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Chemical and Microbiological Contamination of Natural Water Resources in Saedinenie, Bulgaria

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Abstract: Human impact on the quality of freshwater source creates a risk of long-term pollution in water bodies intended for drinking and irrigation purposes. In the present study, the chemical and microbiological contamination of natural water from two wells and Potoka River of the town Saedinenie, Bulgaria were studied. All groundwater samples showed very high NO_3^- and Cd^{2+} content – 400 mg/l and 0,167 mg/l for Well 1 together with 431 mg/l and 0,188 mg/l for Well 2, respectively. Strong contamination with *Escherichia coli*, *Enterococcus faecalis* and sulfite-reducing microorganisms was also observed. In both cases limits for drinking purposes were exceeded. NO_3^--N in the Potoka River was around 4,47 mg/l, i.e. less than the maximum threshold value. However, the amount of Cd^{2+} was found to be highest in all of the conducted experiments, reaching 0,300 mg/l at permissible values of 0,010 mg/l. This shows all tested samples require pre-treatment before using for any purpose.

Keywords: *Cadmium, Freshwater, Microbiological indicators, Nitrates, Pollution*

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1. Introduction

Pollution of natural water resources is one of the major problems of the modern world as 1/3 of the humanity do not have access to safe water used for drinking, household or agricultural activities (Abascal et al., 2021). Anthropogenic factors such as old sewage systems, overuse of fertilizers, and unregulated waste disposal have a stronger negative impact on water quality than geogenic ones (European Economic Community, 1991; Krishnan & Indu, 2006; Sasakova et al., 2018; Kirschke et al., 2019; Li et al., 2021; Modak & Basu, 2022). In the last years, many countries, particularly those from the European Union (EU), have increased their efforts to develop long-term sustainable solutions in order to reduce the influence of the freshwater contamination challenge (Kirschke et al., 2019; Akhtar et. al., 2021). The goals are directed to the decrease of persistent soil nutrients and heavy metals, such as NO_3^- and Cd^{2+}, which have a major impact over agricultural lands and human communities in Europe directly or imputedly (Moloantoa et. al., 2022).

Different regulatory mechanisms stated that the quality of water resources in Europe is not good (A blueprint to safeguard Europe's water resources, 2012), especially in those areas with intensive agriculture which leaded to high NO_3^- water content (Hansen et. al., 2017; Eurostat, 2018; Musacchio et. al., 2020; Aquamonitrix, 2022). For example, in Europe, nearly 4% of freshwater losses are due to nitrate pollution (Moloantoa et. al., 2022). As a result, for each European country, nitrate vulnerable zones are created (Nitrate vulnerable zones, 2015). For Bulgaria these zones are two, each of it covers most of the country area with intensive agricultural focus. However, the problem is that the large rivers and those of international importance were mainly studied and monitored, while for small reservoirs in small settlement areas similar information is extremely insufficient or even missing (Varbanov & Garciyanova, 2016; Rizk et al., 2022). But there are many Bulgarian and even European rural areas without or with old plumbing installation where drinking water is taken directly from wells and used without any treatment. A possible connection is also found between the presence of NO_3^- and different pathogenic microorganisms in groundwater sources (Abascal et al., 2021). In this case health concerns can occur for the local population due to the long term application of polluted water (Eurostat, 2018; Ba et. al., 2022). It was found that the high concentration of nitrates in drinking water of small Bulgarian villages can cause thyroid dysfunction in children and pregnant women (Nitcheva et al., 2020).

The problem may get worse if natural water basins are contaminated with Cd, which is defined as the seventh most toxic metal in the world (Jaishankar et al., 2014). Direct sewage discharge from different industrial activities in rivers or infiltration from soil are the main sources of this toxic element. In many parts of the world Cd^{2+} occur in drinking waters over the recommended World Health Organization values. Moreover, Cd^{2+} is closely related to Zn^{2+} presence in different water sources (Guidelines for drinking water quality, 2022). Bulgaria has reduced significantly the Cd environmental pollution. However, the levels of this heavy metal still remain high (Heavy metal emissions in Europe, 2021). For that reason Cd^{2+} in natural sources must be monitored, recognized and regulated (Akhtar et. al., 2021).

Little communities without sewerage and a prevalent population who live single-family houses with old septic latrines and active agricultural activities, such as the town of Saedinenie, Bulgaria, may have contaminated freshwater sources, which create a risk of water pollution with chemical and microbiological impurities (Chernev, 2014). As elevated levels of NO_3^- and Cd^{2+} can occur in natural waters from such settlements, this requires a mandatory analysis of the water sources to determine its proper application.

In the present study the suitability of groundwater from the southern part of the town of Saedinenie for drinking needs was studied, and its possible use for irrigation purposes was compared with that of the Potoka River.

2. Materials and methods

Samples of groundwater from two wells and surface running water from the Potoka River, based in the southern part of the town of Saedinenie, located in the Upper Thracian Plain of southern Bulgaria were studied. The wells are situated 10 m and 500 m from the Potoka River, respectively. The depth of the groundwater is 18 m for the first well (Well 1) and 14 m for the second (Well 2). At 50 m from Well 2 there is an abandoned septic latrine. In the immediate vicinity of the wells are the remains of an old cow farm.

As one of its left tributaries, the Potoka River falls into the nitrate vulnerable zone along the Maritsa River. It belongs to the category of small and medium rivers with fine substrate (Application 1-4). Treated wastewaters from the factory for concrete products Rubikon Beton Ltd and Saedinenie Wine Cellars are discharged directly into the river basin. According to data from the East Aegean River Basin Directorate, the state of the Potoka river is defined as moderate, without exceeding the threshold allowable concentrations of pollutants (Application M4-11).

Chemical indicators

The chemical parameters of the water were analyzed photometrically by tests from the Spectroquant® series, Merck Millipore, USA. The determination of heavy metals: Zn^{2+}, Cd^{2+} as well as different sources of inorganic N: NH_4, NO_2^-, NO_3^- are performed respectively by Zinc Cell Test (Spectroquant®-Zinc cell test), Cadmium Cell Test (Spectroquant®-Cadmium cell test), Ammonium Test (Spectroquant®-Ammonium test), Nitrite Test (Spectroquant®-Nitrite test), Nitrate Test (Spectroquant®-Nitrate test).

Microbiological indicators

In the study of microbiological indicators, standard methods for analysis of water intended for drinking purposes were used. The concentration of *Pseudomonas aeruginosa* was determined according to ISO 16266:2008 (ISO, 2008). Detection and enumeration of fecal enterococci was performed by following the instructions of ISO 7899-2:2003 (ISO, 2003). The total number of mesophilic aerobic and facultative anaerobic microorganisms was made as per ISO 6222:2002 (ISO, 2002), respectively at 22^0C and 37^0C, and the sulfite-reducing bacteria – based on the information of ISO 6461-2:1986 (ISO, 1986).

3. Results and discussion

3.1. Evaluation of well water for drinking purposes

Groundwater is one of the most widely used water sources for domestic purposes (Hartmann et al., 2021; Rahman el al., 2021). In Table 1, the data from the chemical analysis of the well water are presented. The values of the indicators are compared with the current permissible norms for groundwater quality intended for drinking (Regulation 1 on research, use and protection of groundwater, 2007).

The results show that the Zn^{2+} content of the water from Well 1 is 2,55 mg/dm^3 or 1,25 times over the regulative threshold of 2 mg/dm^3. Higher Cd^{2+} are found in both wells – 0,167 mg/l for Well 1 and 0,188 mg/l for Well 2, i.e. 33 times more than the allowed amount. The main source of Cd contamination in waters may be the concrete plant, located along the Potoka River before it enters the city of Saedinenie as Cd emissions from cement dust were widely found in nature (Işikli et al., 2006). Other sources of Cd pollution can be the dust from the burned fossil fuels which are a commonly used for heating in small settlements (Ali et al., 2019), as well as older plumbing systems (Guidelines for drinking water quality, 2022). Excessive use of phosphorus-containing and manure fertilizers is supposed to be the one of the main causes of soil contamination with Cd and Zn and their subsequent migration into water (Kubier et al., 2019; Ullah et al., 2009; Roberts, 2014; Wei et al., 2020).

The two wells confirm the typical for the whole country pollution of the groundwater with NO_3^- (Water resources management and water quality, 2020). For the waters from Well 1 it is 8 times higher, and for Well 2 – 8,62 times higher than the allowed concentration. The closer location of Well 2 to the septic tank in comparison to Well 1 can be the reason for the higher values of inorganic N in its water. The studied groundwater is of unacceptable quality for drinking purposes (Regulation 1 on research, use and protection of groundwater, 2007).

According to the current Bulgarian legislation, the extraction of groundwater in private property is possible only when they are of guaranteed drinking quality (Water act, 1999). The conducted analyzes show that the permitted values for NO_3^- and Cd^{2+} in the waters have been exceeded, which prohibits their application for direct consumption or other household activities (Regulation 9 on water quality, intended for drinking and household purposes, 2001).

High microbial contamination was reported in all well water samples (Table 2). The results from both wells show strong microbial contamination with hygienic microorganisms. The total plate count of mesophilic aerobic and facultative anaerobic microorganisms (TPC) at 22^0C in Well 1 is 8,7 times more than in Well 2, and at 37^0C – 31,8 times more. The concentration of viable *Escherichia coli* cells in the two wells shows close values. The number of living cells of *Enterococcus faecalis* in Well 1 is 11,1 times higher than its content in Well 2. The presence of hygienically indicative *Escherichia coli* and *Enterococcus faecalis* is unacceptable in drinking water, as they are indicators of fecal contamination. *Pseudomonas aeruginosa* was not detected in the studied waters, unlike the sulfite-reducing bacteria. The last are 1,32 times higher in Well 2 than those in Well 1.

Many authors have found the presence of *Escherichia coli*, Enterococci and total coliforms in groundwater samples, rich in NO_3^- as both enteric bacteria and nitrates are associated with domestic leaks, mainly from latrines and septic tanks (Abascal et al., 2021). The stray monitoring of the manure storage facility to the abandoned cow farm and the poorly constructed septic latrine, which are located next to each other, create preconditions for the release of microbiological and chemical contaminants into the groundwater of the wells (Megha et al., 2015; Swain & Senapati, 2021). And if in developing countries the high nitrate content is caused by a lack of adequate sanitary actions, such results are unacceptable for Bulgaria, as an EU member, where it is supposed that only nitrate pollution should occur in natural waters due to intensive agriculture (Abascal et al., 2021; de Vries et al., 2022). In order to improve the quality of water and, in particular, its nitrate regime, it is imperative EU to carry out activities such as the modernization of the sewage network in populated areas, better monitoring of the waste streams from industrial activities, liquidation of illegal landfills and the pre-disposal of old ones. Particular attention should be paid to the old and active livestock complexes, in which there is no possibility to store the released manure properly or to use it at the wrong time (Garciyanova, 2016).

3.2. Evaluation of well and surface running water for irrigation purposes

Freshwater applied in agriculture for irrigation activities must ensure the production of high quality crops on which the safety and health of the population directly depend (Regulation 18 on the quality of water for irrigation of agricultural crops, 2009). In Table 3 chemical indicators determining the irrigation possibilities of well and river water are summarized. The results are compared with the permissible norms for waters intended for irrigation of agricultural crops.

All analyzed samples show elevated values of Cd^{2+}, as in the Potoka river is reported the highest concentration reaching 0,300 mg/l or 30 times over the allowed for irrigation – 0,01 mg/l. Cd^{2+} for Well 1 is 0,17 mg/l and for Well 2 – 0,19 mg/l. Only Zn^{2+} content Well 1 is found to be over the irrigation limits with 0,55 mg/l more. Here, it is possible old plumbing systems to release Zn^{2+} in the groundwater from Well 1. In Bulgaria, the main contribution of Cd over land is based on atmospheric deposition with 0,86 g Cd/ha.y, which is 2 times higher than the average levels for EU, followed by 0,36 g Cd/ha.y from fertilizers and 0,09 g Cd/ha.y from manure and biosolids application (Basin Directorate "Eastern White Sea Region", 2016). As heavy metals are persistent pollutants with strong migratory ability their high concentrations in natural water sources create a risk of accumulation in cultivated crops, and hence in humans (Afzaal et al., 2022). This makes the studied waters unsuitable for direct irrigation in agriculture. A possible alternative can include pre-treatment of waters for heavy metal removal and their subsequent use (Da'ana et al., 2021).

As a part of EU regulated Bulgarian vulnerable zones the concentration of N in freshwater basins is essential to be monitored, especially it is most soluble and ecologically significant form – nitrate ions (Ali et al., 2019; Nitcheva et al., 2020). NO_3^- in water attended for irrigation must not exceed 20 mg/dm^3 (Regulation 18 on the quality of water for irrigation of agricultural crops, 2009). The analyzed groundwater is 20,2 times richer in NO_3^- than river water or 4,5 times higher than the permitted value for watering. The nitrate content in the Potoka River is within the permissible limits, which makes it suitable for irrigation on this indicator. Obtained results confirm the statement that in recent years in Bulgaria, along with other European countries, a lower content of nitrate ions is observed in rivers compared to groundwater (Eurostat, 2018) due to the targeted policy for sustainable development of the EU for the implementation of environmentally friendly agricultural practices supporting the restoration of natural waters (Kirschke et al., 2019; Akhtar et. al., 2021). However, efforts should be directed basically to the elimination of nitrates in groundwater, where problems still remain unresolved. The long-term deposition of nitrates in deep underground water bodies poses a risk of the emergence of the so-called "nitrate bomb" that may affect water quality in the future for decades (Hansen et. al., 2017; Aquamonitrix, 2022). A stronger integration of

water and agriculture policies is needed, as well as the introduction of better economic instruments to improve the quality of natural waters in the future (Kirschke et al., 2019).
Moreover, if groundwater from the two wells is used for watering, elevated nitrate concentration in it is a prerequisite for the crop production with extended development stage, reduced yield and low quality of the finished product (Renseigné et al., 2007; Abascal et al., 2021).

Table 1: Comparison of chemical indicators in groundwater of Well 1 and Well 2 for drinking purposes

Chemical indicator	Sample of groundwater Unit	Well 1	Well 2	Permissible values*	Permissible values**
Zn^{2+}	mg/dm^3	2,55	0,29	1,00	4,00
Cd^{2+}	mg/dm^3	0,167	0,188	0,005	0,005
NH_4^+	mg/dm^3	2,0	2,0	0,5	0,5
NO_2^-	mg/dm^3	0,25	0,36	0,50	0,50
NO_3^-	mg/dm^3	400	431	50	50

Legend: *Permissible values for chemical indicators in groundwater (Regulation 1 on research, use and protection of groundwater, 2007); **Permissible values for chemical indicators in water intended for drinking and household purposes (Regulation 9 on water quality, intended for drinking and household purposes, 2001);

Table 2: Comparison of microbiological indicators in groundwater of Well 1 and Well 2 for drinking purposes

Microbiological indicator	Sample of groundwater Unit	Well 1	Well 2	Permissible values*
TPC** at 22°C	cfu***/cm^3	2.10^3	$2,8.10^2$	<100
TPC at 37°C	cfu/cm^3	$2,2.10^3$	69	<20
Escherichia coli	cfu/100 cm^3	1.10^2	$1,4.10^2$	0
Enterococcus faecalis	cfu/100 cm^3	1.10^2	9	0
Pseudomonas aeruginosa	cfu/100 cm^3	0	0	0
Sulfite-reducing microorganisms	cfu/50 cm^3	$1,02.10^2$	$1,35.10^2$	0

Legend: *Permissible values for chemical indicators in water intended for drinking and household purposes (Regulation 9 on water quality, intended for drinking and household purposes, 2001); **Total plate count; ***Colony forming units

Table 3: Comparison of chemical indicators in groundwater of Well 1, Well 2 and surface-flowing water from the Potoka River for irrigation purposes

Chemical indicator	Unit	Sample of groundwater Well 1	Well 2	Potoka River	Permissible values*
Total iron ions	mg/dm^3	0,05	0,05	0,05	5
Zn^{2+}	mg/dm^3	2,55	0,29	0,20	2
Cd^{2+}	mg/dm^3	0,17	0,19	0,30	0,01
NH_4^+-N	mg/dm^3	2	2	2	5
NO_3^--N	mg/dm^3	90,36	97,36	4,47	20

Legend: *Permissible values for water quality for irrigation purposes (Regulation 18 on the quality of water for irrigation of agricultural crops, 2009).

4. Conclusion

The study reached following conclusion after conducting the study:

1. High concentrations of Cd^{2+}, NO_3^- and strong microbial contamination make the groundwater from Well 1 and Well 2 unfit for direct drinking.
2. The tested groundwater could not be used for irrigation due to the excessive amount of Cd^{2+}, NO_3^- and Zn^{2+} (for Well 1).
3. The excessive content of Cd^{2+} defines the water from the Potoka River as unsuitable for irrigation purposes.
4. Both groundwater and river water from the southern part of Saedinenie must be properly treated before any future application for drinking, household and irrigations needs.

The conclusions drawn can be used to enrich the information system on the quality and purity of water in small settlements in Bulgaria and serve as a basis for creating events related to better awareness of the local population about health and safety from the application of these waters.

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