Annales Universitatis Paedagogicae Cracoviensis
Studia Naturae, 8: 137–154, 2023, ISSN 2543–8832
DOI: 10.24917/25438832.8.7

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Comparison of the microbiological quality of water from selected dug and deep wells in Nowy Sącz district (Southern Poland)

Introduction

Microbiological purity of water is an important aspect affecting the safety of its consumers. Microbiological water analysis aims to precisely estimate the numbers of bacteria present in the tested sample and allow for the recovery of microorganisms in order to identify them. Indicator bacteria surrogates used to measure the potential presence of fecal material and associated fecal pathogens. Indicator bacteria such as *Escherichia coli* T. Escherich, coliform bacteria, *Enterococci*, *Clostridium perfringens* Veillon & Zuber, coagulase-positive staphylococci, *Pseudomonas aeruginosa* Schröter, Migula, *Legionella* sp. are part of the intestinal flora of warm-blooded animals. They do not reproduce in the aquatic environment.

Legal regulations relating to the microbiological purity of water are presented in the Polish regulation of the Minister of Health of December 7, 2017 (*Dz.U. 2017, poz. 2294*), regarding the quality of water intended for human consumption. In Poland and in other EU countries, the guidelines regarding water do not differ. According to these guidelines, there cannot be even one indicator bacterial cell in one hundred millilitres of tap water. An important indicator of water purity is the determination of the total number of psychrophilic and mesophilic bacteria present in 1 ml of water.

Many buildings in smaller towns in Poland do not have a water supply network, therefore individual underground water intakes are created. According to data from the Central Statistical Office, in Nowy Sącz County (49°37′26′N 20°41′50′E), 44% of residents, despite the development of water supply infrastructure, still do not connect to the network.

In order to maintain appropriate conditions and ensure the health and safety of people using water, systematic water quality monitoring should be carried out. Currently, people with their own water intakes become more aware of the existing threat resulting from the presence of bacteria in water. Unfortunately, consumers wrongly assess water quality based on organoleptic characteristics such as taste, smell, colour, which are not always consistent with microbiological contamination of water. Users of private water intakes often do not comply with the rules related to the construction and sealing of wells. This is a serious problem, especially since, according to the regulations, water that comes from individual intakes and is not used as part of a business activity or for buildings of collective residence (over 50 people) is not subject to control by the State Sanitary Inspectorate (*Dz.U. 2017/ 2294*; Tymczyna et al., 2003).

Fig. 1. Dug wells in the Nowy Sącz district (Southern Poland): A-C – different forms of dug wells (Photo. N. Nosal)

The area of the Małopolska Voivodeship (Southern Poland) has a highly diversified geological structure, relief and soil conditions. Some geological layers allow partially the rainwater to pass through, creating groundwater. Groundwater occurs up to 5 meters deep and is constantly fed by atmospheric precipitation and has the ability to self-clean. The deeper the water is taken, the better the self-purification process is. Unfortunately,

agricultural, municipal and industrial pollutants get into them. Groundwater is covered by a continuous, impermeable layer of soil, which limits the ingress of pollution from outside, and is therefore well protected. Additionally, they are characterised by low temperature, which limits the multiplication and metabolism of microorganisms (Alley, 2009; Gromiec, 2014; Matuszewska et al., 2018; Ostrowska et al., 1999; Widłak, Łukawska, 2014; Zawadzki, 1999).

Fig. 2. Deep wells in the Nowy Sącz district (Southern Poland): A-D – different forms of deep wells (Photo. N. Nosal)

In the Nowy Sącz district, you can find ring wells (dug) supplied with groundwater, as well as deep wells (drilled) that draw water from aquifers. Dug wells, from which water sample were taken were made of concrete rings with a diameter of 1 m covered with a concrete or metal cover. The depth of the examined dug wells ranged from 1.5 to 7 meters (Fig. 1). 30% of the dug wells were located close to fertilised fields, 20% were located close to home sewage treatment plants with seepage drainage. According to the information obtained, 28% of buildings are not connected to the municipal sewage network. Deep wells up to 30 meters deep were characterised by very good protection

against contamination (Fig. 2). Water was collected via a deep-well pump. All wells from which water was collected for testing were used as a source of drinking water or for domestic purposes. Out of 100 facilities, 28 wells were disinfected with chlorine using generally available disinfectants in the three months preceding the collection of water samples. In order to improve the microbiological quality of water taken from wells, UV lamp irradiation was used in three buildings. Water samples taken immediately before the lamp and after exposure to ultraviolet rays were analysed.

In accordance with the requirements contained in the Regulation of the Minister of Health of 2017 (*Dz.U. 2017/ 2294*), 100 ml of water sample cannot contain a single colony-forming unit (CFU) of indicator bacteria: *E. coli* and coliform group. An additional criterion is the analysis at 22 ± 2°C of 1 ml of the sample for the total number of microorganisms, which in water taken from the consumer's tap cannot exceed 200 CFU in 1 ml, while in water introduced into the network it should not exceed 100 CFU in 1 ml.

The aim of the work was to perform a microbiological analysis of 100 selected samples of water intended for drinking purposes from individual dug or deep wells located in the Nowy Sącz district (Southern Poland).

Methods

Determination of coliform bacteria and *Escherichia coli* by membrane filtration MF (in accordance with the PN-EN ISO 9308–1:2014–12 standard)

100 ml of the tested water sample was passed through a sterile membrane filter made of mixed cellulose esters (MCE), with a pore size small enough to retain bacterial cells (typically 0.45 μm). The filter was placed on a sterile metal frit in a filter apparatus generating a negative pressure (Fig. 3 – Appendix 1). Then, the membrane filter was transferred aseptically to the surface of an agar plate (Chromogenic Coliform Agar CCA) inhibiting the growth of gram-positive bacteria (Fig. 4A). The plate was incubated in aerobic conditions at 36 ± 2°C for 21 hours. After incubation, the growth was checked and all bacterial colonies growing on the membrane filter were counted. *E. coli* colonies are coloured dark blue to purple (positive reaction to β-D-galactosidase and β-D-glucuronidase). This reaction is demonstrated by lactose-positive bacilli growing in the colonies (Fig. 4B).

Coliform colonies ranging in colour from pink to red show a positive reaction to β-D-galactosidase (Fig. 4C). To confirm the presumed coliform bacteria, an oxidase detection test was performed. Coliform colonies grown on chromogenic CCA medium were transplanted using a sterile loop onto tryptic soy agar (TSA) and incubated for

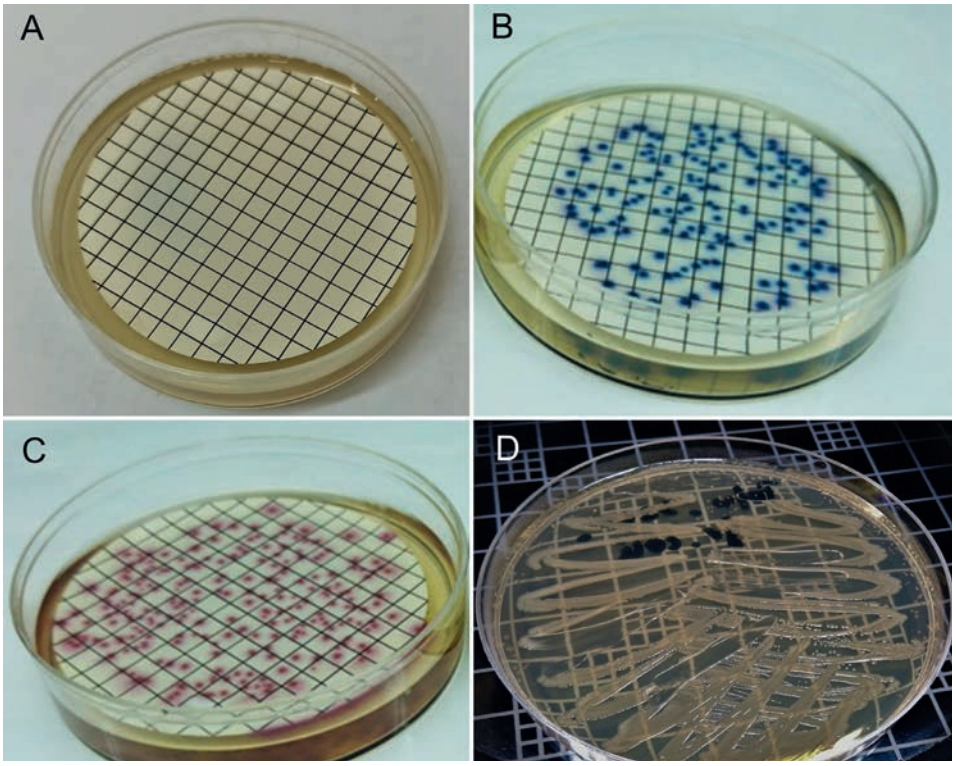

Fig. 4. Membrane filter on a plate of Chromogenic Coliform Agar – A, dark blue colonies of *E. coli* on Chromogenic Coliform Agar – B, pink to red coliform colonies on Chromogenic Coliform Agar – C, coliform colonies on tryptic soy agar (TSA) (Photo. N. Nosal)

22±2 hours at 36 ± 2°C (Fig. 4D). After incubation, the colonies were placed on filter paper and spotted with two drops of indole. The appearance of a pink colour within 10 seconds confirmed the presence of coliform bacteria in the tested water sample (Fig. 5A-B).

Determination of the total number of microorganisms using the plate count method in accordance with the PN-EN ISO 6222:2004 standard

1 ml of the tested water sample was transferred to a Petri dish with a diameter of 90 mm, then poured over a liquefied agar medium with yeast extract without glucose. After mixing and solidification, the medium was incubated in aerobic conditions at 22 ± 2°C for 72 hours. All grown colonies were counted using a colony counter, which constituted the CFU number in 1 ml of the tested water sample (Fig. 5C).

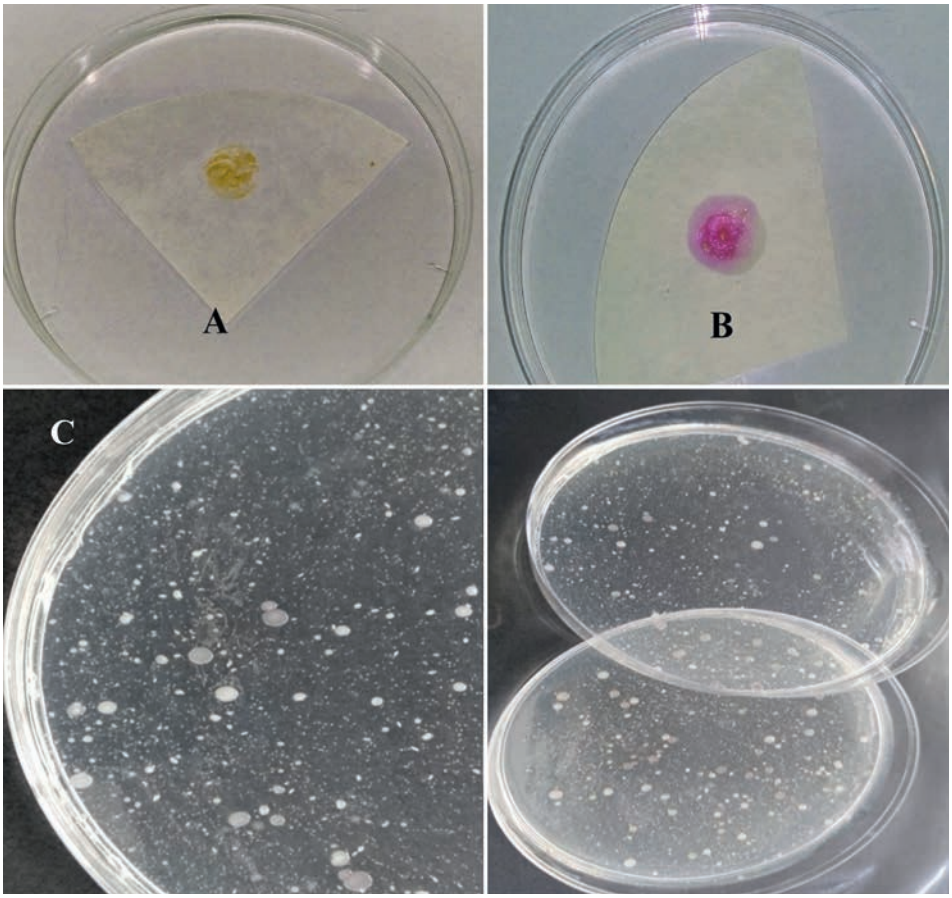

Fig. 5. Coliform bacteria: before the indole test – A, after the indole test – B; appearance of microorganisms visible to the naked eye on an agar medium – C (Photo. N. Nosal)

Results and discussion

Permissible plate count standard for the total number of microorganisms is less than 200 CFU in 1 ml of tested water (Tab. 1).

Tab. 1. Microbiological requirements for water should meet the requirements for consumption in accordance with the *Regulation of the Minister of Health* (2015)

Tested microbiological indicator	Volume of water sample [ml]	Permissible number of CFU in a water sample
Coliform bacteria	100	0
Escherichia coli	100	0
Total number of microorganisms	1	< 200

CFU – colony-forming unit

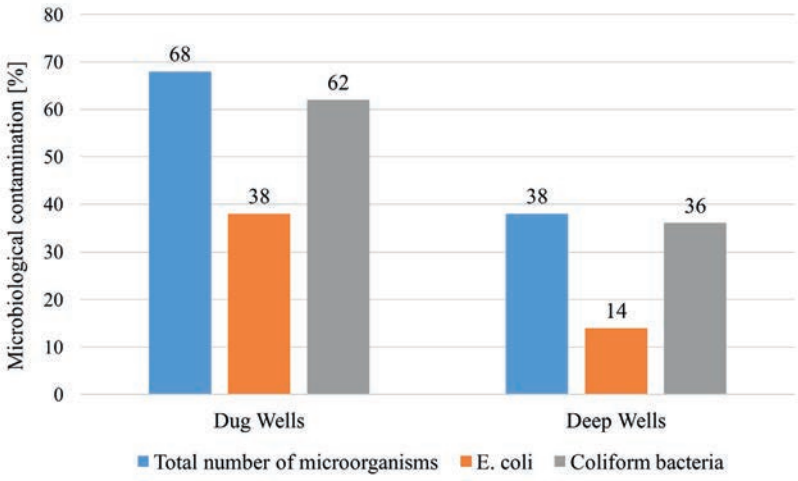

Fig. 6. The percentage comparison of the tested microbiological indicators is clearly in favour of drilled wells

The results of microbiological analyses of water samples collected from 50 dug wells showed that in 34 samples (68%) the total number of microorganisms exceeded. The presence of coliform bacteria was found in water samples from 31 wells (62%) and *Escherichia coli* was detected in water from 19 facilities (38%), which indicates a significant microbiological contamination exceeding the permissible standards (Fig. 6, Tab. 2 – Appendix 2).

Water from 50 drilled wells was less microbiologically contaminated than water from dug wells. In 31 facilities, water analysed to determine the total number of microorganisms met the required standards and the value of 200 CFU was not exceeded in 1 ml of tested water samples. The presence of *E. coli* was found in water from 7 facilities (14%) and *E. coli* bacteria were recorded in water samples from 18 wells (36%), while the requirements that water intended for drinking should meet definitely exclude the presence of these bacteria (Fig. 6, Tab. 2 – Appendix 2). In water samples from dug wells of poor technical condition, the presence of microorganisms, coliform bacteria and *E. coli* was detected. Dug wells made of concrete rings were covered with a concrete or metal cover, of which cover leakage was observed in 47% of cases.

Disinfection was carried out in 15 wells dug a few days before taking water samples. Chlorine disinfection significantly improved the microbiological quality of water. After disinfection, the presence of *E. coli* and coliform bacteria was not recorded in almost all water samples (Tab. 2 – Appendix 2). Only 6 samples of water taken from disinfected wells contained single *E. coli* CFU. Disinfection turned out to be ineffective in the case of two dug wells (no. 42 and 49, Tab. 2 – Appendix 2), which could be due to the

use of a too small dose of chlorine in relation to the amount of water in the well, lack of prior cleaning of the bottom and walls of the well, or faulty construction of the well and possibility of contact of well water with sewage. The use of inappropriate materials for the construction of dug wells and improper maintenance of the water system are favourable conditions for the formation of biofilm (Yasoniri et al., 2007).

Due to the small depth, dug wells are exposed to contamination caused by seepage of surface water from spring thaws and atmospheric precipitation causing high variability of water inflow in the well (Talalaj, 2008). Microbiological contamination was recorded in 11 dug wells, which were relatively shallow, ranging from 1.5 to 2.8 meters deep. The quality of groundwater is influenced by the poor condition or complete lack of water and sewage infrastructure. The subsurface layers of groundwater, the water table of which is at a depth of less than 5 m, are most exposed to groundwater degradation (Widłak, Łukawska, 2014).

In deep, drilled wells, water comes from aquifers located from several to even 70 meters underground. Out of 50 water samples tested, the presence of *E. coli* was found in eight cases, with a small number of bacteria not exceeding 8 CFU. A small content of *E. coli* in water (a few cells in 100 ml of water), in the absence of exceedances of other microbiological and physicochemical indicators (including oxidation, ammonium nitrogen, nitrates, chlorides, sulphates), may result from incorrect collection of water samples for testing (bottle without proper sterilisation, improper collection activities, e.g. improper sterilisation of the tap from which the water sample is taken). In such a case, the water sample should be taken again to fully exclude microbiological contamination of *E. coli* in the water (*Water from wells…*, 2019).

Only in one facility (sample no. 49, Tab. 2 – Appendix 2) the water was very microbiologically contaminated and more than 80 CFU of *E. coli* and coliform bacteria and more than 300 CFU of other microorganisms were found. This water was definitely unfit for use. The disinfection of drilled wells significantly improved the microbiological quality of water. No presence of *E. coli* was detected in the tested samples and only in one sample (sample no. 28, Tab. 2 – Appendix 2) coliform bacteria were recorded. The presence of these bacteria may indicate the likelihood of the presence of other groups of bacteria, including pathogenic ones, but in the absence of the simultaneous presence of *E. coli* in the water, contamination of the intake with sewage is usually ruled out. Coliform bacteria often occur in water supply systems as a result of the development of biofilm on the surface of water pipes or deposits on the internal surfaces of pipes (sediments resulting from the precipitation of such contaminants as iron in water, manganese or the so-called scale deposits in cases where the water hardness is high). Coliform bacteria also often multiply in distribution networks and water supply installations of buildings with irregular and small water consumption, where water stagnation often occurs in the pipes (*Water from wells…*, 2019).

Conboy and Goss (2000) proved that the location of the water intake had a huge impact on water quality. They showed that in rural areas, microbial contamination depended on soil characteristics, geological structure and the location of wells. Wells located in limestone rocks and clay soil are high-risk sources. Geological layers behave differently in relation to rainwater that naturally seeps into the ground. Some layers allow water to pass through (soil, sands, gravels, some clays, etc.) and others do not (massive rocks, clays, etc.). Thanks to the first impermeable layer, located at a depth of about 25 m, groundwater accumulates on its surface and can be drawn from wells (Zawadzki, 1999).

Wells located in urban areas are clean and free from microbiological contamination. Increased values of microbiological contamination in water (Tab. 2 – Appendix 2) in rural areas may result from cattle and pig breeding, as well as the use of natural fertilisers. There are also bacteria that do not originate from unfavourable human activities, but they occur in their natural environment such as soil, soil water and groundwater (Talalaj, 2008; Gromiec 2014).

Large amounts of bacteria were detected in water samples taken from wells located in rural areas near a home sewage treatment plant with seepage drainage. It can therefore be concluded that the presence of *E. coli* bacteria and faecal coliform bacteria in water is the result of contamination from a leaky septic tank. It was observed that septic tanks in the examined area were in poor technical condition.

Paul et al. (2004) conducted groundwater research in a small city in Germany. Based on the analyses in which faecal bacteria were detected, they concluded that groundwater contamination was related to, among others, leaks in sewage systems in the nearby vicinity. Based on the results of water analyses in Canada by Goss et al. (1998), where approximately 75% of water comes from surface water and 30% comes from groundwater intakes, it can be concluded that potential sources of groundwater pollution households have leaky or poorly maintained sewage treatment systems (septic tanks), as well as if manure runoff seeps through the soil near the well or when manure is spread on agricultural fields.

In accordance with the provisions contained in § 31 section 1 of the Regulation of the Minister of Infrastructure of April 12, 2002 (*Dz.U. 2002, nr 75 poz. 690*) on the technical conditions to be met by buildings and their location, the distance from the well to the axis of the well should be at least 5 m to the plot boundaries, 30 m to the nearest individual sewage infiltration pipe, 70 m to the enclosures for farm animals on unpaved terrain, 15 m to livestock buildings and related tight silos as well as tanks for collecting waste or compost. Casing for dug wells must be made of impermeable materials and well-sealed. When using concrete blocks to build wells, they must be grouted from the outside to the depth, at least 1.5 m from the ground level and from the inside at the entire height. At a minimum distance of 1 m around the dug and drilled well, the area must be hardened and have a 2% slope towards the outside.

The presented test results of water from private water intakes located in the Nowy Sącz district indicate that all water used for drinking purposes should be subjected to treatment processes, which involve adapting their properties and composition to the requirements resulting from their intended use. For this purpose, it is recommended to clean and disinfect the well. In wells dug to improve water quality, the bottom of the well should be deepened and cleaned, and protected against flowing surface water. If bacteria appear in the water, disinfection using chemical or physical methods is necessary to eliminate them. Due to its low costs, the most commonly used water disinfectant is chlorine (sodium hypochlorite). This method eliminates microorganisms, but the water changes its taste and smell for some time. Some of the chlorine is combined with water admixtures, and the remaining part remains as the so-called useful chlorine, acts on microorganisms and when reacting with water it may be in the form of hypochlorous acid, hypochlorous ion or molecular chlorine. In order to avoid secondary contamination of water with bacteria, a constant chlorine concentration should be maintained. Unfortunately, hypochlorite or isocyanurate compounds produce organochlorine products that are mutagenic and carcinogenic. The organic compounds most hazardous to health are trihalomethanes (THM), haloacetic acids and chloramines. Halogen organic compounds are formed as a result of the reaction of agents used to disinfect water with natural matter found in water, which is a specific precursor to their formation (Wyczarska-Kokot, 2009; Zmysłowska, 2009; Michalski, Łyko, 2012).

Ozone can also be used to disinfect water, since it has a bactericidal effect and is 50 times more effective than chlorine. It decomposes spontaneously in water and thus enriches the water with oxygen as well as improves the colour, taste and smell of water. It is biodegradable from pure oxygen. Ozone is a strong oxidant that acts on the bacterial cell wall by increasing cell adhesion and lysis. It causes damage to the cell membrane through lipid oxidation. Due to high operating costs, it is very rarely used as a disinfectant (Wichrowska et al., 2002).

Currently, ultraviolet radiation of water is commonly used for disinfection. This is a physical method and has a bactericidal effect. It involves irradiating water flowing through a chamber in which the lamp emits UV radiation of a specific power. Quartz mercury lamps emit invisible UV rays with a wavelength of 100–400 nm. The bacteriological effect is demonstrated by waves with a length of 254–265 nm. During constant irradiation of water, no secondary contamination occurs. This method is safe if it is not combined with water chlorination, because then disinfection by-products are formed: groups of trihalomethanes, halogenoacetonitrile acids, haloacetic acids, halogenoketones, chloropicrin, hydrate and chloral (Dera, 1997; Włodyka-Bergier, Bergier, 2013).

In order to minimise the risk of microbiological contamination of water, it is necessary to conduct regular water tests to determine the water quality, because the consumption of contaminated water poses a great epidemiological threat to humans and

animals. Facilities that are subject to constant sanitary supervision have an annual water testing schedule and are constantly inspected and cleaned by the owners. Therefore, it is recommended that individual water intakes should also be analysed for water quality at least once a year in order to monitor the occurrence of possible contaminations, which, considering the results presented in this study, were relatively high. However, in order to predict the susceptibility of the shot for contamination in a given location, it is useful to know the history of the well or area, where the well is to be located (Tymczyna et al., 2003; *Water from wells…*, 2019).

Conflict of interest

The authors declare no conflict of interest related to this article.

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Appendix 1

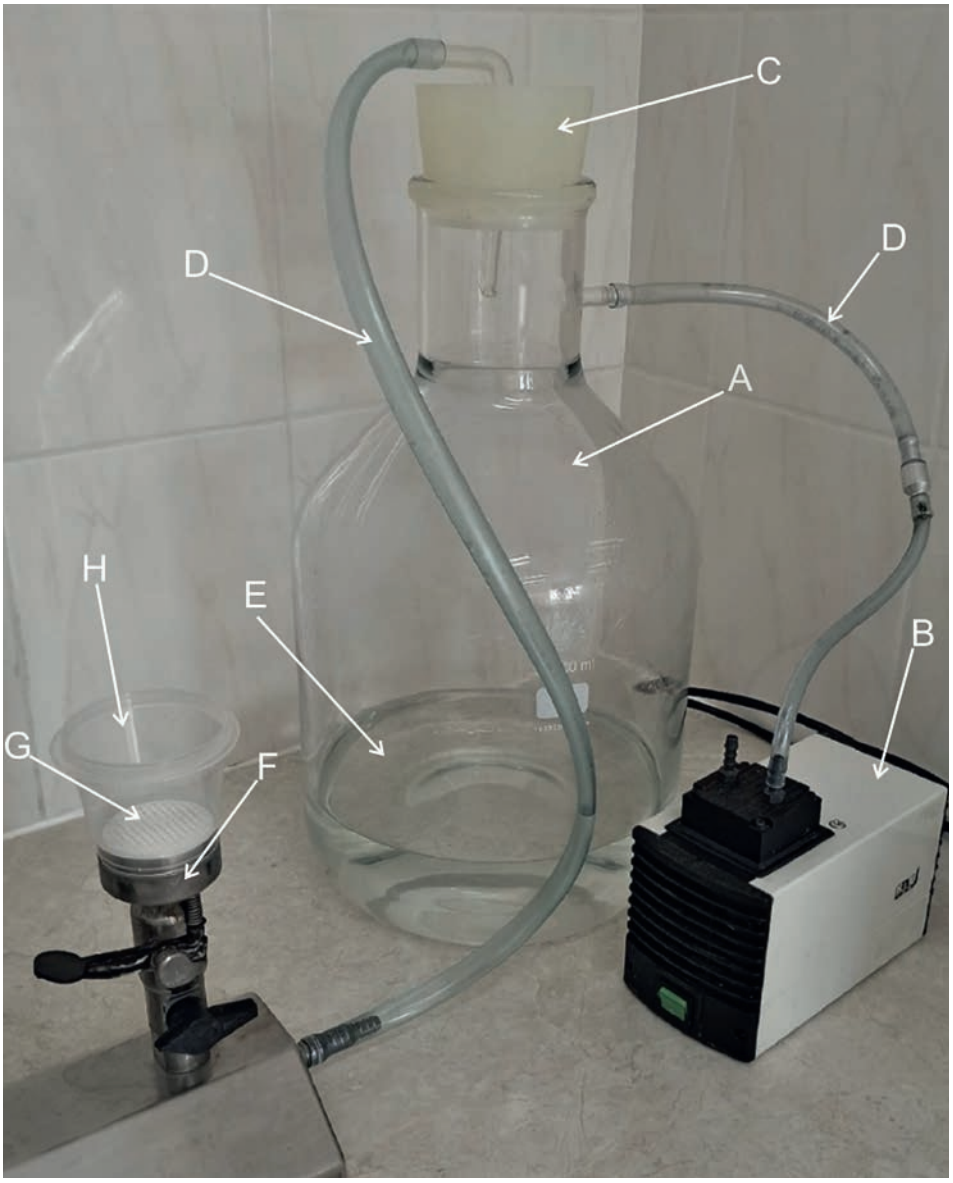

Fig. 3. Membrane filtration set: A – Büchner flask with a ground joint and a side tube (suction flask), B – suction from the pump creates a negative pressure in the flask, C – rubber stopper, D – rubber hose, E – filtrate accumulating in the flask, F – metal sinter, G – sterile membrane filter with a pore diameter of 0.45μm, H – plastic funnel (100 ml) (Photo. N. Nosal)

Tab. 2. Results of microbiological analyses in water samples taken from dug and drilled wells; in grey, exceeding the permissible number of CFU (colony-forming unit) in a water sample is marked

Sample number	Chlorinated water	Number of bacteria		
		Coliform bacteria	Escherichia coli	Total number of microorganisms
		Dug wells		
1.	No	9	0	>300
2.	No	0	0	0
3.	Yes	0	0	4
4.	Yes	0	0	2
5.	No	0	0	49
6.	No	0	0	>300
7.	Yes	0	0	5
8.	No	62	0	286
9.	No	6	0	>300
10.	No	37	0	>300
11.	Yes	0	0	61
12.	No	>100	2	28
13.	No	0	0	>300
14.	No	16	0	>300
15.	Yes	5	0	>300
16.	No	0	0	88
17.	No	118	3	224
18.	No	>100	4	>300
19.	No	>100	9	>300
20.	No	17	0	>300
21.	No	4	1	>300
22.	No	0	0	122
23.	Yes	0	0	8
24.	No	5	5	>300
25.	No	>100	12	>300
26.	No	>100	10	>300
27.	No	0	0	>300
28.	No	>100	20	>300
29.	Yes	0	0	>300
30.	Yes	13	1	161
31.	No	>100	128	>300
32.	Yes	6	6	>300
33.	No	6	0	30
34.	No	>80	0	>300
35.	No	93	1	>300

36.	No	69	15	>300
37.	No	0	0	>300
38.	No	0	0	>300
39.	No	>80	2	>300
40.	No	>80	>80	>300
41.	No	134	0	>300
42.	Yes	42	42	>300
43.	Yes	0	0	>300
44.	Yes	0	0	5
45.	Yes	0	0	7
46.	Yes	0	0	42
47.	Yes	9	0	>300
48.	No	>80	78	>300
49.	Yes	>80	>80	>300
50.	No	56	4	40

Tab. 2, continued

Sample number	Chlorinated water	Number of bacteria		
		Coliform bacteria	Escherichia coli	Total number of microorganisms
		Drilled (deep) wells		
1.	No	0	0	104
2.	No	0	0	9
3.	No	13	0	21
4.	Yes	0	0	179
5.	No	35	5	>300
6.	No	28	2	187
7.	No	0	0	0
8.	No	0	0	0
9.	No	20	6	>300
10.	No	28	0	187
11.	No	12	8	>300
12.	No	>100	3	>300
13.	No	0	0	0
14.	Yes	0	0	0
15.	No	4	0	>300
16.	No	0	0	90
17.	No	0	0	>300
18.	Yes	0	0	>300
19.	No	0	0	116
20.	No	0	0	88
21.	No	0	0	23
22.	Yes	0	0	0
23.	No	0	0	>300

24.	No	0	0	3
25.	No	11	0	>300
26.	No	11	0	>300
27.	No	0	0	0
28.	Yes	32	0	>300
29.	Yes	0	0	222
30.	No	43	0	37
31.	No	0	0	0
32.	Yes	0	0	0
33.	Yes	0	0	0
34.	No	0	0	>300
35.	No	>80	0	>300
36.	No	0	0	140
37.	No	0	0	27
38.	Yes	0	0	0
39.	No	0	0	33
40.	No	0	0	0
41.	No	14	8	158
42.	Yes	0	0	173
43.	Yes	0	0	>300
44.	No	>80	0	>300
45.	No	14	0	117
46.	No	0	0	>300
47.	Yes	0	0	23
48.	No	19	1	>300
49.	No	>80	>80	>300
50.	No	0	0	0

Abstract

In the Nowy Sącz district (Southern Poland) the water supply network is not sufficiently developed and many farms use individual dug or deep wells. Groundwater supplying dug wells comes from atmospheric precipitation and may be contaminated with agricultural fertilizers, municipal and industrial sewage. Groundwater present in deep wells is well protected against pollution and low water temperature limits the multiplication of microorganisms. Microbiological tests of water are aimed at determining the total number of microorganisms in 1 ml of the tested sample and checking the presence of indicator bacteria, i.e. coliform bacteria and *Escherichia coli* in the water. The aim of this work was to perform a microbiological analysis of 100 selected samples of water intended for drinking purposes from individual dug or deep wells located in the Nowy Sącz district. In the tested water samples from individual intakes an increase in the total number of microorganisms, the presence of coliform bacteria and *Escherichia coli* bacteria were noted. Water from dug wells, that do not have proper insulation, is characterised by significant bacteriological contamination compared to water from deep wells. In the most cases water from deep wells meets the required microbiological standards.

Keywords: dug and deep wells water, total number of microorganisms, coliform bacteria, *Escherichia coli*, membrane filtration, plate count method

Received: [2023.11.01]

Accepted: [2023.12.21]

Porównanie jakości mikrobiologicznej wody z wybranych studni kopanych i głębokich na terenie powiatu nowosądeckiego (Polska Południowa)

Streszczenie

Na terenie powiatu nowosądeckiego (południowa Polska) sieć wodociągowa nie jest dostatecznie rozbudowana i wiele gospodarstw korzysta z indywidualnych studni kopanych lub głębinowych. Wody gruntowe zasilające studnie kopane pochodzą z opadów atmosferycznych i mogą być zanieczyszczone nawozami rolniczymi, ściekami komunalnymi oraz przemysłowymi. Wody podziemne obecne w studniach głębinowych są dobrze chronione przed zanieczyszczeniami, ponadto niska temperatura wody ogranicza namnażanie mikroorganizmów. Badania mikrobiologiczne wody mają a na celu określenie ogólnej liczby mikroorganizmów w 1 ml badanej próby oraz sprawdzenie obecności bakterii wskaźnikowych czyli bakterii grupy coli i *Escherichia coli* w wodzie. Celem pracy było wykonanie analizy mikrobiologicznej 100 wybranych prób wody przeznaczonej do celów pitnych pochodzących z indywidulanych studni kopanych lub głębinowych zlokalizowanych na terenie powiatu nowosądeckiego. W badanych próbach wody z indywidualnych ujęć odnotowano wzrost ogólnej liczby mikroorganizmów, obecność bakterii grupy coli oraz bakterii *Escherichia coli*. Woda ze studni kopanych, które nie mają właściwej izolacji, charakteryzuje się znacznym zanieczyszczeniem bakteriologicznym w porównaniu do wody pochodzącej ze studni głębinowych. Woda z ujęć głębinowych w większości przypadków spełnia wymagane normy mikrobiologiczne.

Słowa kluczowe: woda kopana i głębinowa, całkowita liczba mikroorganizmów, bakterie z grupy coli, *Escherichia coli*, filtracja membranowa, metoda zliczania płytkowego

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