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BIOTESTING OF QUARRY LAKES AS AN ALTERNATIVE SOURCE OF DRINKING WATER SUPPLY

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Quarry water quality was assessed by biotesting using microalgae *Chlorella vulgaris* Beijer and crustaceans *Daphnia magna* Straus. The results of the study of the toxicity of quarry waters are presented, indicating the safety of their use as sources of drinking water supply. Sufficiently high water quality of the considered reservoirs is connected with their origin. As a result of limestone mining, underground waters were opened, which are cleaned of impurities, being filtered by porous rocks. The possibility of using the waters of quarry lakes as an alternative source of water supply for the Sevastopol region is being considered. It is noted that the use of flooded quarry waters should be substantiated by long-term hydrogeological studies.
Keywords: quarry lakes, drinking water, biotesting.

INTRODUCTION

Crimea has experienced the problem of water shortage for a very long time [1]. In 2020, the situation with the water supply of the city of Sevastopol became critical. Insufficient rainfall and a winter with little snow led to drought. The reserves of the Chernorechensky reservoir, which provides the city with water, decreased to 22.5 million m^3, which amounted to a third of its volume.

As urgent measures aimed at maintaining the water regime of the city and preventing emergencies, it was decided to use the resources of water bodies available in the region for water supply: lakes near Mount Gasforta, flooded Inkerman and Kadykovsky quarries. Previously [2, 3] the potential of mentioned quarries was studied and the possibility of their use as sources of irrigation was substantiated.

The Inkerman lime-shell quarry had been developed since 1944 for the extraction of building materials. In 1978, after blasting, the quarry began to fill with groundwater. The depth of the formed reservoir is up to 60 m, the water level is constant, the area of the water surface is about 23 thsnd. m^2.

The reservoir near Mount Gasforta was formed in 1977 by flooding a quarry of fluxing limestones with water due to surface and underground runoff. The development of the rock reached the level of groundwater, after which the mountain springs were opened. The reservoir has the following characteristics: the water surface area is 35 thsnd. m^2, the depth is up to 10 m, and the volume is more than 2,4 million m^3. Its water is clean and transparent.

The Kadykovsky quarry is a multi-stage formation with ledges from 15 to 36 m wide increasing as they reach the water surface. The quarry shape is close to an oval with the largest diameter (1.69 km) from west to east. The smaller diameter, oriented from north to south, is 0.76 km. The depth of the quarry is more than 40 m. The reservoir is replenished by underground sources, its volume is more than 5 million m^3. For several decades of the last century, limestone was mined here, which was necessary to produce ferroalloys. The reason for the flooding of the Kadykovsky quarry was that it was filling with groundwater.

The purpose of current work is to determine the toxicity of quarry waters in order to assess the possibility of its use as additional sources of drinking water supply in the Sevastopol region.

MATERIALS AND METHODS

Currently, one of the most widely used methods for assessing the toxicity of natural water pollution is biotesting [4, 5]. The use of this method has several advantages over physical and chemical analysis, which often fails to detect unstable compounds or quantitatively determine ultra-low concentrations of toxicants, as well as to take into account their combined effect. Biotesting makes it possible to quickly obtain an integral assessment of toxicity at a specific point in time [6, 7]. Toxicity assessment is carried out using at least two organisms from different systematic groups. The value of a more sensitive organism found in the study is taken as the final result.

Planktonic crustaceans (*Daphnia magna* Straus) were used as the first test organism for biotesting of quarry waters [8, 9]. The assessment was carried out based on measurements of the number of crustaceans by the method of direct counting, which is based on the determination of the mortality of daphnia under the influence of toxic substances present in the test sample [10]. The criterion for acute toxicity is the death of 50 % or more of daphnia in 48 h in the test sample, provided that in the control experiment all crustaceans retain their viability.

An algologically pure culture of microalgae (*Chlorella vulgaris* Beijer) [11] for biotesting was at the stage of exponential growth. To assess the toxicity of quarry waters, the optical density of the test culture was measured. The technique is based on recording differences in the optical density of algae grown on the control medium and in the tested water samples, which may contain toxic substances [12]. The criterion for water toxicity is a decrease by 20 % or more (growth inhibition) or an increase by 30 % or more (growth stimulation) of the optical density of the algae test culture grown for 22 h.

In accordance with the methodology [10, 12], during the experiments, comparisons were made between the control and tested water samples diluted by 1 (without dilution), 3, 9, 27, and 81 times. Mathematical methods of data processing were used to evaluate the experimental results.

RESULTS AND DISCUSSION

The results of assessing the toxicity of quarry water samples using crustaceans (*D. magna*) as a test organism are shown in Table 1. To determine the acute toxicity of the studied waters, the percentage of daphnia that died in the tested water (A, %) was calculated compared to the control:

$$A = \frac{\overline{X}_C - \overline{X}_T}{\overline{X}_C} \cdot 100\%,$$

where $\overline{X}_C$ – number of surviving daphnia in control (average of three parallel determinations);

$\overline{X}_T$ – number of surviving daphnia in test water (average of three replicates).

Since there was observed no death of more than 10 % of the organisms in any of the samples, there was no need to calculate the harmless dilution ratio and acute toxicity.

Table 1

Biotesting of quarry waters using the test organisms of crustaceans (*Daphnia magna* Straus)

Water sample dilution, times	Inkerman quarry lake			Gasforta quarry lake			Kadykovsky quarry lake		
	X_T	$\overline{X}_T$	A,%	X_T	$\overline{X}_T$	A,%	X_T	$\overline{X}_T$	A,%
control	10	10.0	0	10	10.0	0	10	10.0	0
	10			10			10		
	10			10			10		
81	10	10.0	0	10	10.0	0	10	10.0	0
	10			10			10		
	10			10			10		
27	10	10.0	0	10	10.0	0	10	10.0	0
	10			10			10		
	10			10			10		
9	10	10.0	0	10	10.0	0	10	10.0	0
	10			10			10		
	10			10			10		
3	10	10.0	0	10	10.0	0	10	10.0	0
	10			10			10		
	10			10			10		
1 (without dilution)	10	9.7	3	10	10.0	0	9	9.3	7
	10			10			10		
	9			10			9		

When assessing the toxicity of quarry waters using microalgae (*Ch. vulgaris*) as a test culture (Table 2), we calculated the relative difference (%) of the average optical density for each dilution compared to the control (*l*):

$$l = \frac{\left(\overline{D}_C - \overline{D}_0\right)}{\overline{D}_C} \times 100,$$

where $\overline{D}_C$ и $\overline{D}_0$ – average values of optical density in the control and in the experiment samples from four parallel determinations, respectively.

Since samples without dilutions had deviations in optical density in the form of growth suppression by more than 20 %, the toxic dilution factor (TDF) was calculated using the formula:

$$TDF = 10^{\frac{(\lg D_b - \lg D_l)\times(l_l - 0{,}2)}{l_l - l_b} + \lg D_l},$$

where D_b – dilution value at which the deviation index was below the toxicity criterion;

D_l – dilution value (lower) at which the deviation index was above the toxicity criterion;

l_b and l_l – the values of the indices l corresponding to these dilutions, expressed in fractions.

As D_b and D_l, that pair of the highest dilutions was used, between which there was a transition of the index l of the value of the established toxicity criterion.

The calculated values of the toxic dilution ratio for the Inkerman flooded quarry were 2.5; Lake Gasforta – 2.2; Kadykovsky flooded quarry – 2.9.

Table 2

Biotesting of quarry waters using the test organisms of microalgae (*Chlorella vulgaris* Beijer)

Water sample dilution, times	Inkerman quarry lake		Gasforta quarry lake		Kadykovskiy quarry lake	
	Optical density, $\overline{D}$	l, %	Optical density, $\overline{D}$	l, %	Optical density, $\overline{D}$	l, %
control	0.155	-	0.155	-	0.155	-
1 (without dilution)	0.064	58.7	0.057	63.2	0.010	93.5
3	0.136	12.3	0.148	4.5	0.128	17.4
9	0.152	1.9	0.155	0.0	0.137	11.6
27	0.155	0.0	0.155	0.0	0.145	5.8
81	0.155	0.0	0.155	0.0	0.155	0.0

In the study of quarry waters using *D. magna* test organisms, the number of deaths in the tested samples of daphnia did not exceed 10 %, which indicates the absence of toxic effects. The test culture *Ch. vulgaris* had higher sensitivity, however, when the test samples were diluted 3 times, the toxicity criterion was also not exceeded.

In all undiluted samples of quarry water, inhibition of the growth of *Ch. vulgaris* exceeding 20 % was observed. The relative difference in the average optical density for samples without dilution compared to the control was 93.5 % for Kadykovskiy quarry lake, 63.2 % for Gasforta quarry lake and 58.7 % for Inkerman quarry lake. Thus, the transition through the criterion of toxicity in the form of 20 % growth inhibition was between 1 and 3 times dilutions of the test water.

The calculated values of the toxic dilution ratio make it possible to rank the considered water bodies in order of increasing possible toxic effect of their waters: Inkerman quarry lake, Gasforta quarry lake and Kadykovsky quarry lake. However, already at a 3-times dilution, the tested samples of all water bodies became completely harmless.

CONCLUSIONS

The obtained results substantiate the possibility of using the quarry lakes of the Sevastopol region as a backup source of water supply for the city. Quarry water quality assessment confirmed that the samples taken are harmless when diluted 3 times and do not have an acute toxic effect on living organisms from two distant systematic groups.

Sufficiently high-water quality from the considered water bodies can be explained by their origin as a result of limestone mining. Apparently, groundwater is being cleared of impurities, while being filtered through porous rocks.

When implementing the strategic task of solving the problem of water shortage in the city of Sevastopol in the winter of 2021, the daily water intake from Kadykovsky quarry lake amounted to 15 thsnd. m^3, from Gasforta and Inkerman quarry lake about 5 thsnd. m^3, which made it possible to reduce the load on the Chernorechensky reservoir, which is the main source of water in the region (50 thsnd. m^3/day).

It should be noted that the use of water from flooded quarries have to be justified by long-term hydrogeological studies confirming the renewability of groundwater supplying the quarry. Otherwise, the withdrawal of water can lead to the achievement of an irreplaceable level of the reservoir, swamping and death of the ecosystem of the flooded quarry lake.

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Кучерик Г. В. Биотестирование обводнённых карьеров как альтернативного источника питьевого водоснабжения / Г. В. Кучерик, Ю. А. Омельчук, Д. М. Сытников // Ученые записки Крымского федерального университета им. В. И. Вернадского. Биология, химия. – 2022. – Т. 8 (74), №2. – С. 87–92.

Качество карьерных вод оценивали с помощью биотестирования с использованием микроводорослей *Chlorella vulgaris* Beijer и ракообразных *Daphnia magna* Straus. Представлены результаты исследования токсичности карьерных вод, свидетельствующие о безопасности их использования в качестве источников питьевого водоснабжения. Достаточно высокое качество воды рассматриваемых водоемов связано с их происхождением: в результате добычи известняка вскрылись подземные воды, которые очищаются от примесей, фильтруясь пористыми породами. Рассматривается возможность использования вод обводнённых карьеров в качестве альтернативного источника водоснабжения Севастопольского региона. Отмечено, что использование вод затопленных карьеров должно быть обосновано многолетними гидрогеологическими исследованиями.

Ключевые слова: карьерные озера, питьевая вода, биотестирование.