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Estimation of metal content and bacteria in drinking water

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Abstract
Metals reach drinking water through various ways, and at certain concentrations they are toxic to the body. In addition to anemia, cancer, kidney problems, these are hepatotoxic. Microorganisms are an indicator for the degree of potability of water. Their presence can lead to gastrointestinal disorders.
Taking these shortcomings into account, in this paper the bacterial load after 24 hours of incubation on nutrient agar was evaluated and the presence of contaminating bacteria, considered dangerous for the human body, was analyzed. Several special differential media were used to highlight them. Microbiological analysis was performed on water from ten wells. The water that raised microbiological problems was also chemically analyzed. Thus, in addition to the content of sodium (Na) and calcium (Ca), the content of metals in four wells was also monitored. Copper (Cu), cadmium (Cd) and lead (Pb) were absent from the four wells, but the concentration of the other metals varied. Also, the bacterial load varied depending on the pollution sources. In some wells, bacteria with a risk for the consumer have been identified.

Keywords: microbial indicators, nickel, copper, cadmium, minerals, manganese

Introduction

In rural areas, drilled wells are a source of drinking water for human and animal consumption, but also for agriculture. Worldwide, underground sources provide more than 40% of the total amount of water for human consumption and agriculture [35]. Underground water is considered a safe source, because it is separated from the soil surface by several layers with reduced permeability and with a protective role against physico-chemical and microbiological contaminants. The underground water is characterized by a reduced biological and quantitative diversity. Microorganisms in groundwater are oligotrophic. Common bacteria are accustomed to low temperatures. Microorganisms present underground are actinomycetes, yeasts, sulphurous, ferrous bacteria and manganobacteria. The representative genera are *Achromobacter* and *Flavobacterium* [12].

Due to the recent increase in pollution, the need for safe drinking water is becoming a priority and a challenge for researchers and international decision-making bodies. Water quality depends on physical, chemical and biological parameters. Their modification is accompanied by the deterioration of quality and the appearance of health problems. Groundwater contamination is the result of chemical treatments from agriculture, abandoned mining areas, industrial activities [3, 24, 34] and improperly stored garbage. Pesticides can be a source of pollution [10, 22]. Through leaching, the chemical and microbiological composition of the underground water can be changed. Frequent chemical pollutants are metals that accumulate in the food chain, which are difficult to biodegrade and present a high risk to human health and the environment [2, 20, 25, 27].

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Metals have a carcinogenic effect when they are in high concentrations and interfere with enzyme activities, cause cardiac, renal, nervous disorders, tooth decay, heart disease and changes in the genetic material. In small quantities, some metals can be beneficial to the body, while others can be harmful even in small amounts [34]. Also, according to WHO (2003) [39] data, mortality caused by infectious diseases from water contaminated with microorganisms represents 50% [14]. Polluted water also has a negative effect on animals, plants and the soil.

In Romania, drinking water is defined and regulated by Law no. 458 of July 8, 2002 - regarding the quality of drinking water [23], supplemented and amended by Law no. 311 of June 28, 2004 and Ordinance no. 22/2017 for the amendment and completion of Law no. 458/2002 on the quality of drinking water, which transposes Directive (EU) 2020/2184 on the quality of water intended for human consumption [5, 13, 41, 42].

In rural areas, physical-chemical and microbiological analyzes of drinking water are completely absent. This study aims to determine the bacteria count in water from ten wells that have been drilled and to identify the types of metals present in some wells that were chosen according to depth. Also, an attempt was made to establish the possible sources of pollution and to inform the owners about the potential health hazards of metals and bacteria.

Material and Method

Collection and transport of water samples

Water samples were collected from ten households, from the perimeter of Timis county, Romania. The water was collected in clean bottles, hermetically sealed, labeled and transported under appropriate conditions, at low temperatures, to the chemical and microbiological analysis laboratory of the University of Life Sciences "King Mihai I" in Timisoara.

Microbiological and chemical analysis

The cultural method was used to estimate the bacteria in the water samples. The cultural method is one of the most recommended methods when it comes to a routine evaluation of microorganisms. It is not an expensive method. The bacteria were isolated on nutrient agar, in three repetitions. Undiluted water was inoculated in a quantity of 1 ml/Petri plate. The inoculated Petri dishes were incubated at 37°C for 24 hours. The results obtained show the number of mesophilic germs and were expressed in CFU•mL-1[38].
Analyses of metals and minerals water content was made using ContrAA-300, Analytik-Jena device, as described by Bordean et al, 2010 [9].

Results and Discussion

The results indicate an increase in the number of mesophilic bacteria (Fig.1) in some water samples above the limits imposed by the standards in force (table 1).

Table 1. Microbiological parameters according to Law no. 458/2002 [23] supplemented and amended by Law no. 311 of June 28, 2004 and Ordinance no. 22/2017 [32]

Parameter	Maximum value allowed
Number of colonies at 22˚C	**100/ml**
Number of colonies at 37˚C	20/ml
Clostridium perfringens	0/100 ml
Coliform bacteria	0/100 ml
Escherichia coli	0/100 ml
Intestinal enterococci	0/100 ml
Pseudomonas aeruginosa	0/250 ml

These increases are between 11.4-70% above the values allowed by Law no. 458 of July 8, 2002 [23] regarding the quality of drinking water, supplemented and amended by Law no. 311 of June 28, 2004 and Ordinance no. 22/2017 [32] for the amendment and completion of Law no. 458/2002 regarding the quality of drinking water, in accordance with Directive (EU) 2020/2184 [13].

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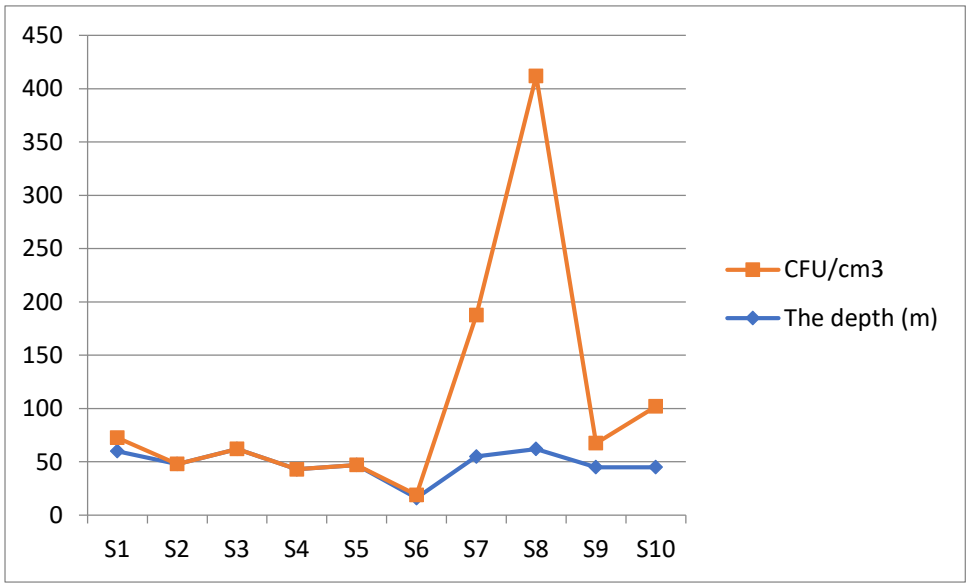

Fig. 1 Estimation of the number of mesophilic bacteria after 24 hours of incubation

The highest number of bacteria was recorded in samples S8 > S7 > S10, the values being between $3.5x10^2$-$0.57x10^2$ (fig. 1). Based on the information provided by the well owners, it was concluded that the primary sources of these increases are human activities, including livestock farming, agricultural practices, and household waste. Other studies also highlight these pollution methods [4, 18]. The lowest number was recorded in samples S1 and S6, the values being between 0.12 $x10^2$ and 0.03 $x10^2$ CFU/ml, values below the legally regulated values. Bacterial growth was not detected in the remaining five water samples. Similar increases were reported by other authors [6]. Similar to our experiments, these cases also show that the sources of contamination stem from household human activities.

The presence and growth of bacteria in drinking water is also reported by other authors. For example, Karwowska et al., (2023) [19] observed the existence of psychrophilic and mesophilic bacteria in water sources considered to be of very good quality. The number of mesophilic bacteria was considered low (3.0 ± 3.0 CFU/cm^3). The authors observed that the number of mesophilic bacteria increases at room temperature, during the standard storage of 48 hours. Under these conditions, the number of bacteria reached values of 10^3–10^4 CFU/cm^3. Household and agricultural waste is also responsible for the contamination of water with metals [1]. Water contaminated with metals, such as As, Cd, Cr (hexavalent), Cu, Hg, Ni and Pb is responsible for many human diseases (such as cancer, liver, mental, kidney problems) [4, 7, 16, 17, 37].

Table 2. The concentration of chemical elements in water, according to Law no. 458/2002 (republished) [23] and Directive Directive (EU) 2020/2184 [13].

Indicators	Drinking water (mg/l)
Calcium (Ca^{2+})	-
Sodium (Na^{+})	200
Total chromium (Cr^{3+} + Cr^{6+})	0.05
Copper (Cu^{2+})	0.1
Zinc (Zn^{2+})	5
Lead (Pb^{2+})	0.01
Total iron (Fe^{2+} + Fe^{3+})	0,2
Total manganese (Mn^{2+} + Mn^{7+})	0,05
Nichel (Ni^{2+})	0.02
Cadmium (Cd)	0.005

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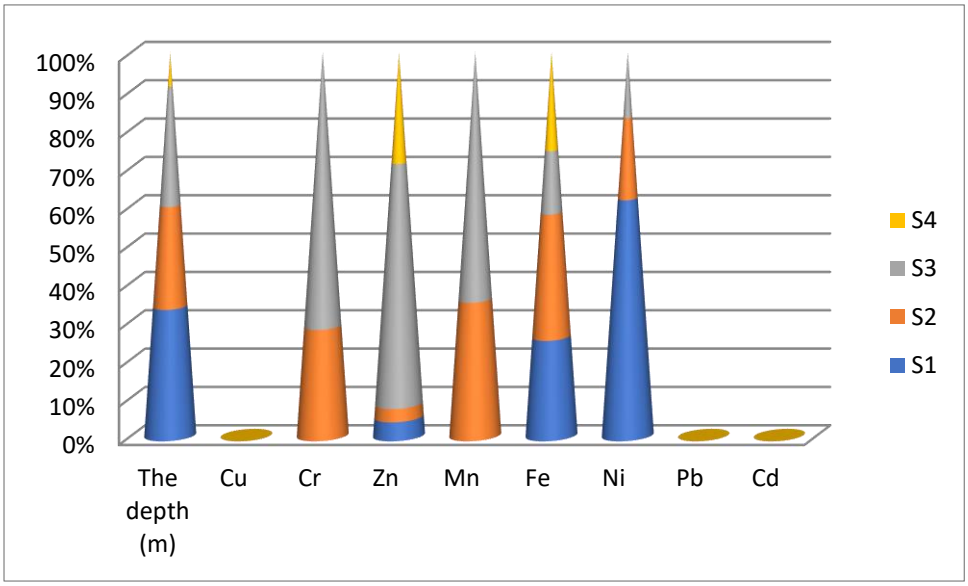

Fig. 2 The concentration of metals (mg/ml) in the water samples

The presence of heavy metals in drinking water may be natural, depending on the geology of the region, but in many cases it is caused by human activity. According to the rules in force, the Pb concentration can be 0.01 mg/l, Cu 0.1 mg/l and Cd 0.005 mg/l. These three metals were not detected in the analyzed water samples. The metals that exceeded the legal concentrations were Cr (in samples S2, S3), Ni (in samples S1-S3), Mn (in samples S2 and S3) and Fe. The latter is present in all the analyzed samples. Fe and Mn are among the essential elements for humans. To avoid excessive storage of iron in the body, the EU regulated the iron concentration at 5 mg/l [13]. The water's taste and smell are altered due to the high concentration of iron, making it unsuitable for consumption. Drinking water, which contains 0.3 mg/l of iron, contributes approximately 0.6 mg/day to the body's iron needs. Iron is found naturally in plants and animals. It exceeds 2-3 times the allowed concentration. In most fruits and vegetables the iron content is low (1–10 mg/kg), only green vegetables have a higher content (20–150 mg/kg) [43]. Contrary to our results, some recent studies recommend the enrichment of water with iron [26,31]. These recommendations are based on the global prevalence of severe anemia problems[36]. These anemias are also favored by microbial infections and a deficient immune system. Iron deficiency is widespread worldwide and affects around 5 billion people, especially children and women [15,21]. The phenomenon is mostly found in underdeveloped countries.

High concentrations of iron, manganese and lead were also found by other researchers [29]. According to these data, the sources of contamination with Mn and Fe are of geogenic nature, they are found naturally in soil, clay, rocks, but they are also the result of human activities, on the other hand, the Pb contamination is a result of anthropological activity (industry, batteries, vehicles, pipelines). High concentrations of Fe cause cardiovascular problems, diabetes, pulmonary embolism, hemochromatosis, etc. Increased levels of Mn produce emotional and memory disorders, hallucinations, Parkinson's, etc. [8, 11]. Zn was present in all samples, but below the standard limits.

Studies have indicated that drinking water with high levels of zinc (3.0 mg/L) has a metallic taste [30]. Zinc deficiency affects growth, the immune and nervous system [3].

Chromium is another metal that exceeded the legally regulated concentration. Compared to trivalent chromium which is essential for carbohydrate metabolism, hexavalent chromium has a negative impact on the human body in the short and long term, it is mutagenic and carcinogenic.

Water brings a significant supply of minerals to the body. Among the essential minerals are calcium and sodium. Calcium (Ca) was present in all analyzed water samples. The highest concentration was observed in sample S1. Sodium was not highlighted in sample S2. All concentrations are reduced, compared to those estimated by the norms in force. Both minerals have an effect on the health of the body. Some studies show that sodium in large quantities increases blood pressure, and Ca reduces cases of sudden death [28]. Calcium plays a role in physiological processes in the body (nerve transmission, vascular and muscular contraction, blood coagulation). Calcium deficiency determines the fragility of the bone system, increased risk of cerebrovascular accidents, kidney stones, hypertension and cardiovascular diseases [40].

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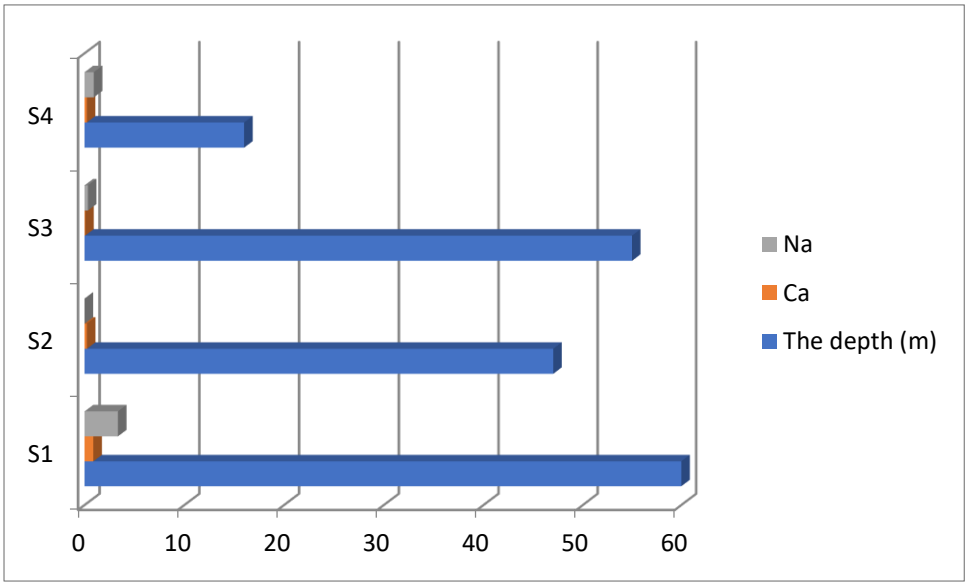

Fig. 3 Analysis of minerals from water samples

Conclusions

The potability of water is crucial to the safety of humans, which is why routine microbiological analyses, by cultural methods, without excessive costs, and metal analysis have become a necessity. Water intended for human consumption, loaded with bacteria and metals above the permitted limits must be treated before consumption.

The presence of some iron, manganese, chromium and nickel in the water from wells drilled above the permitted limit requires monitoring over a longer period of time, long-term observation and establishing the risks associated with them, in order to avoid the negative effects of underground water on the health of residents.

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