

An assessment of the physicochemical and microbiological stability of bottled natural medicinal waters from Polish health resorts

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Abstract: Balneotherapy, utilising natural medicinal waters, gases, and peloids, is a clinically effective method for complementing the treatment of various medical conditions. One specific type of balneotherapy is crenotherapy, which involves the oral administration of natural medicinal waters. This study aimed to assess the impact of storage time on the stability of the chemical composition and microbiological quality of bottled natural medicinal waters, as well as to assess the feasibility of using packaged natural medicinal waters at home to prolong the effect post-sanatorium treatment, as recommended by the chief physician responsible for health resorts. Fourteen bottled medicinal waters sourced from seven Polish health resorts were analysed. The study examined the following samples: one HCO₃-Cl-Na-Ca, four HCO₃-Cl-Na, one HCO₃-Mg-Na-Ca, two HCO₃-Na-Ca, two HCO₃-Ca, two HCO₃-Na and two Cl-Na waters. Physicochemical and microbiological parameters were measured at the following times: t_0 (immediately after packaging), t_1 (midway through the declared shelf life) and t_2 (at the end of the shelf life). Physicochemical analysis of the medicinal waters demonstrated the stability of the chemical composition in terms of basic minerals and physicochemical parameters over the product's shelf life, as defined by the manufacturer. Faecal indicator bacteria or *Pseudomonas aeruginosa* were absent in all of the samples tested. However, coliform bacteria, potentially of biofilm origin, were detected in one sample. An initially high number of microorganisms, determined at 22°C, observed in three water samples, was no longer detected at the final testing stage. The findings indicate that the medicinal waters analysed maintain stability in chemical composition, organoleptic parameters and microbiological purity throughout the manufacturer's specified shelf life.

Keywords: natural medicinal water, balneotherapy, crenotherapy, medicinal resources

INTRODUCTION

Balneology is the scientific discipline that studies the therapeutic properties of natural medicinal resources used for medical purposes. Within balneology, researchers examine not only the chemical composition and microbiological parameters of these resources but also their effects on human health and their application in various therapeutic procedures, such as baths, compresses, inhalations, or drinking therapies (Cheleschi et al. 2022, Carbajo et al. 2024, Fioravanti et al. 2024). Balneotherapy is one of the most widely practised non-pharmacological treatment methods, employed across many European countries, including Italy, Spain, France, Germany, Hungary, Lithuania and Poland, as well as in Japan, Turkey, Israel and China (Cheleschi et al. 2022, de Oliveira et al. 2023, Fioravanti et al. 2024). According to the Polish Ministry of Health, Poland is home to 47 spa towns, characterised by natural medicinal resources and favourable climatic conditions (Ministry of Health of the Republic of Poland website, n.d.). Furthermore, the National Health Fund's (NHF) annual report indicates that approximately 400,000–500,000 patients in Poland benefit from sanatorium-based treatments funded by the NHF each year (National Health Fund [NHF] website, n.d.).

Natural medicinal resources encompass gases and minerals, such as medicinal waters and peloids (peats), whose therapeutic properties have been confirmed in line with the principles set out in the Act on Spa Treatment (Ustawa 2005, Ponikowska & Kocharński 2017, Cheleschi et al. 2022). These resources possess diverse therapeutic effects, determined by their natural primary chemical and microbiological composition (Ponikowska & Kocharński 2017, Ziemska et al. 2019). In Poland, these natural resources must adhere to quality standards for balneochemical classification and meet health, safety and suitability criteria, as validated by a certificate of therapeutic properties (Rozporządzenie 2006).

In accordance with the regulations of the Polish Ministry of Health (Rozporządzenie 2006) medicinal waters in Poland are classified as follows:

- 1) Mineral waters – containing at least 1000 mg of dissolved components per cubic decimetre, including chlorides, sulphates, and bicarbonates of sodium, calcium, and magnesium.
- 2) Specific waters (low-mineralised) – containing fewer than 1000 mg of dissolved solid components per cubic decimetre, including one or more therapeutic-specific components at defined or higher concentrations:
 - a) Iodide water – containing at least 1 mg of iodides per cubic decimetre,
 - b) Sulphide water – containing at least 1 mg of sulphides or other sulphur(II) compounds per cubic decimetre,
 - c) Fluoride water – containing at least 2 mg of fluorides per cubic decimetre,
 - d) Ferruginous water – containing at least 10 mg of iron(II) per cubic decimetre,
 - e) Silicic water – containing at least 70 mg of metasilicic acid per cubic decimetre,
 - f) Carbonic water – containing at least 1000 mg of free carbon dioxide per cubic decimetre,
 - g) Mildly carbonic water – containing from 250 to 999 mg of free carbon dioxide per cubic decimetre or
 - h) Thermal water – characterised by a temperature of at least 20°C at the spring,
 - i) Radon water – with a radioactivity level of at least 74 Bq/dm³.
- 3) Specific mineral waters – mineral waters containing one or more of the distinctive components listed above.

In balneotherapy, various types of medicinal waters are utilised in therapeutic baths, including thermal chloride-sodium waters, sulphide-hydrogen sulphide waters, carbonic waters, carbon dioxide-rich waters and radon waters (Ponikowska & Kocharński 2017, Reger et al. 2022).

Aerosol therapy makes use of chloride-sodium waters, as well as specific iodide, sulphate-sodium, magnesium-calcium, bicarbonate-calcium-magnesium and radon waters (Ponikowska & Kocharński 2017, Zajac 2021).

Crenotherapy, a prominent form of balneotherapy, involves the ingestion of medicinal water directly from the spring at a spa pump room. The effectiveness of the drinking therapy is influenced

by factors such as the type of medicinal water, its temperature, volume, type and concentration of individual minerals, specific substances and method of administration.

In the treatment of various conditions, particularly those affecting the gastrointestinal tract (liver and biliary tract, stomach, duodenum and intestines), the urinary tract and metabolic diseases (diabetes, obesity), medicinal bicarbonate and acidic waters are commonly used. Additionally, chloride-sodium waters (with concentrations up to 1.5%) and sulphide-hydrogen sulphide and sulphurous waters are frequently employed (Ponikowska & Kochański 2017, Costantino et al. 2019, Ziemska et al. 2019, Chaves et al. 2020, Dumitrescu et al. 2022). Recent studies by other authors indicate that crenotherapy, particularly with sulphurous waters, also offers significant benefits, such as improved pain perception, reduced depression and insomnia and an overall enhancement in the quality of life for patients suffering from chronic low back pain (CLBP) associated with lumbar osteoarthritis (Costantino et al. 2019, 2022, de Macedo Antunes et al. 2019). Furthermore, the oral intake of sulphate waters has been shown to alleviate pain and improve physical function (disability) in overweight and obese patients suffering from CLBP associated with lumbar osteoarthritis (Costantino et al. 2022). Ongoing research is further investigating medicinal waters' use in treating chronic sinusitis (Nimmagadda et al. 2022).

The effects of crenotherapy can be categorised into topical (occurring in the gastrointestinal tract) and general effects. The topical effect refers to the physical and chemical impact on the mucosal lining of the gastrointestinal tract, depending on factors, such as water's temperature, osmotic pressure or degree of mineralisation. The CO₂ content in the waters promotes vasodilation, which enhances secretion, accelerates peristalsis and aids gastric emptying. Hypotonic, isotonic or slightly hypertonic mineral waters are recommended for treating conditions of the gastrointestinal tract, as well as the liver and biliary tract (Ponikowska & Kochański 2017, Dumitrescu et al. 2022). In summary, as described above, medicinal

waters have a wide range of applications in the treatment of various ailments.

After completing the sanatorium treatment, the drinking therapy can be continued or repeated at home, as prescribed by the physician, using commercially available bottled medicinal waters. However, current regulations (Rozporządzenie 2012, Chelesi et al. 2022) do not provide specific guidelines for the production and bottling of natural medicinal waters. They only permit the availability of bottled medicinal waters at the spa pump room. Despite this, bottled medicinal waters are available commercially outside of health resorts. As a result, these medicinal resources are currently not subject to the control by local sanitary and epidemiological stations or pharmaceutical inspectorates, which could pose potential health risks to consumers. There is limited literature data to confirm the stability of the chemical and microbiological composition of bottled natural medicinal waters. Changes in the quality of these waters can affect both physicochemical parameters, such as pH or the precipitation of sediments and microbiological parameters, such as increased contamination. For certain substances (e.g. iron and calcium) precipitation from solutions can result in the loss of their therapeutic properties, as these elements become unavailable for absorption by the human body. The growth of undesirable bacteria associated with changes in the physicochemical properties of the product and storage conditions can adversely affect its therapeutic efficacy and suitability for use.

The primary objective of this study was to assess the effect of storage time on the stability of the chemical composition and microbiological quality of bottled natural medicinal waters sourced from Polish health resorts. Additionally, the study sought to determine the shelf life of the evaluated medicinal products, considering any observed changes in the physicochemical and microbiological parameters within the shelf life declared by the manufacturer. A further aim of the study was to evaluate the suitability of these waters for home use to prolong the effect of sanatorium treatment, as recommended by the chief physician responsible for the health resorts.

METHODS

Fourteen bottled medicinal waters, provided by seven health resorts (Szczawnica, Wysowa-Zdrój, Krynica-Zdrój, Polanica-Zdrój, Szczawno-Zdrój, Kołobrzeg, Połczyn-Zdrój) were tested. The locations of sampling sites are shown in Figure 1, and a brief description of the tested medicinal waters is provided in Table 1. Samples were obtained through cooperation with the health resorts, which consented to provide samples of medicinal waters in packaging immediately after production. This arrangement ensured that analyses could be conducted directly after packaging (at time t_0). Physicochemical and

microbiological parameters were determined at three time points: t_0 (immediately after packaging), t_1 (midway through the declared shelf life) and t_2 (at the end of the shelf life). For thirteen out of the fourteen waters tested, the manufacturer declared a 1-year shelf life, while for one water (bicarbonate-calcium type, 9) had a declared shelf life of 9 months. The water samples tested were supplied and stored in their original packaging throughout the study. The samples were provided in PET bottles (light blue, green or brown) and bag-in-box packaging to minimise the influence of light or atmospheric exposure on the contents. All samples were stored at room temperature in a light-free environment.

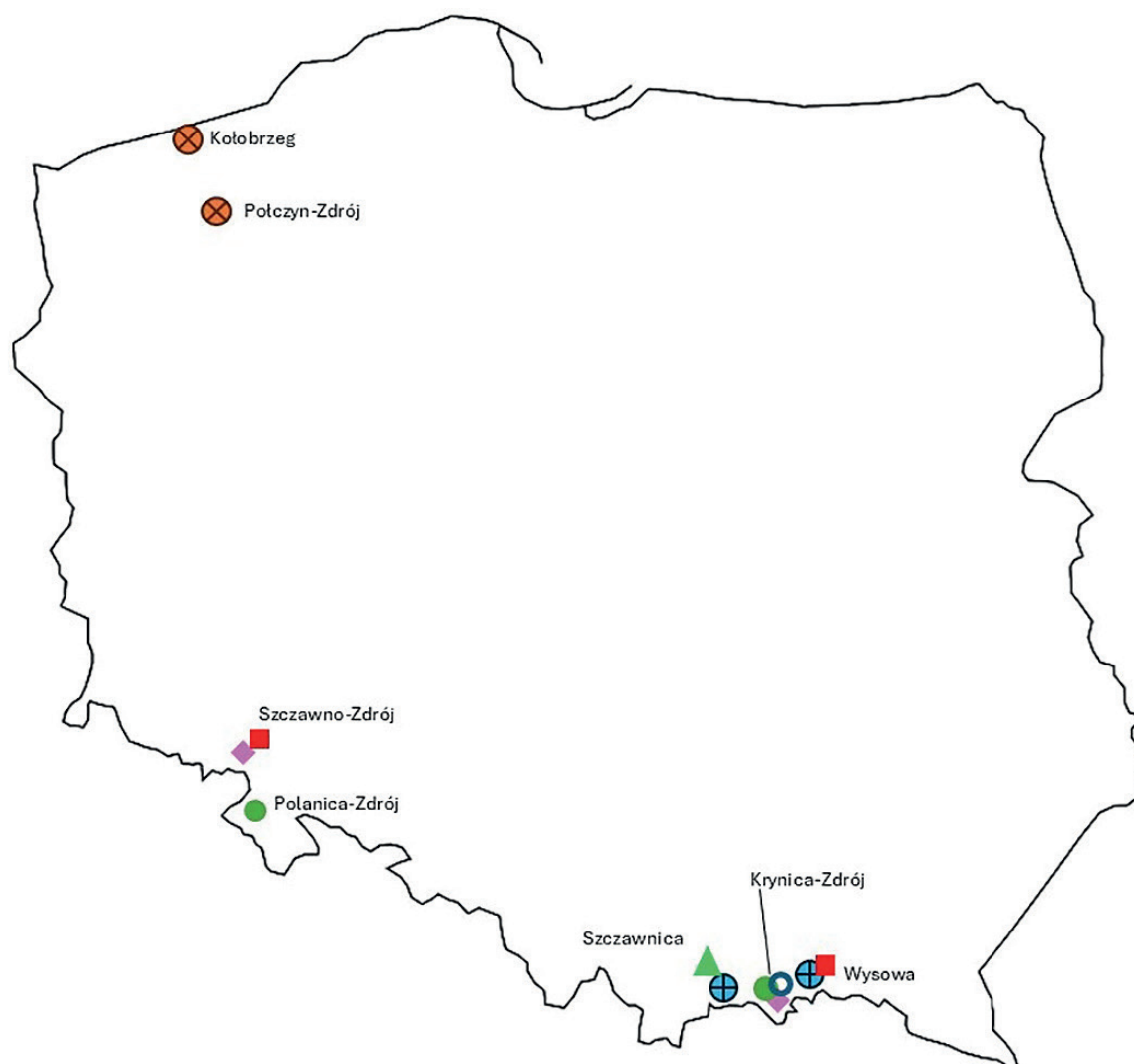


Fig. 1. Location of sampling sites in Poland. Explanation of symbols included in Table 1

Table 1
Characteristics of tested medicinal waters

Sample number	Sampling place	Type of water	Identification on the map	Shelf-life	Package
1	Szczawnica	HCO ₃ -Cl-Na-Ca	▲	12 months	bag-in-box
2	Szczawnica	HCO ₃ -Cl-Na	⊕	12 months	bag-in-box
3	Wysowa-Zdrój	HCO ₃ -Cl-Na	⊕	12 months	brown PET bottle
4	Wysowa-Zdrój	HCO ₃ -Cl-Na	⊕	12 months	brown PET bottle
5	Szczawnica	HCO ₃ -Cl-Na	⊕	12 months	bag-in-box
6	Krynica-Zdrój	HCO ₃ -Mg-Na-Ca	○	12 months	brown PET bottle
7	Szczawno-Zdrój	HCO ₃ -Na-Ca	■	12 months	green PET bottle
8	Wysowa-Zdrój	HCO ₃ -Na-Ca	■	12 months	brown PET bottle
9	Polanica-Zdrój	HCO ₃ -Ca	●	9 months	green PET bottle
10	Krynica-Zdrój	HCO ₃ -Ca	●	12 months	brown PET bottle
11	Szczawno-Zdrój	HCO ₃ -Na	◆	12 months	blue PET bottle
12	Krynica-Zdrój	HCO ₃ -Na	◆	12 months	brown PET bottle
13	Kołobrzeg	Cl-Na	⊗	12 months	brown PET bottle
14	Połczyn-Zdrój	Cl-Na	⊗	12 months	brown PET bottle

For the sake of data clarity, in Figure 1 the same sign was applied to waters of the same type. If there are several water intakes of the same type in a particular locality, the mark has only been applied once.

Physicochemical testing included the determination of the following parameters: colour, odour, electrical conductivity, oxidation-reduction (redox) potential and the content of ammonium, sodium, potassium, calcium, magnesium, iron(II) and iron(III), manganese, chloride, fluoride, bicarbonate, sulphate(VI) and nitrate(III) and nitrate(V) ions, as well as metasilicic acid and orthoboric acid. The total dissolved substances were calculated on the basis of concentrations of these ions. Microbiological testing involved the detection of *Escherichia*

coli, coliform bacteria, intestinal enterococci, *Pseudomonas aeruginosa*, anaerobic spores of sulphite-reducing anaerobes, and the total count of microorganisms at 22°C and 36°C. The methodologies and references are outlined in Table 2. The number and types of physicochemical and microbiological parameters tested were determined according to the requirements for medicinal waters as set out by national regulations.

In the absence of criteria for assessing the stability of physicochemical parameters for packaged medicinal waters, a value of ±20% of the value measured at t_0 was adopted, in accordance with Article 20(2) of the Regulation of the Minister of Health of 31 March 2011 (Rozporządzenie 2011).

Table 2
Description of physicochemical and microbiological methods

Range	Parameter	Method	References
Physicochemical parameters	electrical conductivity	conductometric method	PN-EN 27888:1999
	pH	potentiometric method	PN-EN ISO-10523:2012
	redox potential E_h	potentiometric method*	an in-house test procedure
	colour	spectrophotometric method	an in-house test procedure based on the standard PN-EN ISO 7887:2012+Ap1:2015-06, method C
	water hardness	titration method, calculation method	an in-house test procedure based on the standard PN ISO 6059:1999
	sum of dissolved substances	calculation method	value expressed as the sum of the parameters given in Table 3 (on the interleaf)
	ammonium	ion chromatography	PN-EN ISO 14911:2002
	sodium	flame photometry	an in-house test procedure based on the standard PN-C-84081-22:1980
	potassium	flame photometry	PN-C-84081-22:1980
	calcium	titration method	PN-ISO 6058:1999
	magnesium	titration method	PN-ISO 6058:1999, PN-ISO-6059:1999
	iron	inductively coupled plasma ionisation mass spectrometry (ICP-MS)	PN-EN ISO 17294-2:2016-11
	manganese	inductively coupled plasma ionisation mass spectrometry (ICP-MS)	PN-EN ISO 17294-2:2016-11
	fluoride	potentiometric method using ion-selective electrode	an in-house test procedure based on the standard PN-C-04588-03:1978
	chloride	titration method	PN-ISO 9297:1994
	bicarbonate	titration method	an in-house test procedure based on the standard PN-EN-ISO-9963-1:2001
	sulfate(VI)	gravimetric method	PN-ISO 9280:2002
	nitrate(III)	ion chromatography	PN-EN ISO 10304-1:2009+AC:2012 LC
	nitrate(V)	ion chromatography	PN-EN ISO 10304-1:2009+AC:2012 LC
	Microbiological parameters	metasilicic acid**	inductively coupled plasma optical emission spectrometry (ICP-OES)
orthoboric acid***		inductively coupled plasma ionisation mass spectrometry (ICP-MS)	PN-EN ISO 17294-2:2016-11
relative error of the analysis		calculation method	PN-C-04638-02:1989
<i>Escherichia coli</i>		membrane filtration	PN-EN ISO 9308-1:2014
coliforms		membrane filtration	PN-EN ISO 9308-1:2014
intestinal enterococci		membrane filtration	PN-EN ISO 7899-2:2004
<i>Pseudomonas aeruginosa</i>		membrane filtration	PN-EN ISO 16266:2009
spores of sulphite-reducing anaerobes (clostridia)	membrane filtration	PN-EN 26461-2:2001	
TMC 22, TMC 36****	pour plate method	PN-EN ISO 6222:2004	

* The measurements were made using a differential potential meter connected by a compound electrode (a platinum electrode connected to a chlorosilver comparison electrode) and a temperature sensor. The determination involves measuring the electromotive force of a cell composed of platinum and comparison electrodes immersed directly in the test sample. The measurement was considered completed if within ten minutes the meter readings did not vary more than 10 mV. The potential value determined against the saturated chlorosilver electrode was corrected and reported against the potential of the hydrogen electrode. Certified reference materials and calibration standards were used during measurements.

** The content of metasilicic acid was calculated based on the results of the determined silicon content.

*** The concentration of orthoboric acid was calculated, based on the content of labelled boron.

**** TMC – total microbial count (total counts of microorganisms).

RESULTS AND DISCUSSION

Poland is home to rich deposits of mineral medicinal waters, particularly sodium-chloride, bicarbonate, hydrogen sulphide, radon and thermal waters (Ponikowska 2001, Ziemska et al. 2020). Crenotherapy is often used as a complementary treatment in conjunction with conventional medicine for various health conditions (Dumitrescu et al. 2022). A growing body of research supports the use of medicinal waters in the management of various chronic diseases. In this study, fourteen samples of medicinal waters from seven health resorts were examined. The results of the physicochemical analyses of these waters at t_0 , t_1 and t_2 are presented in Table 3 (on the interleaf). The results of microbiological analyses of the medicinal waters at t_0 , t_1 and t_2 are listed in Table 4.

Physicochemical parameters

Certain physicochemical and organoleptic parameters are frequently used as indicators of water quality due to their rapid measurement times and low-cost instrumentation (Rusydi 2017). Electrical conductivity, for example, is primarily influenced by the concentration of dissolved mineral salts and temperature (Gapparov & Isakova 2023). The results of this study confirmed a linear relationship between electrical conductivity and the sum of the dissolved substances in the tested water samples, especially in waters 1–12 (samples 13, 14 are much more mineralized salines) (Fig. 2). This relationship was observed across all types of tested waters. The values for electrical conductivity and pH showed stability over time for individual samples, ranging from 96.0% to 117.0% of the initial t_0 values, with a median of 103.7% (Fig. 3).

Several parameters, such as redox potential, colour and pH, exhibited changes at t_1 , t_2 compared to t_0 (Table 3, Fig. 3), with no clear dependence on water type. It is important to note that the characteristics of water are determined not only by the primary components, such as bicarbonates, sulphates, chlorides, calcium, magnesium, and sodium, which classify the water type, but also by transient components, gaseous substances, and easily precipitable compounds. It should be remembered that the third measurement at t_2 was

conducted approximately one year after the water was sourced and bottled. Despite the samples being stored in their original sealed packaging, they were exposed to air during the bottling process and stored at room temperature, not under the conditions of the spring (e.g., temperature and pressure) (Macioszczyk & Dobrzyński 2007, Macioszczyk 2017). These changes in redox conditions and temperature after bottling likely contributed to the observed differences in parameters such as redox potential, colour, and pH.

Water hardness is primarily determined by the presence of alkaline earth metals, particularly calcium and magnesium. It is a key parameter for assessing water contamination, with hardness values typically increasing when contamination is present (Macioszczyk & Dobrzyński 2007). In this study, the total hardness for one water sample (1) showed a lower value (CaCO_3 at $t_1 = 355 \text{ mg/L}$), a 22.1% decrease from the value determined at t_0 . The remaining hardness values were within the range of 89.9% to 119.3% of the initial value at t_0 .

Key components such as sodium, potassium, calcium, bicarbonate, chloride, fluoride and sulphate showed stability at t_1 and t_2 across all samples tested. Any minor changes in the concentrations of these components were not dependent on the water type and were likely due to analytical errors. No changes in the chemical type of the waters were observed at t_1 or t_2 , as illustrated in Thicket-type graph (Fig. 4 on the interleaf). The sum of dissolved substances, including cations, anions and dissolved components, ranged from 94.9% to 111.0% with medians of 102.8%, 99.6% and 100.2%, respectively.

Iron is present in water in two oxidation states: Fe^{2+} and Fe^{3+} . The former is more easily absorbed by the human body. However, the concentration of iron in its various forms is influenced by factors such as pH, redox potential, the presence of organic matter and the activity of iron-reducing bacteria (which was not examined in the present study) (Macioszczyk & Dobrzyński 2007). During water storage, insoluble iron compounds (Fe^{3+}) typically precipitate out, a phenomenon observed in eight of the water samples (Fig. 5). Eight samples (1, 2, 5–6, 9–11, 13) showed a decrease in iron concentration, accompanied by a change in manganese concentration only in sample 13.

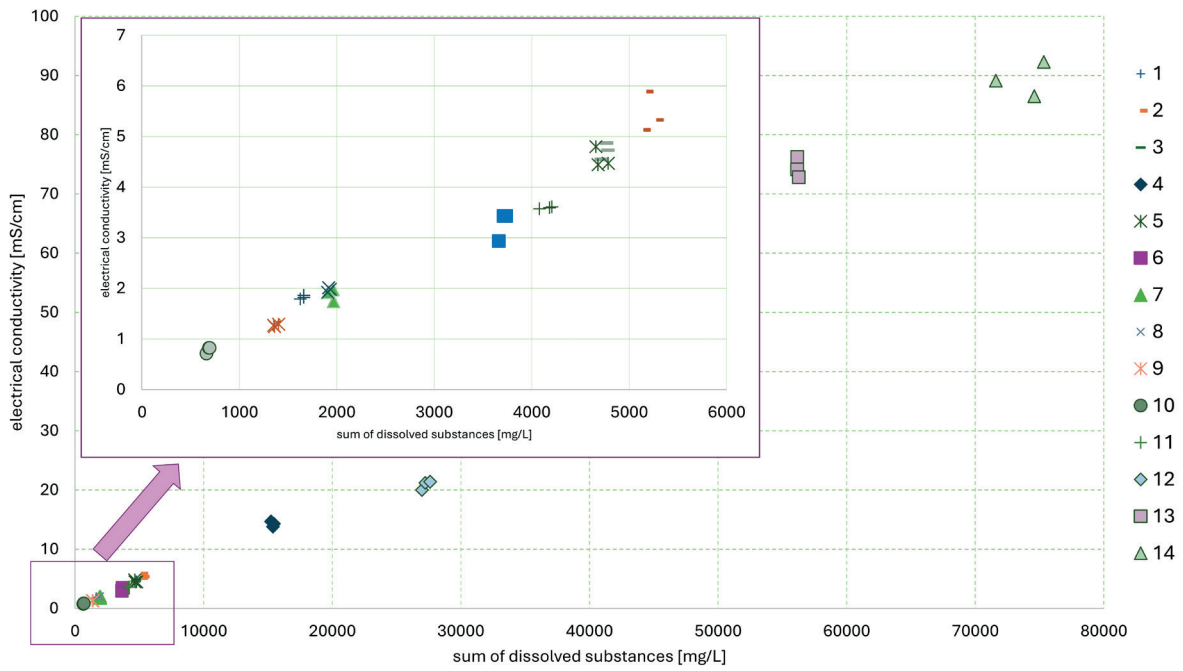


Fig. 2. Electrical conductivity versus sum of substances in tested medicinal waters

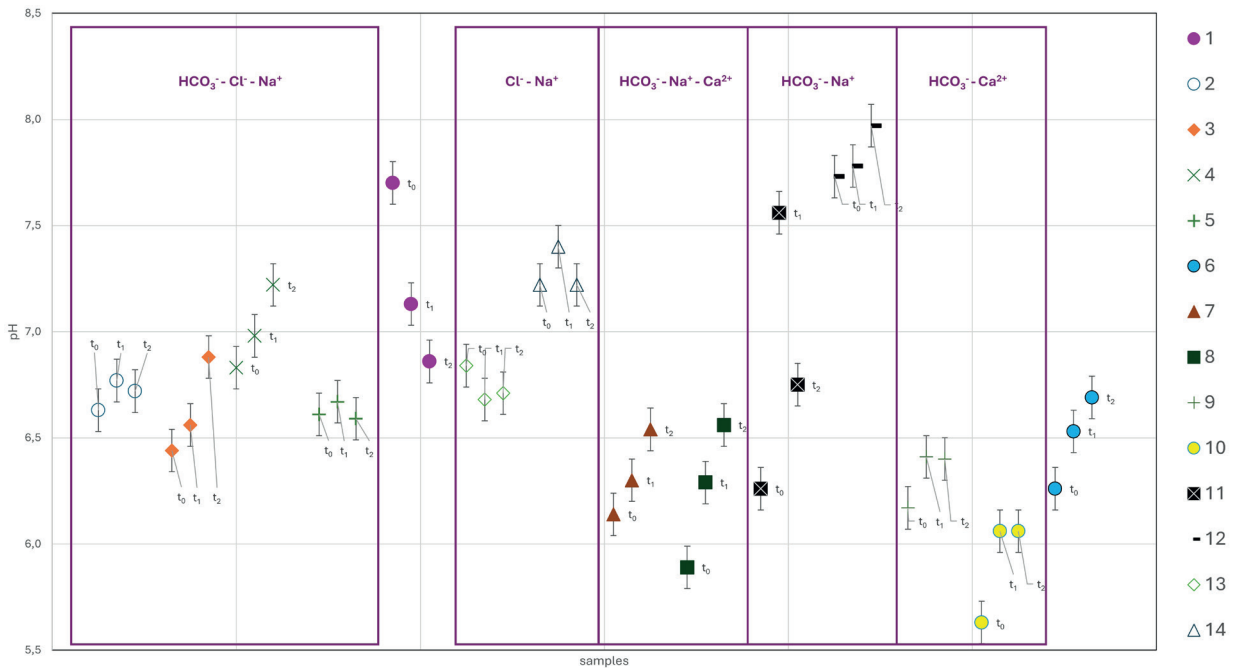


Fig. 3. pH values of tested medicinal waters at t_0 , t_1 , and t_2

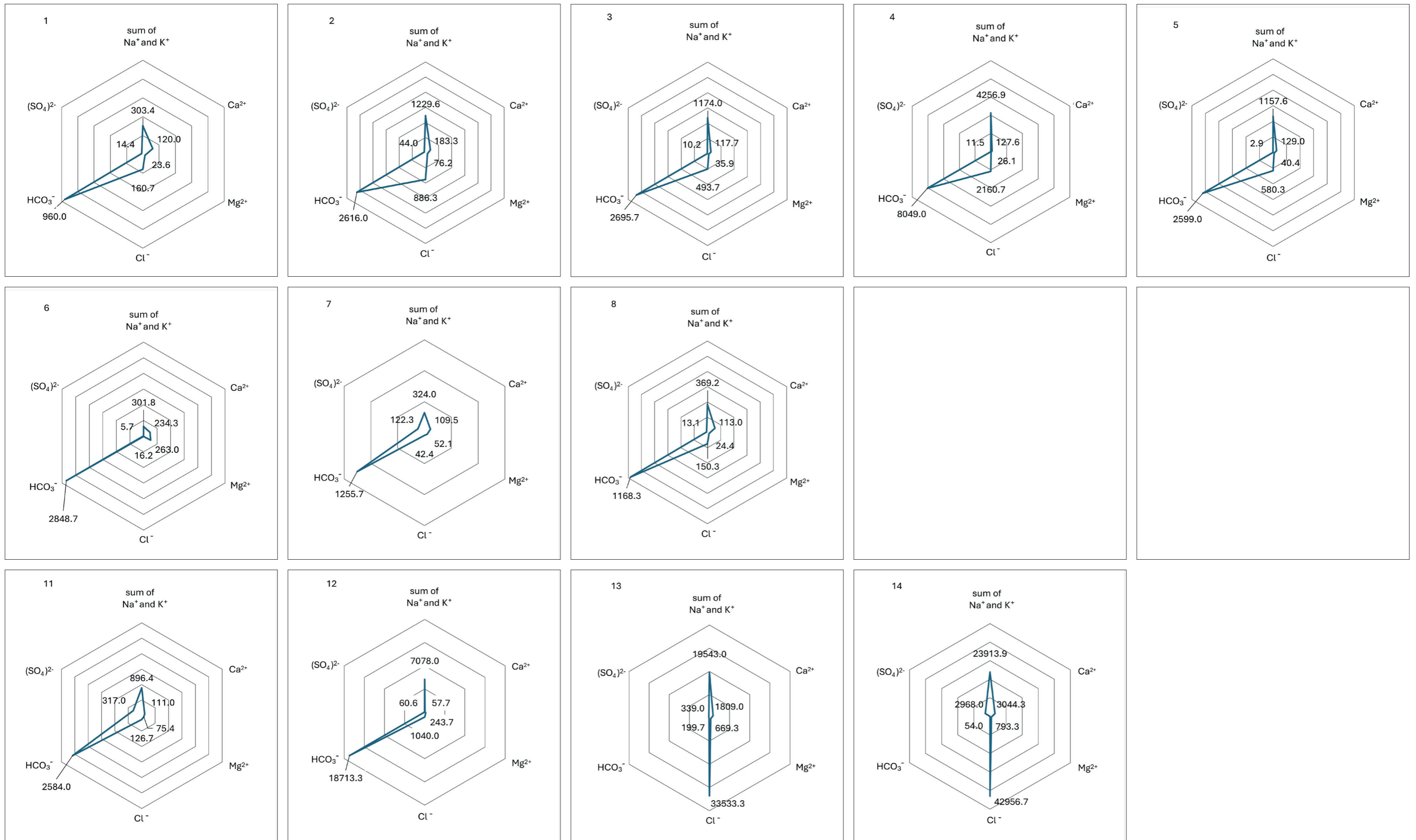


Fig. 4 Average contents of the major components of samples 1-14 in ickel-type graph

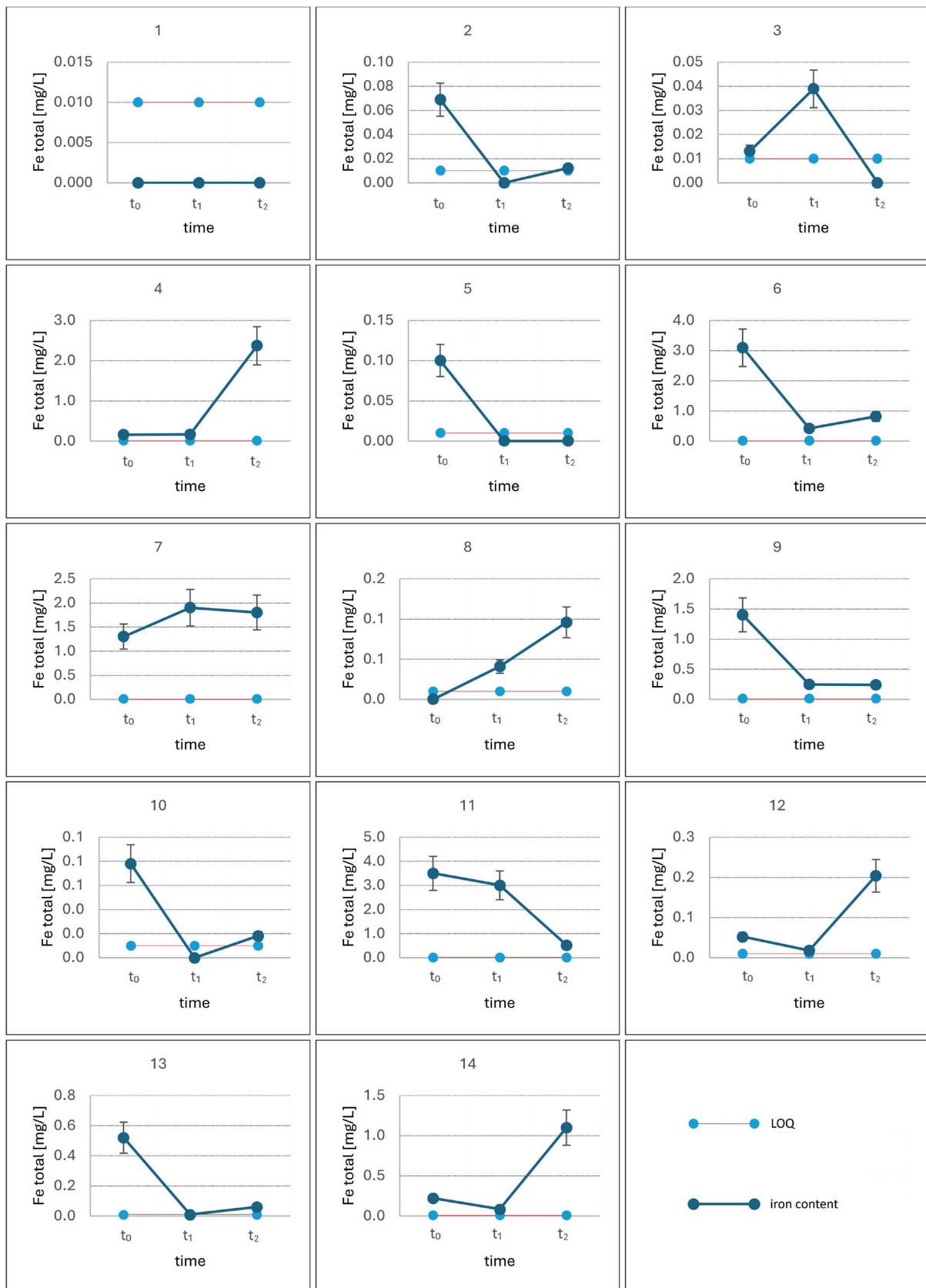


Fig. 5. Changes of iron concentration in tested medicinal waters at t_0 , t_1 and t_2

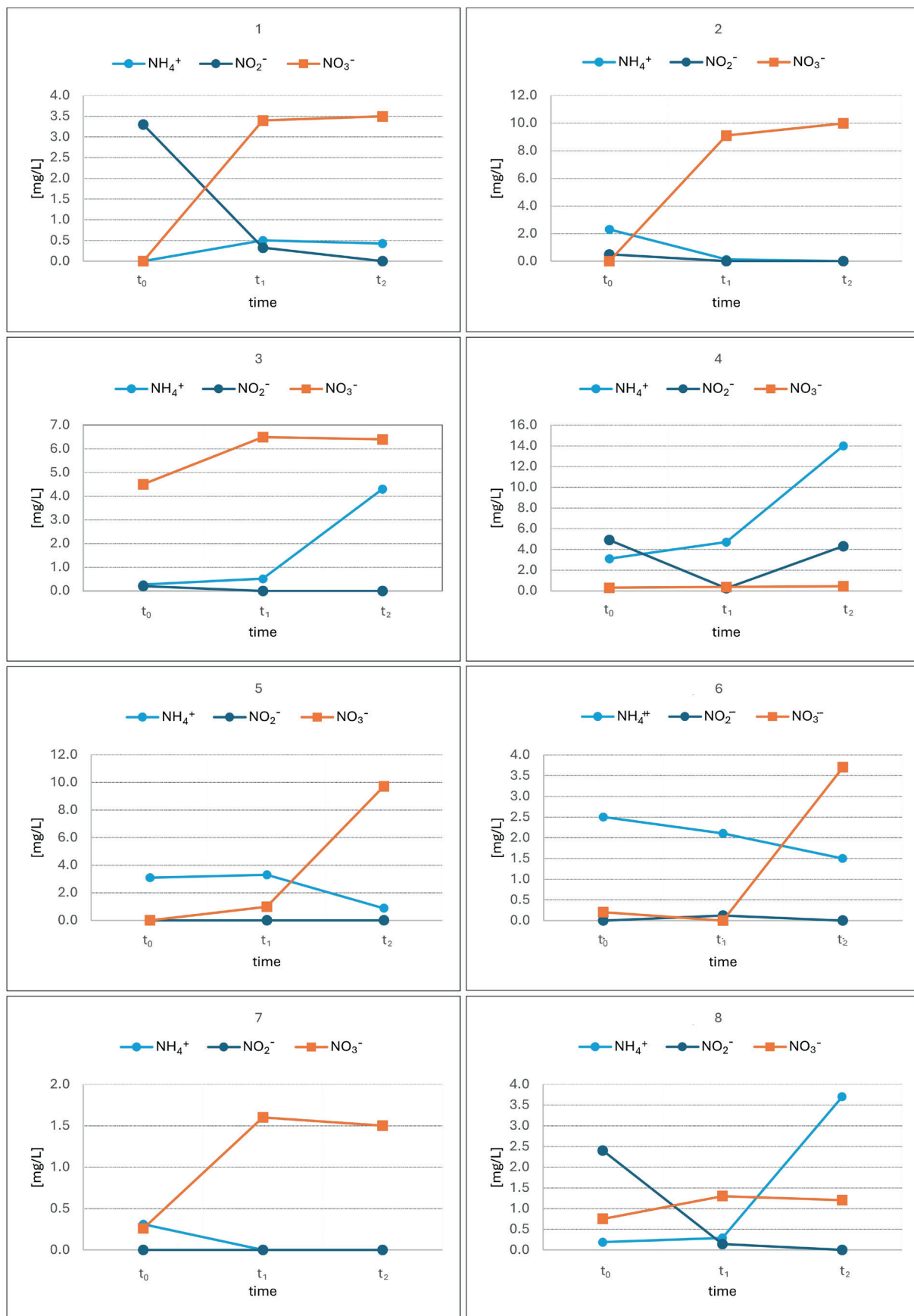


Fig. 6. Changes of nitrogenous compounds concentrations in tested medicinal waters at t_0 , t_1 and t_2 in samples 1-8

The physicochemical tests revealed changes in the concentrations of nitrogenous compounds (i.e. ammonium, nitrate(III) and nitrate(V) ions) at t_1 and t_2 in thirteen out of fourteen waters tested, with twelve showing a significant increase in nitrate(V) ion levels (above 20.0%). In the eight samples (2–8, 14) where an increase in nitrate(V) ion levels was observed over time, a decrease in redox potential values was noted at the same time.

The $\text{HCO}_3\text{-Na-Ca}$, $\text{HCO}_3\text{-Cl-Na-Ca}$ and $\text{HCO}_3\text{-Mg-Na-Ca}$ -waters, apart from the aforementioned increase in nitrate(V) levels, showed no significant differences in most of the other tested parameters. These observed changes may be related to nitrogen transformations – nitrification, denitrification, and ammonification, which occur easily with the involvement of nitrifying bacteria (though not part of the current study) (Figs. 6, 7).

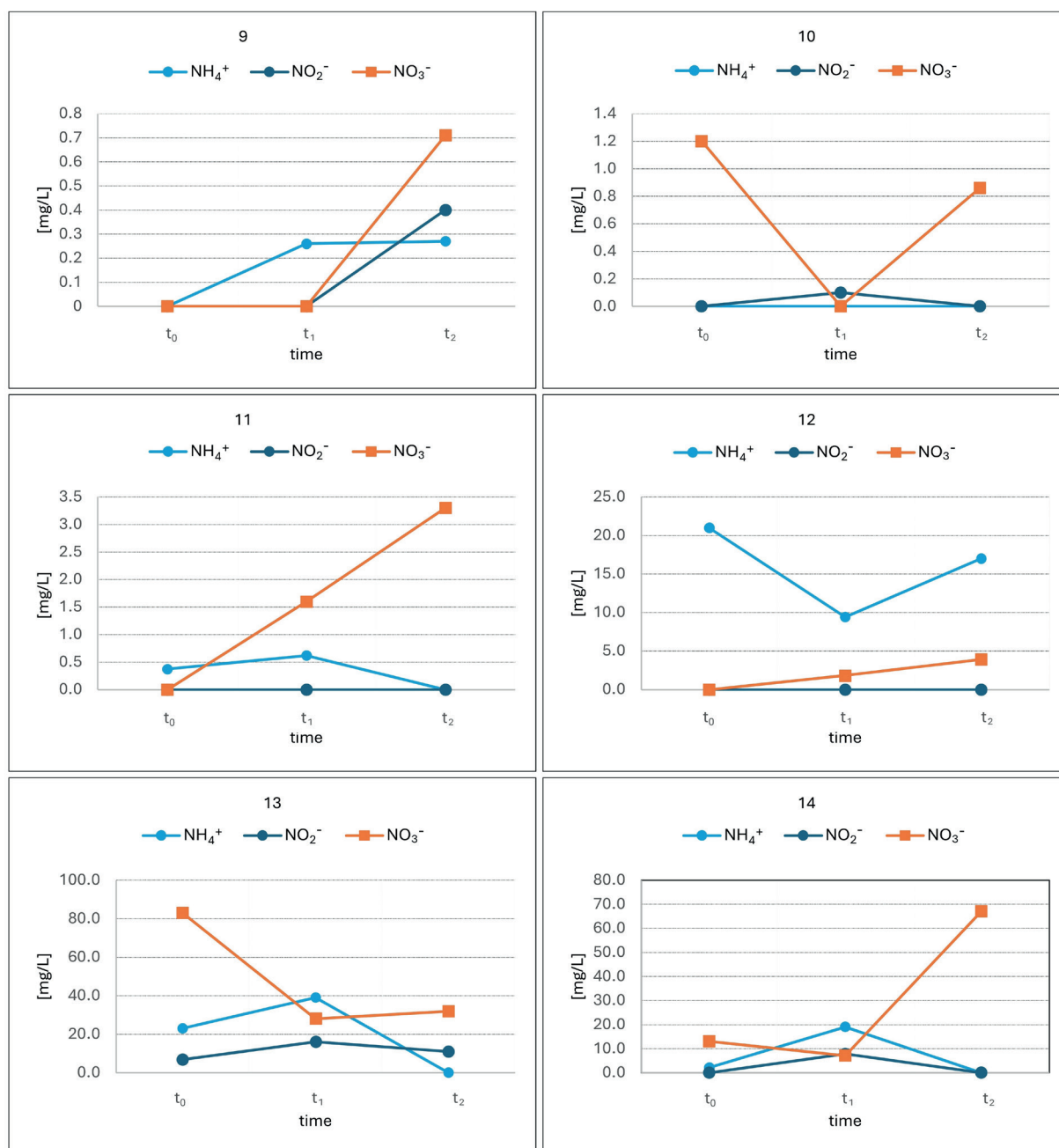


Fig. 7. Changes of nitrogenous compounds concentration in tested medicinal waters at t_0 , t_1 and t_2 in samples 9–14

In eight samples (3–4, 6–10 and 12), the concentrations of metasilicic acid and orthoboric acid remained stable over time. A decrease in orthoboric acid concentration (up to 60%) was observed in three water samples of different types, with a predominant content of sodium Na^+ , bicarbonate HCO_3^- and chloride Cl^- ions (3, 5, 11), while the metasilicic acid content was stable.

The results indicate that the medicinal waters tested show stability in chemical composition and organoleptic parameters throughout the shelf life, as specified by the manufacturer.

Microbiological parameters

The natural microbiota of medicinal waters plays an important role in shaping their unique properties and potential therapeutic benefits. Natural mineral waters, spring waters, and those recognised as medicinal cannot be subjected to disinfection or other processes that may alter their original composition and properties (Leclerc & Moreau 2002, Rozporządzenie 2006, 2011). At production facilities, microbiological control is conducted at all stages of the production process (from source to final product) to ensure the safety of the finished product.

Tests for indicator bacteria in medicinal waters were conducted to verify hygiene conditions in bottling plants and to monitor processes. This is a crucial element of quality control, determining potential risks for patients undergoing therapy. No indicator microorganisms indicating faecal contamination (e.g., *E. coli*, intestinal enterococci, spores of sulphite-reducing anaerobes) or problems related to biofilm (e.g., *P. aeruginosa*) were detected in any of the tested samples. These results confirm proper process oversight and the health safety of the final product.

Among all the tested samples, only one sample showed the presence of coliform bacteria (10 CFU/250 mL), with no other indicators detected. It is worth noting that coliform bacteria are not a specific or reliable indicator of faecal contamination, as they are also found in the environment and may originate from soil or plant material. Psychrotrophic bacteria, e.g. *Serratia fonticola*, *Rahnella aquatilis*, are examples of this (Leclerc & Moreau 2002). Unlike faecal indicators, coliform bacteria, like total count of microorganisms (TMC), are useful parameters that can serve as criteria for assessing the

cleanliness and integrity of water systems, including biofilm potential in water bottling installations.

Tests for total microbial counts (TMC) showed that microorganisms were more frequently detected at 22°C than at 36°C. This result confirms the presence of autochthonous microbiota in the tested waters. At t_0 , microorganisms were detected at 22°C in 8/14 samples (57%), while total microbial counts at 36°C were detected in 5/14 samples (36%). The values for this parameter were much lower – ranging from 1 CFU/mL to 7 CFU/mL – compared to microbial counts at 22°C, which exceeded 300 CFU/mL.

Autochthonous bacteria in water do not pose a significant sanitary risk and do not threaten human health as they do not grow at human body temperature. These bacteria are typically psychrotrophic species (Allen et al. 2004) and appear in low numbers at water sources (Schmidt-Lorenz 1976).

In contrast, bacteria detected at incubation temperature of 36°C \pm 2°C, could indicate potential contamination with sewage or intestinal microorganisms, which could suggest the presence of potentially pathogenic bacteria (Leclerc & Moreau 2002). However, the low growth levels and absence of more specific microbial indicators suggest this is not the case.

In the study, water samples 1, 3, and 14 showed a high total count of microorganisms at 22°C, exceeding 300 CFU/mL. In sample 1, this was observed at both t_0 and t_1 ; in sample 2, only at t_0 ; and in sample 14, only at t_1 (at t_0 , sample 14 had 51 CFU/mL). The high overall microbial count at 22°C recorded in three waters may be a natural phenomenon resulting from the presence of specific autochthonous microflora, and in the case of storage tests, it is related to the natural increase in bacterial numbers present in the water from the source. For all waters, with one exception, at t_2 , at the end of the storage period, the overall microbial counts at 22°C and 36°C yielded zero results. In none of the waters at t_2 – i.e., at the end of the storage period – was the presence of the tested microorganisms detected, indicating the stabilisation of the microflora during storage.

It is difficult to definitively identify the cause of the high microorganism levels (>300 CFU/mL) obtained from the total microbial count at 22°C without access to results related to specific stages of the production process. Several factors, such

as the immediate environment of the bottling machine, the microbiological quality of the process air, and the cleanliness of the preforms and caps, can influence the microbiological quality of the final product. Contamination of the packaging may occur during its production, transport to the factory, or during improper storage and transport to the bottling machine. Given other microbiological results, improper production oversight in this area is not likely to be the cause of these increases. It is important to note that bottling water causes a drastic change in the environment for the bacteria living in it (Lorenz 1990, Morita 1997, Leclerc & Moreau 2002). This phenomenon, referred to as the “bottle effect,” causes the number of microorganisms to increase dramatically after bottling, reaching levels of up to 10^4 – 10^5 CFU/mL (Leclerc & da Costa 1998, Leclerc & Moreau 2002). This increase is due to nutrients present in low concentrations that are adsorbed and concentrated onto the surface, thus becoming more available to the bacteria (Bischofberger et al. 1990, Morita 1997, Leclerc & Moreau 2002).

The results of the microbiological tests are presented in Table 4.

With regard to the research conducted and the results obtained, the use of bottled medicinal waters after the completion of a stay at a health resort should be discussed. A patient’s stay at a health resort lasts between 21 and 30 days, during which they receive specific treatments prescribed by their doctor, including crenotherapy. Due to the chronic nature of the diseases treated, it is often recommended that the patients continue the drinking treatment at home, as prescribed, to maintain the therapeutic effects post-sanatorium. There is a lack of scientific literature on the stability of the chemical composition and microbiological properties of bottled natural medicinal waters. It is important to highlight that current national legal regulations regarding medicinal waters do not set upper limits for the concentrations of key components such as magnesium, calcium, sodium, potassium, bicarbonates, sulfates, chlorides and fluorides. However, the decision on the choice of treatment and its duration lies with the health resort physician. National regulations, such as the Health Ministry’s decree, do set limits for undesired components at excessive concentrations, such as nitrates(III) – 0.02 mg/L,

and nitrates(V) – 10 mg/L, as well as organoleptic and physicochemical requirements for medicinal waters intended for drinking treatments (e.g., colour as Pt content <5 mg/L, pH dependent on the chemical composition of the water). These requirements apply to the water provided at the treatment site in the sanatorium, not the packaged product (Rozporządzenie 2006). Nevertheless, the tested medicinal waters met most of these requirements, with the exception of the limit for nitrates(III), which can be explained by the detection methodology (detection limit of 0.1 mg/L) and the transformation of nitrogen compounds during storage.

Current national and EU food legislation on bottled waters (Directive 2009/54/EC, Rozporządzenie 2011) does not include the medicinal waters category. In Poland and the EU, three types of water can be bottled and marketed as food products, namely: natural mineral water, spring water and table water (Directive 2009/54/EC, Rozporządzenie 2011). Bottled spring and mineral waters show significant differences in mineral composition between different EU countries (Stoots et al. 2021). Published studies mainly focus on chemical and microbiological contaminants in bottled waters (Bradley et al. 2023, Schalli et al. 2023, Tihanyi-Kovács et al. 2023).

The physicochemical tests carried out by the authors on bottled medicinal waters from seven Polish health resorts at t_0 , t_1 and t_2 allowed the conclusion that no significant changes in water quality occurred over time. Assuming permissible variations in the content of characteristic constituents of no more than $\pm 20\%$, it can be concluded that the mineral composition of the studied waters is stable over time. The observed changes in nitrogenous compound concentrations may be the result of natural transformations, such as those related to the presence of nitrifying bacteria. The shelf life proposed by the manufacturer ensures the maintenance of physicochemical properties and adequate microbiological purity, thus ensuring the safety of the product. No typical trends in changes in physicochemical properties or constituent content were identified for medicinal waters of the same type. Although significant health risks were not found concerning the examined parameters, other potential risks (e.g. microplastic, plasticisers) should also be considered in future investigations (Welle & Franz 2018).

Table 4
Results of microbiological analyses of the medicinal waters (ND – not detected)

Sample identification		Time	Microbiological parameters						
number	type of water		TMC 22	TMC 36	coliforms	<i>E. coli</i>	intestinal enterococci	<i>P. aeruginosa</i>	spores of sulphite-reducing anaerobes
			[CFU/mL]	[CFU/mL]	[CFU/250 mL]	[CFU/250 mL]	[CFU/250 mL]	[CFU/250 mL]	[CFU/50 mL]
1	HCO ₃ -Cl-Na-Ca	<i>t</i> ₀	>300	7	ND	ND	ND	ND	ND
		<i>t</i> ₁	>300	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	ND	ND	ND	ND	ND	ND	ND
2	HCO ₃ -Cl-Na	<i>t</i> ₀	3	2	ND	ND	ND	ND	ND
		<i>t</i> ₁	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	1	ND	ND	ND	ND	ND	ND
3	HCO ₃ -Cl-Na	<i>t</i> ₀	>300	1	ND	ND	ND	ND	ND
		<i>t</i> ₁	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	ND	ND	ND	ND	ND	ND	ND
4	HCO ₃ -Cl-Na	<i>t</i> ₀	14	ND	ND	ND	ND	ND	ND
		<i>t</i> ₁	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	ND	ND	ND	ND	ND	ND	ND
5	HCO ₃ -Cl-Na	<i>t</i> ₀	1	1	ND	ND	ND	ND	ND
		<i>t</i> ₁	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	ND	ND	ND	ND	ND	ND	ND
6	HCO ₃ -Mg-Na-Ca	<i>t</i> ₀	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₁	3	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	ND	ND	ND	ND	ND	ND	ND
7	HCO ₃ -Na-Ca	<i>t</i> ₀	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₁	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	3	ND	ND	ND	ND	ND	ND
8	HCO ₃ -Na-Ca	<i>t</i> ₀	1	ND	ND	ND	ND	ND	ND
		<i>t</i> ₁	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	ND	ND	ND	ND	ND	ND	ND
9	HCO ₃ -Ca	<i>t</i> ₀	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₁	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	ND	ND	ND	ND	ND	ND	ND
10	HCO ₃ -Ca	<i>t</i> ₀	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₁	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	ND	ND	ND	ND	ND	ND	ND
11	HCO ₃ -Na	<i>t</i> ₀	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₁	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	ND	ND	ND	ND	ND	ND	ND
12	HCO ₃ -Na	<i>t</i> ₀	10	4	10	ND	ND	ND	ND
		<i>t</i> ₁	9	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	ND	ND	ND	ND	ND	ND	ND
13	Cl-Na	<i>t</i> ₀	11	ND	ND	ND	ND	ND	ND
		<i>t</i> ₁	ND	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	ND	ND	ND	ND	ND	ND	ND
14	Cl-Na	<i>t</i> ₀	51	ND	ND	ND	ND	ND	ND
		<i>t</i> ₁	>300	ND	ND	ND	ND	ND	ND
		<i>t</i> ₂	0	ND	ND	ND	ND	ND	ND

CONCLUSIONS

Recent research results indicate the beneficial effects of balneotherapy treatments in the therapy of diseases such as obesity, metabolic syndrome, sleep disorders, mental health disorders, long COVID-19 and rehabilitation after cancer treatment (Reger et al. 2022, Antonelli et al. 2024, Fioravanti et al. 2024, Galvez et al. 2024). Further fundamental and applied research into the chemistry and natural microflora of medicinal resources is essential to deepen the understanding of the mechanisms behind the effects of individual resources, including medicinal waters, in various balneotherapy treatments.

The results from tests assessing the effect of storage time on the stability of the chemical composition and the microbiological quality of bottled medicinal waters from seven Polish health resorts (Szczawnica, Wysowa-Zdrój, Krynica-Zdrój, Polanica-Zdrój, Szczawno-Zdrój, Kołobrzeg, Połczyn-Zdrój) allow us to conclude that these products remain stable throughout the shelf life declared by the manufacturer, i.e. within 1 year (9 months for sample 1) from date of manufacture. Furthermore, the results indicate that bottled medicinal waters can be used at home to prolong the effects of sanatorium treatment, as recommended by the chief physician responsible for health resorts. The data obtained can be utilised by producers of bottled medicinal waters, as well as in the process of updating regulations regarding natural medicinal resources and health resorts. This also includes the potential for introducing provisions on the production and bottling of natural medicinal waters, along with the obligation to test bottled medicinal waters and define the scope and frequency of such tests.

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