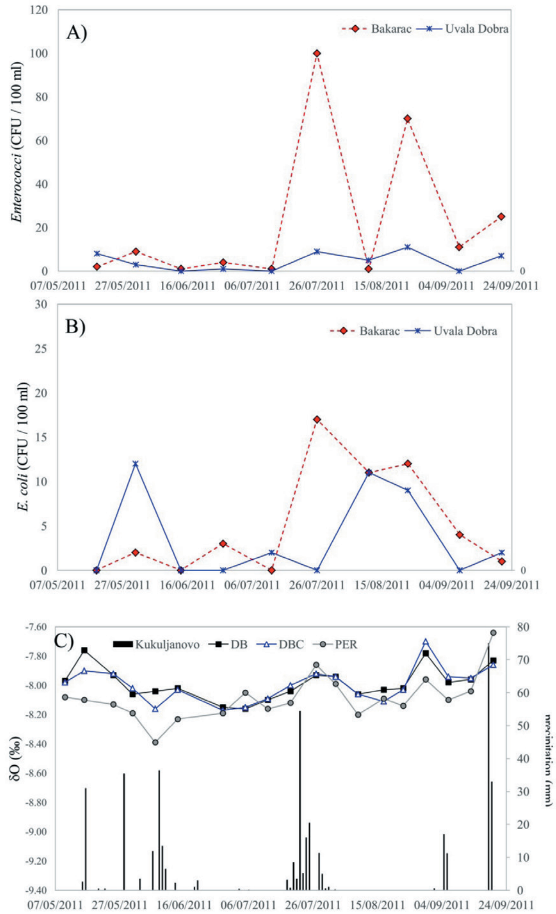


*Fig. 7. Bakar Bay, bathing season 2011: A) enterococci; B) E. coli; C) daily precipitation amount at station Kukuljanovo and spring water δ$^{18}$O time series. BKC – Bakarac; UDB – Uvala Dobra; DB – Dobra; DBC – Dobrica; PER – Perilo*

originating from snow melt (MANCE, 2014). The successive gradual shifts towards less negative values are probably due to the mixing of groundwater with the newly infiltrated precipitation. After the last significant rainfall of June 2010, the summer saw less precipitation and the isotope composition dropped to more negative values becoming almost constant. This, second part of the time series, could indicate a discharging of the groundwater base flow. Thus, the first part of the isotopic time series in Fig. 5C indicates a mixture of the base flow with recent precipitation, while the second part indicates just the base flow. As for bacteria, higher concentrations occurr during the first part of the 2010 time series: between late May and the middle of July (Fig. 5A & 5B).

Isotope data for summer of 2010 in case of ZV and MB show similar behaviour to Bakar Bay springs, but without extremly low values at the beginning of the time series (Fig. 6C). The jump to less negative values occurred after heavy rainfalls in June 2010. As for constantly lower values during the dry season, the second part of the time series is attributed to the base flow. There is no clear association between rainfall and bacteria (Fig. 6A & 6B). The effect of heavy rainfalls during September 2010 is neither visible in the isotopic content nor in the bacteria concentrations for most of the sampled sites. Those rainfalls ended the bathing season 2010 and consequently sea water sampling.

*Table 1. Spearman coefficients of correlation for the Bakar Bay variables*

| | Variable | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ENT BKC | 1.00 | | | | | | | | | |
| 2 | E. coli BKC | 0.63 | 1.00 | | | | | | | | |
| 3 | ENT UDB | 0.49 | 0.71 | 1.00 | | | | | | | |
| 4 | E. coli UDB | | 0.46 | 0.49 | 1.00 | | | | | | |
| 5 | $\delta^{18}$O DBC | 0.64 | | 0.48 | | 1.00 | | | | | |
| 6 | $\delta^{18}$O DB | 0.60 | 0.45 | 0.55 | | 0.94 | 1.00 | | | | |
| 7 | $\delta^{18}$O PER | 0.57 | 0.58 | 0.61 | | 0.74 | 0.81 | 1.00 | | | |
| 8 | rain | | | | | 0.49 | 0.51 | | 1.00 | | |
| 9 | rain (-1) | | | | | | | | 0.54 | 1.00 | |
| 10 | total rain | | | | | | | | | 0.53 | 1.00 |

*NOTE: Table shows statistically significant coefficients only.*
*ENT – enterococci; BKC – Bakarac, UDB – Uvala Dobra, DB – Dobra; DBC – Dobrica; PER – Perilo, rain – rain amount on the day of sea water sampling, rain (-1) – rain amount one day prior to sea water sampling; total rain – total rain amount between two consecutive sea water samplings*

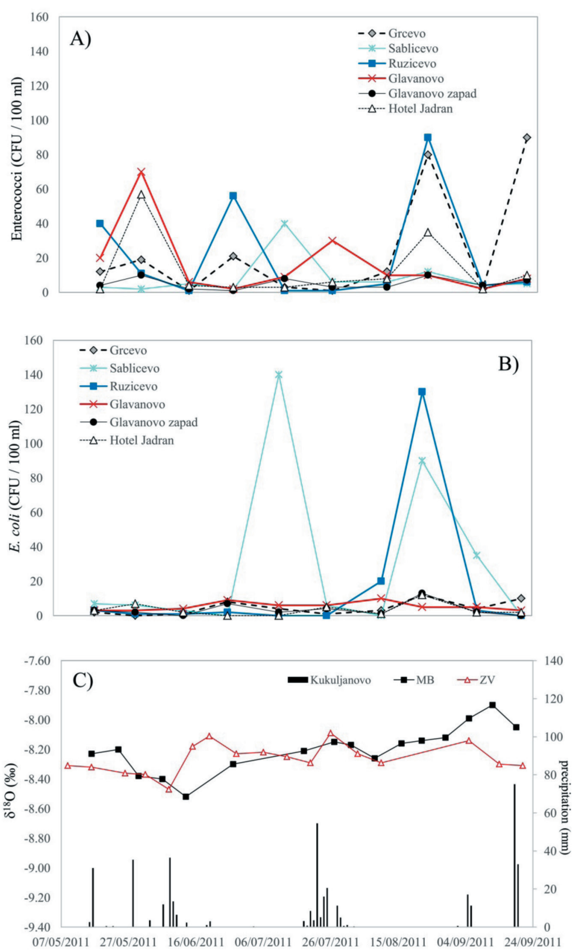


*Fig. 8. Pećine, bathing season 2011: A) enterococci; B) E. coli; C) daily precipitation amount at station Kukuljanovo and well and spring water $\delta^{18}$O time series. GR – Grčevo; RZ – Ružićevo; GL – Glavanovo; GLW – Glavanovo west; SB – Sablićevo; HJ – beach at Hotel Jadran; MB – Martinšćica well; ZV – Zvir*

Summer of 2011 was characterised by a smaller amount of rainfall than summer of 2010, and preceded by a rather dry winter without much snow (METEO.HR, 2018). The isotopic values of Bakar Bay springs water during the period are constant and almost all are within measurement error (Fig. 7C). This means that during the summer of 2011, the most prevalent discharge regime at the springs was the base flow. Somewhat higher $\delta^{18}$O values occured on just three occasions: at the beginning of the sampling, after the July rainfalls, and at the the end of the sampling in September. As for the bacteria in the Bakar Bay, we see clear spikes right after the July rainfall (Fig. 7A & 7B).

The isotopic composition of ZV and MB is almost constant throughout the 2011 bathing season, without significant shifts towards less negative values that were observed in the springs of the Bakar Bay (Fig. 8C). Nevertheless, both types of bacteria that are tested, show

*Table 2. Spearman coefficients of correlation for Pećine variables*

| | Variable | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ENT GR | 1.00 | | | | | | | | | | | | | | | | |
| 2 | E. coli GR | 0.45 | 1.00 | | | | | | | | | | | | | | | |
| 3 | ENT RZ | 0.76 | | 1.00 | | | | | | | | | | | | | | |
| 4 | E. coli RZ | | | | 1.00 | | | | | | | | | | | | | |
| 5 | ENT GL | | | | | 1.00 | | | | | | | | | | | | |
| 6 | E. coli GL | | 0.45 | | | | 1.00 | | | | | | | | | | | |
| 7 | ENT GLW | 0.50 | | 0.62 | | 0.66 | | 1.00 | | | | | | | | | | |
| 8 | E.coli GLW | | 0.65 | | | | 0.50 | | 1.00 | | | | | | | | | |
| 9 | ENT SB | | | | | | | | | 1.00 | | | | | | | | |
| 10 | E. coli SB | | | | | | | | | 0.52 | 1.00 | | | | | | | |
| 11 | ENT HJ | 0.54 | | | | | | | | 0.46 | | 1.00 | | | | | | |
| 12 | E. coli HJ | | 0.54 | | | | 0.57 | | 0.54 | | | | 1.00 | | | | | |
| 13 | $\delta^{18}O$ MB | | | | | | | | | | | | | 1.00 | | | | |
| 14 | $\delta^{18}O$ ZV | | | | | | | | | | | | | 0.54 | 1.00 | | | |
| 15 | rain | | | | | | | | | | | | | 0.44 | | 1.00 | | |
| 16 | rain (-1) | | | | | | | | | | | | | | | 0.54 | 1.00 | |
| 17 | total rain | | | | | | | | | | | | | | | | 0.53 | 1.00 |

*NOTE: Table shows statistically significant coefficients only.*
*ENT – enterococci; GR – Grčevo; RZ —Ružićevo; GL –Glavanovo; GLW – Glavanovo west; SB –Sablićevo; HJ – beach of the Hotel Jadran; ZV – Zvir; MB – Martinšćica well; rain – rain amount on the day of sea water sampling, rain (-1) – rain amount one day prior to sea water sampling; total rain – total rain amount between two consecutive sea water samplings*

several "leaps" during the sampling period (Fig. 8A & 8B).

Before we started the Granger causality tests, we also tested for a statistically significant correlation between variables at different locations. The $\delta^{18}O$ values of all three springs in the Bakar Bay are correlated with bacteria in the Bakar Bay (Table 1). This statistical association gives us additional evidence for the argument that microbial pollution was washed away by the rainfalls and ultmately discharged from the coastal area into the Bakar Bay. In the case of Pećine, no such statistically significant correlation between bacteria and isotopes could be accepted (Table 2). Neither in Bakar, nor in Pećine, the correlation between precipitation and both, bacteria and isotopes, proved to be statistically significant (Table 1 & Table 2).

The main idea of the presented study was to investigate the existence of possible causal links between rainfall, $\delta^{18}O$ and bacteria. We tested this possible link by using a panel data pairwise Granger causality test. The test results are shown in Table 3 & Table 4, where statistically significant results are in emphasis (bold fonts). As shown in Table 3, for Bakar Bay we could establish a statistically significant Granger causality between total rain and $\delta^{18}O$ values, as well as between $\delta^{18}O$ values and *E. coli*. This speaks in favour of our hypothesis of the existence of a precipitation → groundwater stable isotopes → seawater microbial contamination statistical association.

Further, we show a situation for Pećine district, a location that is geographically close enough to the Bakar Bay, nevertheless with somewhat different properties, and thus somewhat different results. Table 4 shows we are able to ascertain direct Granger causalities, for both non-differenced (lag = 2) and differenced (lag = 1), in cases of total rainfall and *E. coli*, total rainfall and $\delta^{18}O$ values, and $\delta^{18}O$ MB values

*Table 3. Results of Panel Data Pairwise Granger Causality Tests for Bakar Bay (statistically significant results in emphasis)*

| Variable | | Non-differenced (lag=2) | | Differenced (lag=1) | |
|---|---|---|---|---|---|
| **Independent (*X*)** | **Dependent (*Y*)** | ***F*-Stat.** | ***P*** | ***F*-Stat.** | ***P*** |
| total rainfall | enterococci | 0.87 | 0.43 | 0.51 | 0.48 |
| total rainfall | *E. coli* | 0.54 | 0.59 | 0.08 | 0.77 |
| total rainfall | $\delta^{18}$O PER | **4.02** | **0.03*** | **4.80** | **0.04*** |
| total rainfall | $\delta^{18}$O DB | **7.07** | **0.002*** | **12.91** | **0.001*** |
| total rainfall | $\delta^{18}$O DBC | **8.78** | **0.001*** | **6.46** | **0.02*** |
| $\delta^{18}$O PER | enterococci | 0.08 | 0.92 | 0.24 | 0.63 |
| $\delta^{18}$O PER | *E. coli* | **4.81** | **0.02*** | **10.80** | **0.002*** |
| $\delta^{18}$O DB | enterococci | 0.2 | 0.82 | 0.58 | 0.45 |
| $\delta^{18}$O DB | *E. coli* | **6.48** | **0.004*** | **19.74** | **< 0.001*** |
| $\delta^{18}$O DBC | enterococci | 0.09 | 0.9 | 0.12 | 0.73 |
| $\delta^{18}$O DBC | *E. coli* | **4.26** | **0.02*** | **9.97** | **0.003*** |

*NOTE: For $X \rightarrow Y$, $H_0$: X does not cause Y. * denotes rejection of the $H_0$. Total rainfall – amount of rain between two consecutive marine water samplings; PER – Perilo; DB – Dobra; DBC – Dobrica.*

*Table 4. Results of Panel Data Pairwise Granger Causality Tests for Pećine (statistically significant results in emphasis)*

| Variable | | Non-differenced (lag=2) | | Differenced (lag=1) | |
|---|---|---|---|---|---|
| **Independent (*X*)** | **Dependent (*Y*)** | ***F*-Stat.** | ***P*** | ***F*-Stat.** | ***P*** |
| total rainfall | enterococci | 1.28 | 0.28 | **4.69** | **0.03*** |
| total rainfall | *E. coli* | **5.74** | **0.004*** | **8.12** | **0.005*** |
| total rainfall | $\delta^{18}$O MB | **26.24** | **< 0.001*** | **5.94** | **0.02*** |
| total rainfall | $\delta^{18}$O ZV | **42.74** | **< 0.001*** | **30.42** | **< 0.001*** |
| $\delta^{18}$O MB | enterococci | **3.64** | **0.03*** | **4.35** | **0.04*** |
| $\delta^{18}$O MB | *E. coli* | 0.16 | 0.85 | 0.93 | 0.34 |
| $\delta^{18}$O ZV | enterococci | 1.52 | 0.22 | 0.21 | 0.65 |
| $\delta^{18}$O ZV | *E. coli* | 2.26 | 0.11 | **5.33** | **0.02*** |

*NOTE: For $X \rightarrow Y$, $H_0$: X does not cause Y. * denotes rejection of the $H_0$. Total rainfall – amount of rain between two consecutive marine water samplings; ZV – Zvir; MB – Martinšćica well.*

and enterococci. In addition, for differenced (lag = 1) values there is causality between total rainfall and enterococci, and between $\delta^{18}$O ZV values and *E. coli*. Therefore, in case of Pećine, a hypothesis of the existence of a precipitation → groundwater stable isotopes → seawater microbial contamination statistical association could not be rejected.

The values shown in Table 3 and Table 4 are the highest results achieved by our calculations and show $\delta^{18}$O and bacteria stock retention times of around 4 weeks (lag = 2 for the non-differenced variables) with stockpiling / depletion times of around 2 weeks (lag = 1 for the differenced variable). Because of the biweekly sampling dynamics, we leave room for values representing shorter periods.

The limitation of this analysis is certainly a small number of data used. Longer and more frequent sampling would give us larger data sets to work with, with possibly more precise findings.

Our goal was to find a statistical association between rainfall as an independent variable, isotope value as a proxy variable of the underground mixing of newly infiltrated precipitation with the groundwater, and finally, microbiological pollution of the sea as a dependent variable. The example of Bakar Bay shows that by introducing $\delta^{18}$O as an intermediate indicator variable between rainfall and marine microbial pollution, we introduced the missing link that might enable us to predict the occurrence of microbiological contamination of the sea and to organise timely interventions to protect public health. Furthermore, the possibility to predict microbiological pollution of the sea may help in the construction of more functional institutional mechanism designs for the economic allocation of common goods and common pool resources such as beaches and groundwater basins.

The results of this study could also serve as a basis for new studies involving the new generation of liquid water isotope analyzers that allow for faster, more frequent, and even continuous water sampling, as well as real-time measurement of water isotopes (FREYBERG *et al.*, 2017). In this way, it would be possible to obtain predictive models with greater speed over traditional culture-based methods. However, one has to bear in mind that the development of new reliable predictive models based on proxy variables (in our case stable isotopes), should include the usage of culture-based methods as well (control purposes).

## CONCLUSIONS

We upgraded the usual black box (rainfall − bacteria) model into a grey box model by adding an intermediate step: isotope content of groundwater discharging into the sea. We tested the proposed model with the Panel Granger test. At both examined sites, we established the existence of a hypothetical predicted statistical relationship between rain, groundwater $\delta^{18}$O values and bacteria concentrations.

Accordingly, stable isotopes, which often serve as indicators of the functioning of karstic systems, could potentially be predictors of faecal contamination of bathing waters in karst littoral areas, as well.

The study of karst is highly interdisciplinary. We retain to interdisciplinarity by connecting meteorological input (precipitation amount) and microbiological output (bacteria concentration) via physical parameters (stable isotopes). Possible applications of the presented findings could be in fields of public health, environmental protection, and ecology as well as in economics in form of a construction of more functional institutional mechanism designs for the allocation of scarce water resources, and their protection from pollution.

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# Okolišni izotop $^{18}O$ u vodi priobalnih krških izvora kao moguća prediktorska varijabla mikrobiološkog onečišćenja mora

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## SAŽETAK

Testirali smo hipotezu o statističkoj povezanosti između kiše, podzemnih voda koje utječu u more i mikrobiološkog onečišćenja mora. Kao varijablu koja opisuje podzemne vode odabrali smo stabilni izotop $^{18}O$. Stabilni izotop $^{18}O$ vode je okolišni obilježivač koji se često korisiti u hidrologiji, osobito u krškim područjima kakav je obalni pojas Jadranskog mora. Testiranje je provedeno na lokacijama u Bakarskom zaljevu i odabranim plažama u gradu Rijeci. Panel analizom "Granger kauzalnosti" utvrdili smo postojanje statistički značajne povezanosti između količine oborina i izdašnosti $^{18}O$ izotopa u podzemnim vodama, kao i statistički značajnu povezanost između izdašnosti $^{18}O$ u podzemnim vodama i koncentracije fekalnih bakterija u moru. U skladu s dobivenim rezultatima možemo zaključti da se stabilni izotop $^{18}O$, u slučaju kada se koristi kao obilježivač za proučavanje krških podzemnih voda, također može koristiti i kao prediktorska varijabla fekalnog onečišćenja mora u koje se te podzemne vode ulijevaju. Pretpostavljamo da bi ova fizikalna metoda mogla biti vrijedan dodatak metodama predviđanja mikrobioloških zagađenja i ekonomske alokacije oskudnih zaliha zajedničkih prirodnih resursa kao što su vodonosnici, izvori pitke vode i plaže.

**Ključne riječi:** mikrobiološko onečišćenje, nuklearna fizika, stabilni izotopi, spektroskopske tehnike, zalihe zajedničkih resursa, prirodni resursi