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MICROBIOLOGICAL GROUNDWATER QUALITY IN SHALLOW WELLS BEFORE AND AFTER DISINFECTION WITH PERACETIC ACID

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Abstract

The microbiological examination of water is used worldwide to monitor and control the quality and safety of various types of water. Peracetic acid (PAA) has garnered increasing attention as an alternative oxidant and disinfectant in water treatment due to the rising demand to reduce chlorine usage and control disinfection byproducts. The main aim of the research was to assess the well water microbiological quality before and after disinfection with PAA. The water samples were taken from 5 wells in the rural areas of Probishtip and Kocani regions of North Macedonia. Sampling was conducted twice (before and after disinfection) per season during the four seasons of the year. Water samples from 5 shallow wells were analysed for microbiological parameters using reference methods. The results were compared with the quality of control water and the permissible values according to the national legislation. Water quality parameters indicated that all well water samples failed to meet safe drinking water limits. A significant improvement in the microbiological quality of the water was observed during the seasons when a PAA working solution with concentrations of 0.05% and 0.1% was used. The regression statistical model revealed that disinfection with PAA and the seasonal variation in its concentration had a statistically significant influence on the microbiological quality of well water ($p<0.001$). Identification and management of groundwater quality are of utmost importance for maintaining freshwater resources, which are essential for sustainable rural development.

Key words: *water, biological contaminants, microbial safety, risk management.*

INTRODUCTION

Clean water is the most precious resource on planet Earth. Water is the most important compound without which there is no life. Water sources can be surface and underground. Surface waters include streams, rivers, natural and artificial lakes, as well as seas and oceans.

From the aspect of hygienic water quality, underground sources are of the greatest importance for supplying high-quality and safe water. Groundwater is used through wells that can be dug or drilled. Groundwater is formed by the percolation of surface or atmospheric water through permeable layers of soil. When it encounters an impermeable layer, the water is retained, and an underground reservoir is created. There, the water is still moving slowly. In addition, depending on the depth to which the water has reached, groundwater can be shallow (<10m) or deep (>10m), i.e. high or low. Groundwater has the best quality compared to other types of water.

Water quality is a key factor in the use of groundwater for households and agricultural production. Moreover, groundwater quality is largely influenced by the natural processes and anthropogenic activities in the surrounding area. The contamination typically results from polluted surface water seeping through the soil and into underground water reserves (Llopis-González et al., 2014). Rainwater runoff further exacerbates the problem by carrying microorganisms from the air, roads, household waste, animal waste, and improperly discarded solid materials into both surface and underground water sources. Safe drinking water is considered to be water that does not contain microorganisms, parasites and their forms in a number (concentration) that poses a danger to human health, does not contain physical and chemical substances and radioactive properties that are harmful to human health, and corresponds in terms of organoleptic properties of drinking water.

Groundwater quality, particularly from shallow wells, poses significant challenges for microbial safety in various applications, including agricultural and domestic use. In many rural regions, natural springs and water sources face significant microbial contamination. This issue becomes more pronounced when the water source is located near villages or in areas where livestock farming is prevalent. Communities living nearby often rely on these springs for drinking water, unknowingly exposing themselves to serious health risks. Groundwater can be contaminated with feces if septic tanks are built uncontrolled, without taking into account the groundwater level. The greatest danger for groundwater contamination is municipal wastewater that is discharged uncontrolled, directly or indirectly, into the recipients (rivers, lakes, septic tanks). From the recipients, through the penetration of the water, harmful substances and microorganisms contaminate the groundwater and well waters, thereby changing the quality of the water.

Chlorine and chlorine-based compounds are the most widely used disinfectants in water treatment due to their antimicrobial properties (Song et al., 2019). However, chlorination concerns over the formation of toxic, carcinogenic, mutagenic and teratogenic disinfection by-products (Doederer et al., 2014). As an alternative, peracetic acid (PAA) is recognised for its efficacy as a broad-spectrum disinfectant, making it suitable for treating microbial contaminants in groundwater. PAA exhibits strong oxidizing properties, allowing it to efficiently target a wide range of pathogens in various environmental contexts. Studies have demonstrated that PAA can significantly reduce bacterial counts even in the presence of organic matter, which typically complicates disinfection processes. For instance, Smither et al. (2018) indicate PAA's broad-spectrum activity and effectiveness against various pathogens, supporting its application in the microbiological disinfection of water sources, including shallow wells. Additionally, Zhang et al. (2021) found that PAA had a faster disinfection effect than other disinfectants, underscoring its rapid action and effectiveness.

Wells used by households and the food industry should be protected from pollution, and the microbiological quality of the water should be regularly monitored. Water quality standards are needed to determine whether groundwater of a certain quality is suitable for its intended use. The main objective of the research was to monitor the microbiological quality of well water yielded from shallow wells in two districts of North Macedonia before and after disinfection with different working concentrations of PAA.

MATERIAL AND METHODS

A field survey was undertaken to monitor the water quality from shallow wells to assess seasonal changes over a period of the year. The impact of disinfection methods involving PAA has been studied.

The shallow wells included in the survey are neither lined nor covered and are located close to the surface, near waste dumps or pit latrines, making the water susceptible to high levels of contamination.

The water samples were taken from 5 wells in the rural areas of Probishtip and Kocani regions of North Macedonia. For the assessment of groundwater hygiene quality before and after disinfection with PAA, the following microbiological parameters were analyzed: total number of coliforms, total bacteria count in 1ml at 37°C, total bacteria count in 1 ml at 22°C, faecally derived enterococci, Pseudomonas aeruginosa, Escherichia coli as number /100ml). The well water samples were tested at the Public Health Center - Kocani.

The research and sampling of well water were carried out during one calendar year by seasons of the year, as follows:

- Season 1 or autumn 2023 (months of September, October and November);

• Season 2 or winter 2023/2024 (months of December, January and February);
• Season 3 or spring 2024 (months of March, April and May);
• Season 4 or summer 2024 (months of June, July and August);

To assess the hygienic quality of well water, samples were collected both before and after disinfection, with testing conducted twice during each season of the year. In the first season, a disinfectant was applied at a concentration of 0.01%, equivalent to 100 ml of PAA per 1,000 liters of water. Seven days after treatment, water samples were taken to evaluate the hygienic condition. In the second season, the disinfectant concentration was increased to 0.025% (250 ml PAA per 1,000 liters). The third season was used a concentration of 0.05% (500 ml PAA per 1,000 liters), and during the fourth season, the highest concentration of 0.1% or 1,000 ml PAA per 1,000 liters was used. This gradual increase in disinfectant concentration aimed to identify the optimal concentration of PAA required to achieve the best disinfection efficiency and improvement in the microbiological quality of well water.

The standard methods used for the examination of microbiological parameters are following the Regulation on Water Safety (Official Gazette of the Republic of Macedonia No. 183 /2018). Sterilized 500 ml laboratory glass bottles were used to take water samples for microbiological analysis.

The following microbial analyses for water samples were performed:

- Most probable number of coliform bacteria in 100 ml of water sample (ISO 9308:2006);
- Coliform bacteria of faecal origin in 100 ml of water sample (ISO 9308:2:1990)
- Total number of microorganisms - number of colonies at a temperature of 37°C (ISO 6222:1990);
- Total number of microorganisms - number of colonies at a temperature of 22°C (ISO 6222:1999);
- Enterococci in 100 ml of water sample (ISO7899-2:2000);
- *Pseudomonas aeruginosa* in 100 ml of water sample (ISO 12780:2002)

The statistical General Linear Model (GLM) for repeated measurement was used to determine the influence of water disinfection with PAA, season of year and well location on water microbiological quality.

RESULTS AND DISCUSSION

The Graphs 1-6 showed the microbiological quality of well water (W1 – W5) including control water samples (C) and Maximum Permitted Concentration (MPC), regarding the period of sampling (1p before disinfection and 2p after disinfection) and seasons of the year (1_23 - autumn 2023; 2_23 - winter 2023/2024; 3_24 - spring 2024 and 4_24 - summer 2024).

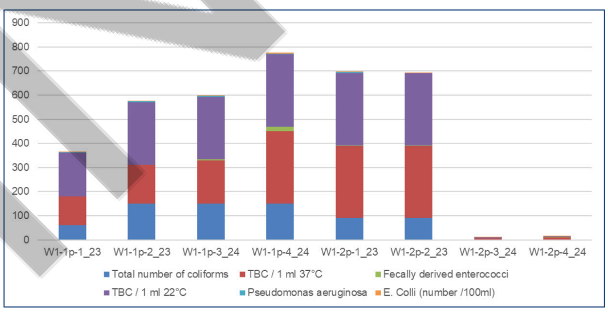

Figure 1. Microbiological quality of water in well 1 before and after disinfection.

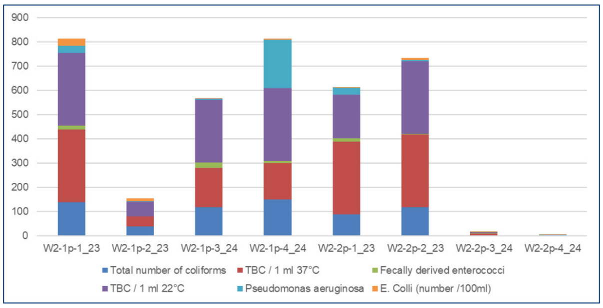

Figure 2. Microbiological quality of water in well 2 before and after disinfection.

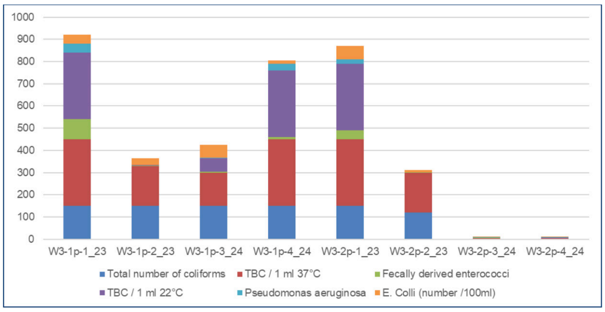

Figure 3. Microbiological quality of water in well 3 before and after disinfection.

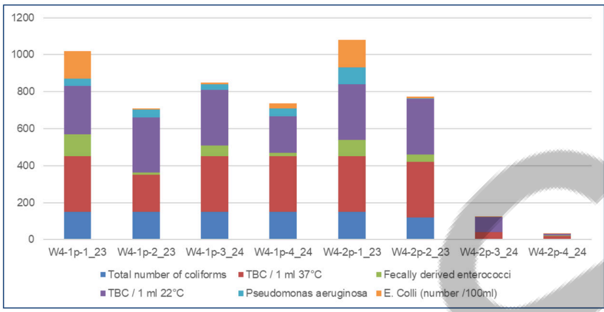

Figure 4. Microbiological quality of water in well 4 before and after disinfection.

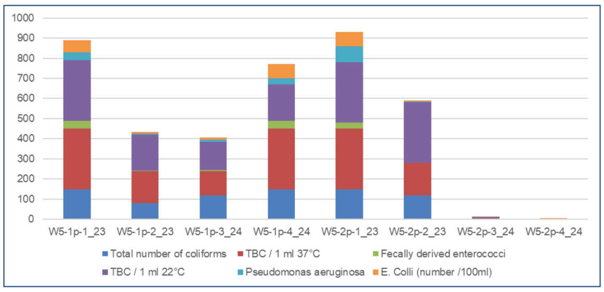

Figure 5. Microbiological quality of water in well 5 before and after disinfection.

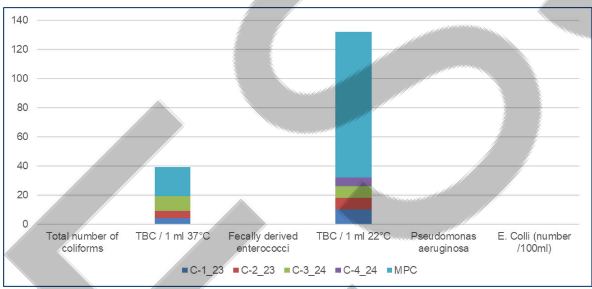

Figure 6. Microbiological quality of control water samples and Maximum Permitted Concentration (MPC).

It can be noted that the microbiological quality of the tested well water samples is not satisfactory from a microbiological point of view, and the obtained values are significantly higher compared to the microbiological quality values of the control water sample and the maximum permitted concentrations (MPC). The results showed that some wells have microbial contamination that can be fatal if the water is consumed untreated. In the water samples from all wells, the highest concentration was determined for the total number of coliform microorganisms and the total number of bacteria, in all four seasons during the year before the well water was disinfected with peracetic acid. The microbiological quality of the control sample in all seasons during the year was within the MPC, which satisfied the needs of drinking water.

Disinfection using PAA and the seasonal variation in its concentration had a statistically significant effect on the microbiological quality of well water ($p<0.001$). In contrast, the location of the shallow wells did not have a significant impact on the microbiological quality of the water.

The microbiological quality of groundwater in shallow wells is a critical public health concern, especially in rural areas where residents often rely on these sources for drinking water. After the disinfection of the well water with an oxidative disinfectant, a significant improvement in the microbiological quality of the water was observed in the seasons when a PAA working solution with a concentration of 0.05% and 0.1% was used. This concentration of PAA used in the third and fourth seasons gave satisfactory results for the tested parameters, so that the sample complies with the rulebook on the safety and quality of drinking water (Official Gazette of the Republic of Macedonia No. 183/18). However, disinfection with 0.01% and 0.025% PAA did not achieve the required microbiological standards.

Table 1 shows the results of the regression statistical model for the impact of the inter-factor variable of the performed disinfection of well water and the fixed factor variables on the microbiological quality of well water.

The presence of various pathogens in untreated groundwater supplies can result in serious health risks. Bacteria, such as *Escherichia coli* and *Salmonella spp.*, frequently emerge as focal points in assessments of shallow well water quality due to their implications for public health (De Giglio et al., 2017; Olorunleke et al., 2022). *Escherichia coli* and *Enterococci* are indicators of human faecal contamination and are possibly associated with human enteric pathogens.

Table 1. Regression model for the influence of disinfection, season and well location on water microbiological quality.

Dependent variable: Microbiological parameters for water quality			
Source of variations	**df**	**Mean square**	**F-value**
Disinfection	1	125691.743	32.832***
Disinfection*seson of year	3	91572.526	23.920***
Disinfection*well	4	584.165	0.153NS
Error	112	3828.326	

*** significant at level p<0.001; NS non-significant;

As a result, testing for coliform bacteria can be a reasonable indication of whether other pathogenic bacteria are present. Therefore, coliform (thermotolerant) bacteria are a commonly used bacterial indicator of the sanitary quality of food and water. Kovačić et al. (2017) noted a documented association between drinking water quality and gastrointestinal disease outbreaks, emphasizing the need for precautions against using untreated groundwater

Effective disinfection methods can mitigate these risks significantly. Moreover, the choice of disinfection method plays a significant role in the post-treatment microbial profile of groundwater. Different methods, including chlorination and PAA treatment, have distinct impacts on microbial populations. Similar to our results, the assessment of microbiological quality in groundwater before and after disinfection with PAA has demonstrated significant improvements in microbial contamination levels (Luukkonen & Pehkonen, 2016). The research of Hwang et al. (2012) has identified PAA as a promising disinfectant, noted for its virucidal and bactericidal properties and its efficacy in degrading potential contaminants without harmful residual effects often associated with chlorine-based water treatment. This effectiveness can be attributed to PAA's ability to penetrate biofilms and inactivate bacteria and viruses upon contact (Shen et al., 2016). Cadnum et al. (2016) highlight that ensuring proper concentration measurements of PAA is necessary for effective disinfection without compromising microbial safety. Queiroz et al. (2013) noted that inadequate concentrations of PAA could lead to reduced effectiveness, similar to other disinfectants like sodium hypochlorite. In addition to its disinfectant capabilities, PAA decomposes into non-toxic byproducts, primarily acetic acid and oxygen, enhancing its appeal as a sustainable disinfectant choice (Candeliere et al., 2016).

Variations in treatment efficacy against specific pathogens depend on factors such as water source characteristics and environmental conditions affecting the target pathogen's viability. Source versus household contamination dynamics can also influence disinfection effectiveness. Therefore, both immediate intervention and long-term management strategies must be implemented to sustain water quality improvements (Ercümen et al., 2017).

CONCLUDING REMARKS

Shallow wells are one of the most important types of water supplies for rural areas, mainly due to their low cost and easy way of construction. The groundwater is vulnerable to microbiological contamination due to risk factors such as human activities, lack of well protection structures and the hydrogeological characteristics in the area. The application of PAA in the disinfection of microbial contamination in groundwater, especially from shallow wells, presents numerous benefits. Its broad-spectrum antimicrobial efficacy, rapid action, minimal ecological footprint, and effective degradation into non-toxic components align well with the urgent need to enhance groundwater microbiological quality in various settings. Bridging to adopting and implementing water safety plans, an integrated strategy addressing infrastructure interventions, hydrotechnical protection of water sources, regular monitoring of water quality, and public education and awareness-raising initiatives is needed.

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REFERENCES

Llopis-González, A., Sánchez, A. L., Requena, P. M., & Suárez-Varela, M. M. (2014). Assessment of the microbiological quality of groundwater in three regions of the Valencian Community (Spain). *International journal of environmental research and public health*, 11(5), 5527-5540.

Cadnum, J. L., Jencson, A. L., O'Donnell, M. C., Flannery, E. R., Nerandzic, M. M., & Donskey, C. J. (2017). An increase in healthcare-associated Clostridium difficile infection associated with use of a defective peracetic acid–based surface disinfectant. *infection control & hospital epidemiology,* 38(3), 300-305.

Candeliere, A., Campese, E., Donatiello, A., Pagano, S., Iatarola, M., Tolve, F., ... & Fasanella, A. (2016). Biocidal and sporicidal efficacy of Pathoster® 0.35% and Pathoster® 0.50% against bacterial agents in potential bioterrorism use. *Health security*, 14(4), 250-257.

Doederer, K., Gernjak, W., Weinberg, H. S., & Farré, M. J. (2014). Factors affecting the formation of disinfection by-products during chlorination and chloramination of secondary effluent for the production of high quality recycled water. *Water research, 48*, 218-228.

Ercumen, A., Naser, A. M., Arnold, B. F., Unicomb, L., Colford Jr, J. M., & Luby, S. P. (2017). Can sanitary inspection surveys predict risk of microbiological contamination of groundwater sources? Evidence from shallow tubewells in rural Bangladesh. *The American journal of tropical medicine and hygiene*, 96(3), 561.

Food and Veterinary Agency (2018). Rulebook on requirements for safety and quality of drinking water, Official Gazette of the Republic of Macedonia 183/2018.

De Giglio, O., Caggiano, G., Bagordo, F., Barbuti, G., Brigida, S., Lugoli, F., ... & Montagna, M. T. (2017). Enteric viruses and fecal bacteria indicators to assess groundwater quality and suitability for irrigation. *International Journal of Environmental Research and Public Health,* 14(6), 558.

Hwang, C., Ling, F., Andersen, G. L., LeChevallier, M. W., & Liu, W. T. (2012). Microbial community dynamics of an urban drinking water distribution system subjected to phases of chloramination and chlorination treatments. *Applied and Environmental Microbiology*, 78(22), 7856-7865.

Kovačić, A., Huljev, Ž., & Sušić, E. (2017). Groundwater as the source of an outbreak of Salmonella Enteritidis. *Journal of epidemiology and global health,* 7(3), 181-184.

Luukkonen, T., & Pehkonen, S. O. (2017). Peracids in water treatment: A critical review. *Critical Reviews in Environmental Science and Technology*, 47(1), 1-39.

Olorunleke, S. O., Okorie-Kanu, O. J., Nwanta, J. A., & Chah, K. F. (2021). Point prevalence and antibiogram of cefotaxime-resistant Enterobacteriaceae isolated from food animals and in-contact humans at abattoirs, animal market, and farms in Southeast, Nigeria. *Nigerian Veterinary Journal,* 42(1), 29-46.

Smither, S. J., Eastaugh, L., Filone, C. M., Freeburger, D., Herzog, A., Lever, M. S., ... & Wahl-Jensen, V. (2018). Two-center evaluation of disinfectant efficacy against Ebola virus in clinical and laboratory matrices. *Emerging infectious diseases*, 24(1), 135.

Song, X., Vossebein, L., & Zille, A. (2019). Efficacy of disinfectant-impregnated wipes used for surface disinfection in hospitals: a review. *Antimicrobial Resistance & Infection Control*, 8, 1-14.

Shen, Y., Huang, C., Monroy, G. L., Janjaroen, D., Derlon, N., Lin, J., ... & Nguyen, T. H. (2016). Response of simulated drinking water biofilm mechanical and structural properties to long-term disinfectant exposure. *Environmental science & technology*, 50(4), 1779-1787.

Queiroz, D. A., Peçanha, M. M., Neves, A. C. C., Frizzera, F., & Tonetto, M. R. (2013). Influence of disinfection with peracetic acid and hypochlorite in dimensional alterations of casts obtained from addition silicone and polyether impressions. *Journal of Contemporary Dental Practice*, 14(6), 1100-1105.

Zhang, N., Guo, J., Liu, L., Wu, H., & Gu, J. (2021). Study on the efficacy of peracetic acid disinfectant (type III) on gastrointestinal endoscopy disinfection. Surgical Laparoscopy Endoscopy & Percutaneous Techniques, 31(4), 395-398.

МИКРОБИОЛОШКИ КВАЛИТЕТ НА БУНАРСКАТА ВОДА ПРЕД И ПО ИЗВРШЕНАТА ДЕЗИНФЕКЦИЈА СО ПЕРОЦЕТНА КИСЕЛИНА

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Резиме

Микробиолошкото испитување на водата се користи за следење и контрола на квалитетот и безбедноста на различните видови вода. Пероцетната киселина привлекува сè поголемо внимание како алтернативно средство за дезинфекција на водата заради трендот на намалена употребата на хлорот кој при дезинфекција на водата формира штетни резидуи. Главната цел на истражувањето беше да се процени микробиолошкиот квалитет на бунарска вода пред и по извршена дезинфекција со пероцетна киселина. Примероците вода беа земени од 5 бунари во руралните области на регионите Пробиштип и Кочани во Северна Македонија. Земањето примероци беше спроведено двапати во секоја од четирите сезони во годината кога беа направени истражувањата, односно во секоја сезона пред и по извршената дезинфекција на водата. Микробиолошките испитувања на примероците бунарска вода беа направени со референтни методи. Резултатите беа споредени со квалитетот на контролните примероци вода и максимално дозволените вредности според националното законодавство. Параметрите за микробиолошкиот квалитет на бунарската вода покажаа дека примероците вода не ги исполнуваат критериумите за безбедна вода за пиење. Значајно подобрување на микробиолошкиот квалитет на бунарската вода беше постигнат во сезоните на годината кога беше извршена дезинфекција на водата со работен раствор на пероцетна киселина во концентрација од 0,05% и 0,1%. Регресиониот статистички модел покажа дека дезинфекцијата и интеракцијата меѓу дезинфекцијата и сезоната во годината имаат статистички значајно влијание врз микробиолошкиот квалитет на бунарската вода ($p<0,001$). Следење и управување со квалитетот на подземните води се од голема значајност за одржување на слатководните ресурси, кои се неопходни за одржлив рурален развој.

Клучни зборови: *вода, биолошка контаминација, микробиолошка безбедност, управување со ризици.*