

Original Research

# Seasonal Potential Transmission of Pathogens Associated with Ground Drinking Water

Wiktor Halecki<sup>1,2\*</sup>, Wiesław Barabasz<sup>3\*\*</sup>

<sup>1</sup>Department of Land Reclamation and Environmental Development, University of Agriculture, Al. Mickiewicza 24/28, 30-059 Kraków, Poland

<sup>2</sup>Department of Biometry and Forest Productivity, Faculty of Forestry, University of Agriculture in Krakow, Al. 29 Listopada 46, 31-425 Kraków, Poland

<sup>3</sup>Department of Microbiology, Faculty of Agriculture and Economics, University of Agriculture, Mickiewicza 24/28, 30-059 Kraków, Poland

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## Abstract

The objective of this investigation was to assess the sanitary status of groundwater to ensure hygienic requirement water standards. Pathogens in water may originate from animals and anthropogenic waste. In a semi-rural area microorganisms are significant indicators connected with human activities in the underground water. Notably, our results showed that a more efficient diagnostic group to determine the level of contamination was proteolytic bacteria ( $p < 0.01$ ). Results showed that pathogenic bacteria such as *Shigella* sp., *Salmonella* sp., *Escherichia coli*, and total coliforms were not detected. The highest number of *Staphylococcus* sp. and fungal spores were found in the spring. Considerable additional research is needed to appropriately measure microbial communities in underground water.

**Keywords:** diagnostic test, microbial indicators, seasonality, underground water, water pathogens

## Introduction

Pathogenic microorganisms are suitable indicators of drinking water [1-4]. Determining underground water is practical for a quality monitoring program [5]. Organic matter, ions, mineral form, nutrients, and toxic substances influence the growth of microorganisms in the underground water [6]. It was shown that the relationship between an individual feature of pathogenic microorganisms and specific hydrological parameters requires appropriate micro-ecological conditions,

likewise the availability of oxygen as well as high content of humus. Otherwise, underground water is sensitive to contamination [7-8], especially in households served with either on-site wastewater systems or private wells in semi-rural areas [9]. Particularly in the spring, water circulation changes temperature and concentration of nutrients. For that reason, the abundance of microorganisms fluctuates in the water throughout the season. Overall, changes of temperature principally limit the growth of pathogenic bacteria in the water [10]. In addition, pathogenic microorganisms play a key role for the quality of water and human consumption [11-12].

Local regulations restrict aquifer pollution. Our paper focuses on reclaiming drinking water as established by the standard approach for common indicators of exposure to

\*e-mail: w.halecki@ur.krakow.pl

\*\*e-mail: rbaraba@cyf-kr.edu.pl

infection. Though disregarded in most empirical studies, the major highlight of this research was to examine potential health risks from the transmission of microbial pathogens.

## Materials and Methods

### Study Area and Microbiological Analysis

Water samples were collected between May 2011 and January 2016 from suburban, semi-rural, and private wells in: Rzezawa (10 m depth), Jodłówka (10 m), Krzeczów (6 m), Borek (6 m), and Łazy (8 m) located in Poland's Małopolska Province. One plastic bottle (500 mL) was chosen for each sample. Samples were tented to limit the amount of intaken microorganisms throughout the season.

Coliform bacteria were determined by presumptive stage, confirmed stage, and completed test in the final reference method. A preliminary test involved inserting a suitable volume of water sample to the substrate with LPB Durham-tubes. Durham-tubes were also immersed in the medium to test for the production of the gas (hydrogen or carbon dioxide). Durham tubes were inserted upside down in the test tubes to detect gas production. Basal medium containing a single disaccharide source – lactose – was used for this purpose. Agar medium was used to determine the number of *Escherichia coli* and total coliforms after 24 hours of incubation at 37°C. To approve results for the isolation of total coliforms, we additionally incorporated samples of a previously prepared technique that exhibited a yellow-green metallic sheen on Endo's medium subjected to final testing. Then we tried to detect colonies by color change of the pH indicator (colonies surrounded by dark pink zone) when a sufficient amount of common end-products of bacterial fermentation acid was produced. The total number of saprophytic fungal spores were incubated at 28°C after 72 hours of incubation.

Short-term experiments have indicated that warmer temperatures can alter fungal biomass production. Spores are associated with responses to warming by conducting selection experiments. Spores are the most important propagules for most fungi, and the impact these organisms produce on their hosts will depend on the ability for fast spore germination and colonization. Spore germination may be affected by many factors – especially temperature – which is required for growth. Fungi are prone to temperature, and may adapt to warming from moderately warm (16°C) and warm (28°C) in spore production [13]. Specifically, we expected that 28°C is considered as representative value during all seasons in order to formulate a predicting model under laboratory conditions. Over 37°C, fungi produce more spores per unit biomass [14] and may not respond to our conclusions in this study. Optimal mycelial growth of conidial germination is initiated by germination of spores, and our standardization can establish field data for thousands of generations within a few months.

Determining total viable count (TVC) of mesophilic bacteria in 1 cm<sup>3</sup> of water was applied onto nutrient agar, MPA, after 48 hours of incubation at 37°C. Gelatin was used to enumerate the total number of proteolytic bacteria after 48 hours of incubation at 20°C. *Salmonella* sp. and *Shigella* sp. were incubated on SS medium at 37°C after 48 hours. Chapman's substratum was used to identify the total number of *Staphylococcus* sp. after 48 hours of being incubated at 37°C. Microbiological examinations were strictly performed in five parallel repetitions from the same sample, and explicitly notified as colony-forming units (CFU/cm<sup>3</sup>).

### Statistical Analysis

We carried out tests based on a diagnostic model that was designed to classify the most accurate microbial communities to detect contaminated water samples. We procedured resampling of residuals data so that they retained the structure of the interpolation of observations that are less than or equal to particular values. Receiver operating characteristic (ROC) was performed to reveal significant differences between sensitivity for mesophilic and proteolytic microbial communities during the season. Coefficient interval was measured by De Long nonparametric method [15]. Comparison between the numbers on each site was obtained using area under curve (AUC). Statistical calculations were surveyed using PQ stat (version 5.1 PI). Canoco for Windows (version 4.51) was applied to evaluate a potential transmission of pathogens using the principal component analysis (PCA) technique. All PCA loading is presented in Table 1. Outlier data were rejected from auxiliary examination.

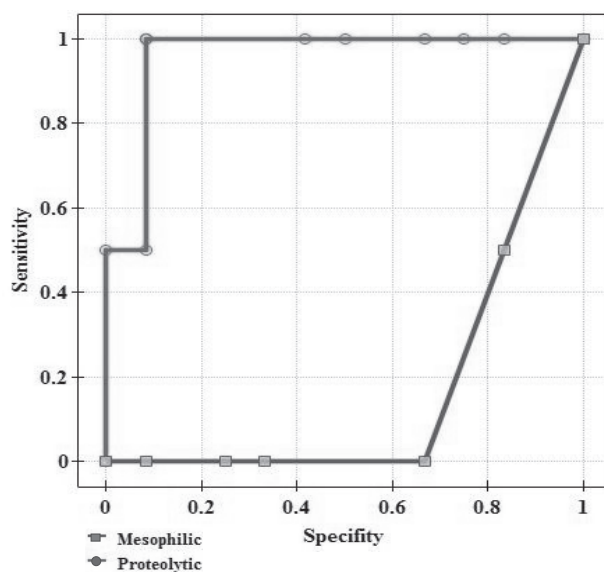


Fig.1. Diagnostic test between mesophilic and proteolytic bacteria.

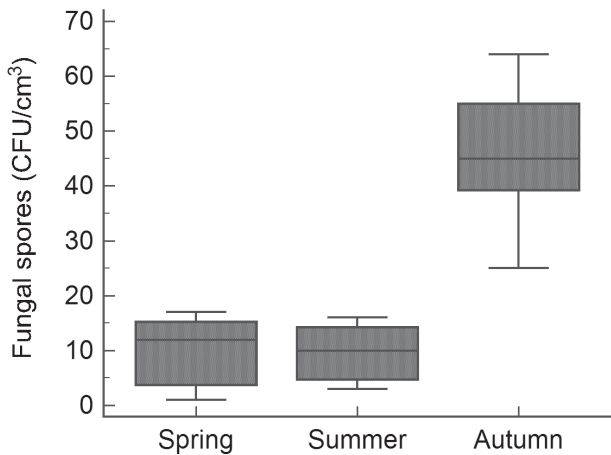


Fig. 2. Box plot for the saprophytic fungal spores in 1 cm<sup>3</sup> of water from the sampling area.

## Results and Discussion

### Diagnostic Test

Only faecal and coagulant substances originating from animal farms could initiate interaction with the microbial community. Here, however, greater emphasis was placed on certain elements of the microbial community growing at 20°C, mostly Gram-negative bacteria. Results showed a response by pathogens to environmental factors. ROC diagnosis (Fig. 1) was more appropriate to identify the sensitivity of proteolytic bacteria, and denoted significant differences at  $p < 0.01$  (AUC = 0.54).

### Seasonal Variability

Human households impact chemical fluctuation, especially for the development of pathogens during anaerobic digestion treatment of cattle manure [16]. Effluents from biodigesters, which are widely spread into farmland for agriculture use, is caused by local people [15]. Microbial communities produce endotoxins such as lipopolysaccharide leaching into underground water. Moreover, the most responsive factor for microbial communities is temperature. Therefore, diagnostic tests require detection of pathogenic bacteria [17-18], and focus on primarily fungal spores, which also should be monitored. Numerical results released by the evaluation of the total number of fungal spores varied during the growing season. The major number of fungal spores varied, particularly in the autumn (Fig. 2). A slightly longer survival period of fungal spores was observed in the underground water [19].

The increase of microorganisms growing at 20°C may transmit available organic matter in the water. Hence, the signaling effects through the high contamination range of animal faecal matter was observed in the study area. The variability of *Staphylococcus* sp. also was examined in our field research and in effect was ascertained at each point in time, which was essential for assessing the

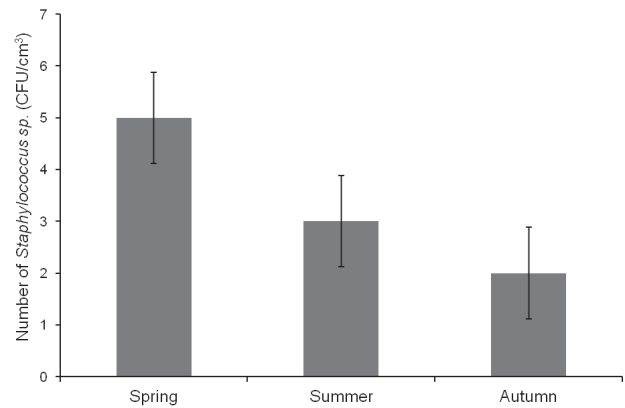


Fig. 3. Mean number of *Staphylococcus* sp. in the sampling area.

level of contamination. We concluded that the amount of *Staphylococcus* sp. was less abundant. Only in the spring was the increase identified (Fig. 3).

PCA analysis revealed that *Staphylococcus* sp. made no important contribution to the prediction of the density of the faecal contamination indicators. 8 m depth has a better value as a predictor of the presence of proteolytic bacteria and mesophilic bacteria (Fig. 4).

Two principal components were useful for presenting the correlation between variables by the observation numbers. The first principal component axis explained 58.15% of variance. The second component of axis explained 21.71% of variance (Fig. 4). Mesophilic bacteria and proteolytic bacteria represented the most important factors (>0.6) quantified by multivariate analysis (Table 1).

Identification of *Staphylococcus* sp. is important for diagnosing potentially pathogenic microorganisms in

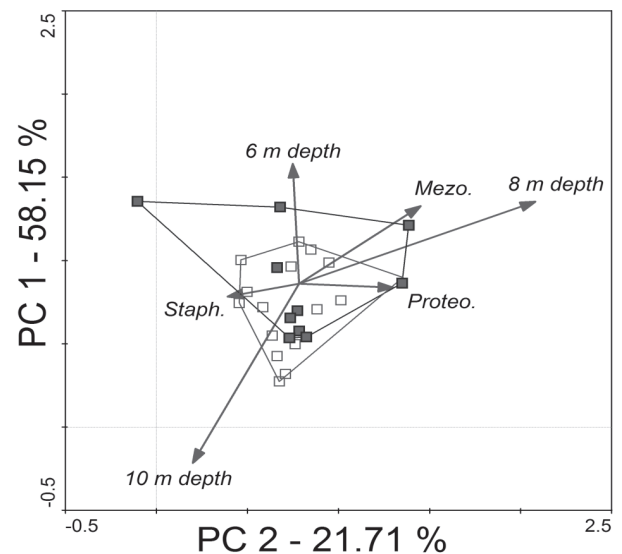


Fig. 4. PCA for potential transmission of pathogens in rural wells. Open squares indicate autumn and closed squares indicate spring. The summation includes the entire experimental data. The first and the second principal components explain 80% of the variance.

Table 1. Rotated component loading matrix obtained from pathogens associated with ground drinking water.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
<i>Staphylococcus</i> sp.	-0.01	-0.06	-0.04	-0.007	0.19	<b>0.97</b>
Mesophilic bacteria	<b>0.76</b>	<b>0.63</b>	<b>-0.71</b>	0.28	0.06	-0.003
Proteolytic bacteria	-0.14	<b>0.73</b>	<b>0.65</b>	-0.07	0.03	0.07
6 m depth	-0.01	0.07	0.08	0.18	<b>0.95</b>	-0.19
8 m depth	0.008	0.12	0.24	<b>0.93</b>	-0.20	0.05
10 m depth	<b>-0.98</b>	-0.15	0.03	0.017	-0.009	-0.0006

Bold numbers indicate the most important factors of each principal component (> 0.6).

water and bottom sediments [20]. The abundance of total coliforms indicate faecal contamination in drinking water [21-22]. In our study, total coliforms *Salmonella* sp. and *Shigella* sp. were not detected. These results are important for determining the quality of underground water in relation to human health. Variability of pathogenes and microbial indicators in a heavily impacted watershed have implications for planning monitoring [22-28].

Nonetheless, more research requires further analysis to determine microbial contamination that is promoted by water distribution systems [29-30]. Because environmental similarity differences in microbial communities were expected to be found, more variable data than in differences in environmental observation were considered in the research area. We recommended quantitative microbial risk assessment (QMRA) as the method to detect potential health risks from exposure to microorganisms.

### Conclusions

We supposed that contaminated groundwater should be explicitly quantified by reducing coagulation and sediment in water wells. That inclination suggested providing safe drinking water for local people, directly to entire domestic purposes. Basic microbiological analysis helped to avoid or decrease the possibility of disease. The routine work of research and conducting sanitary-epidemiological surveillance makes it difficult to test the samples of water toward controlling all possible microbial pathogens. The amount of proteolytic bacteria should be explained by inflows of anthropogenic organic compounds, or manure from animal farms. The abundance of mesophilic bacteria may suggest changes of water's chemical and physical condition throughout the season. Additionally, our finding assumed that seasonal evaluation is required for monitoring pathogens.

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