Integrated Monitoring of the Spring Water Quality in the Mostyska District of Lviv Region

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ABSTRACT

Rural population of Ukraine mainly consume water from wells and individual wells, which (in the vast majority) are in unsatisfactory technical and sanitary condition. For the estimation of the quality of the spring waters in some settlements of Mostyska district, the seasonal samples of were taken in Mostyska, Malniv, Gusiakiv, Pidhat, Volytsia, Nagirne, and Krukenychi. Biotesting of well water was carried out, water was analyzed according to physicochemical and bacteriological indicators. It was established that toxicity is higher than average in the villages Krukenychi, Gusiakiv, Volytsia and Malniv, and in Mostyska high toxicity. The content of nitrites and phosphates did not exceed the MPC in all investigated waters. The content of nitrates and ammonia in the spring waters of the investigated area exceeded the MPC in most settlements, with the exception of samples from the villages Pidhat and Nagirne. In addition, only spring water from the villages Nagirne and Pidhat meets the bacteriological indicators for the quality of drinking water.

Keywords: spring waters, Allium-test, cytotoxicity, physicochemical and bacteriological indicators

1. INTRODUCTION

The anthropogenic load on the environment is increasing every year, and the amount of chemicals released into the environment increases with its growth. For aquatic ecosystems, self-purification and self-regulation are inherent, however the emergence of new compounds disrupts the system's balance and water bodies degrade [2, 4, 14].

The problem of providing the population with quality drinking water is now being given increased attention not only because water is an indispensable substance for human life, but also because pollution of water and drinking water sources determines the degree of environmental safety of entire regions, and drinking poor quality water directly affects the population health [2, 3, 15]. Given the current state of water supply in Ukraine and the quality of drinking water in general, more and more people are opting to use bottled water for consumption [16].

The reasons for the deterioration of the quality of the wells are: incorrect choice of the location for the wells, non-compliance with the sanitary protection standards, inflow of polluted waters from farms, fields, roads, poor sanitary and technical care of wells. This is a significant problem requiring immediate attention at both the state and local levels. It has been researched in many scientific papers, and the issue of water quality is given much attention both in Ukraine and abroad.

2. BACKGROUND

Due to the deterioration of the general ecological state and pollution of water sources, one of the main state tasks is to provide the Ukrainian population with quality drinking water. At present, there is no integrated monitoring of the quality for individual water sources in Ukraine. Assessment of water quality is a key objective of any action in the field of water management, sustainable use of nature and carrying out environmental actions in water bodies. Water quality is evaluated by a wide range of indicators - physico-chemical (hydrochemical, hydrophysical, hydrological) and biological (hydrobiological, bacteriological) [6, 7, 15, 17, 18]. Cytogenetic monitoring of anthropogenic pollution for drinking water possesses an important place in the system of environmental monitoring. The priority of such studies at the cellular and chromosomal levels is determined by the greatest vulnerability of these structures to the body when exposed to mutagens [19].

Mostyska district is located in the western part of the Syansko-Dnistrovsky watershed plain. The overall problem of the area is poor environmental status. Monitoring data of the surface water quality within the area show that, despite the significant decline in industrial production, there is a tendency to deteriorate the ecological
status of surface water bodies in terms of both sanitary, chemical and microbiological indicators in recent years. The main reason for the poor quality of natural waters is the pollution of surface water pools by untreated water [14, 15]. Water of agricultural use flows from the fields saturated with saline solutions and soil particles, residues of chemicals that increase yields. It is established that about 20% of nitrogen, 25% of phosphorus and 30% of potassium are supplied to the reservoirs. Thus, agriculture has become a major pollutant of water bodies with biogenic substances contributing to the intensive development of phytoplankton (flowering of water), stimulate the growth of unwanted aquatic organisms, lead to disruption of the self-purification process [12]. Nitrogen introduced into the soil is converted into easily soluble forms polluting the groundwater.

3. MATERIAL AND METHODS

3.1 Scheme of Monitoring

The monitoring of the spring waters quality in Mostyska district was conducted in the laboratories of the Drohobych Ivan Franko State Pedagogical University. The work was carried out within the framework of the implementation of integrated monitoring of surface water quality in Lviv region [10, 12, 13]. In order to assess the quality of spring waters of some settlements of Mostyska district, seasonal (winter, spring, summer, autumn) samples of waters from some villages and cities of the territory were taken. Water samples were taken in Mostyska, villages Malniv, Husakiv, Pidgat, Volytsia, Nagirne and Krukenychi. These settlements are proportionally located in different parts of Mostyska district. Studying the quality of the water bodies, samples from seven springs of each settlement connected to the pump system were taken. All spring under study have lining from concrete rings, and the depth to the water mirror ranges from 5 to 11 m. The study was conducted from October 2018 to July 2019. The water was collected in sterile vials, labeled and analyzed on the day of sampling. Biotesting of spring water was carried out, water was analyzed according to physico-chemical and bacteriological indicators.

3.2 Research Methods

Biotesting using Allium-test.

Biotesting was performed according to the A.Gorova method [19]. Onion roots (*Allium cepa*) were used as test cultures. This test evaluates only the water-soluble components of the tested water sample. It is simple to conduct and sensitive to determine the overall water toxicity. Toxicity is an inhibition of root growth of *Allium cepa* L., as root growth is suppressed at lower toxicant concentrations than plant germination. The length of the roots of onions was measured using a ruler for 4 days of observations. The average for each test object was calculated. Based on the obtained data, the phytotoxicity index was calculated using the formula:

\[ T = \frac{I_k - I_0}{I_k} \times 100\% \]  

(1)

\[ T \] – the phytotoxicity index of the sample; \( I_k \) – the magnitude of the test reactions in the control sample; \( I_0 \) – the magnitude of the test reactions in the test sample.

The phytotoxicity level scale was used to compare toxicity with the growth test of phytoindicator [19]: 0-20 - no or low toxicity level, 20.1-40 - average level, 40.1-60 - above average level, 60.1-80 - high level, 80.1-100 - maximum level.

The determination of cytotoxicity was performed by calculating the mitotic index in the cells of the onion meristem. For this purpose, several roots of bulbs with a length of about 1-2 cm were cut off and their fixation, staining and microscoping were performed [19]. The severed roots of the bulbs were placed in sealed containers with Clark's solution. The staining of the roots from the bulbs was carried out with a 2% acetoorcein solution. The preparations were analyzed under a microscope «MICROmed XS-5520 LED» at magnification 15x90. Using a microscope attachment connected to a computer and PVR-PLUS, cells were visualized, photographed and counted at different stages of mitosis. Mitotic Index (MI,%) is an indicator (in %) of cells that are divided by the total number of cells analyzed. For the MI determination, 100 cells were examined, of which cells dividing at different stages of mitosis and not dividing were counted.

Physico-chemical studies.

The determination of some physicochemical parameters: the content of nitrates, nitrites, ammonium, phosphates, mineralization. For this purpose, water samples were taken from wells at a depth of 1-2 m. The content of nitrates was determined with phenol disulfonic acid to form a nitro-containing phenol of yellow color [18]. The nitrates content (X) in mg/dm³ was calculated using the formula in terms of nitrate nitrogen:

\[ X = C \times V \]  

(2)

where \( C \) is the nitrate content obtained according to the calibration graph or using the scale of standard solutions, mg/dm³; \( V \) - volume of stained sample (100 or 50 ml), ml.

The content of nitrites was determined based on the ability of nitrites to diazotisate sulfate acid (Griss reagent) with 1-naphthylamine red-violet color [18]. The mass concentration of nitrites \( (X_i) \) in mg/dm3 was calculated using the formula:

\[ X_i = C \times \frac{50}{V} \]  

(3)
The research results show that the water in the waters differs. The effect of seasonal factors is clearly expressed. The research results show that the water in the

\[ X = \frac{m \cdot 1000}{V}, \quad (4) \]

where \( m \) - the mass concentration found using the calibration graph, \( \mu g/dm^3 \); \( V \) - volume of the sample taken for analysis, ml; 50 - volume of standard solution, ml.

The ammonium content was determined using a photometric method by a qualitative reaction with Nessler's reagent [18]. The mass concentration of ammonium nitrogen \( X \) was calculated using the formula:

where the mass of ammonium nitrogen in the sample, \( m \); from the volume of the solution to be analyzed, ml.

Quantitative determination of phosphates was carried out by the interaction of phosphate ions with molybdenum acid ammonium in the presence of tin chloride forming colored solutions [18].

The number of colonies in each of the cups was determined using the calibration graph, \( \mu g\); \( V \) - volume of the sample.

**Statistical analysis**

The received digital material was processed by the variation statistics method using the Student’s t-test. The mean arithmetic values (M) and the mean arithmetic mean errors (± m) were calculated. The changes were considered probable at \( P \leq 0.05 \) and \( P \leq 0.01 \). For calculations, the computer program Statistica 6.0 was used.

**4. RESEARCH RESULTS**

**Spring water biotesting**

The first stage of integrated monitoring of the spring waters quality in Mostyska district was the determination of phyto- and cytotoxicity by biotesting. Based on the growth indicators of Allium cepa L., the seasonal and annual phytoxicity of the spring waters in some settlements within the Mostyska district was established. Distilled water was used as a control. Permissible toxicity of drinking water is not more than 50% [18].

<table>
<thead>
<tr>
<th>Settlement</th>
<th>Autumn Length, mm</th>
<th>Autumn T, %</th>
<th>Winter Length, mm</th>
<th>Winter T, %</th>
<th>Spring Length, mm</th>
<th>Spring T, %</th>
<th>Summer Length, mm</th>
<th>Summer T, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nagirne</td>
<td>7.39±2.13</td>
<td>26.0±3.99</td>
<td>8.54±2.77</td>
<td>33.6±4.85</td>
<td>13.65±4.73</td>
<td>10.2±2.52</td>
<td>15.15±5.73</td>
<td>16.2±3.61</td>
</tr>
<tr>
<td>Pidgat</td>
<td>7.26±2.05</td>
<td>27.0±4.56</td>
<td>7.55±2.02</td>
<td>41.3±5.06</td>
<td>10.7±4.38</td>
<td>29.5±4.32</td>
<td>13.0±3.29</td>
<td>17.1±3.68</td>
</tr>
<tr>
<td>Malniv</td>
<td>8.21±1.96</td>
<td>17.8±3.44</td>
<td>8.81±2.15</td>
<td>31.5±4.63</td>
<td>7.87±3.51</td>
<td>48.1±5.13</td>
<td>6.09±1.55</td>
<td>66.3±5.85</td>
</tr>
</tbody>
</table>

The analysis of the researches results shows that the roots growth in length on the background of different spring waters differs. The effect of seasonal factors is clearly expressed. The research results show that the water in the settlements of Nagirne and Pidgat wells during 2018-2019 have a low average annual level of phytotoxicity. In the villages of Husakiv and Malniv, the phytotoxicity index of spring water fluctuates within the range of 17.5 - 66.3% during the year (p<0.05-0.01). It should be noted...
that the lowest phytotoxicity indicators were recorded in autumn 2018 at 17.5% and 17.8% (p<0.05) respectively. The highest index is typical for the samples taken in summer 2018 - 58.8 and 66.3% respectively, these indicators exceed the acceptable toxicity of 50%. The average annual phytotoxicity indicators in the villages of Husakiv and Malniv do not exceed the maximum permissible toxicity. In the villages of Krukenychi and Volytsia, the phytotoxicity index of spring water fluctuates within the range of 29 - 64.3% during the year (p<0.05-0.01). It should be noted that the lowest phytotoxicity indicators were recorded in the autumn 2018 - 29.0 and 37.3% (p<0.01) respectively, which corresponds to the average toxicity level. The highest index is characteristic of the samples selected in the spring-summer of 2019 and these indicators exceed the acceptable toxicity of 50%.

In Mostyska, a high level of toxicity in the investigated waters has been established within the season, ranging from 51.1 - 66.5% (p<0.05). The average annual phytotoxicity rate is much higher than the permitted toxicity of 50%, these spring water cannot be used for drinking purposes (fig 1).

As a result of the study, it was determined that the toxicity indicators are the lowest in the autumn and winter, while the highest in the spring and summer. Obviously, this is caused by a large amount of rainfall, and, all substances were washed from the soil, roads, etc. with the rains in the surface water.

**Figure 1** Dynamics of the phytotoxicity index for spring waters in the studied settlements

Comparison of the phytotoxicity index for Mostyska district settlements during 2018-2019, seasonally, shows that the average annual phytotoxicity of spring water in the settlements of Nagirne and Pidgat is weak. In the villages Krukenychi, Husakiv, Volytsia and Malniv, toxicity is above average, and there is a high level of toxicity in Mostyska. Drinking water from Mostyska is dangerous for health because the phytotoxicity of these waters exceeds the limit for drinking water.

The cytotoxicity index of the studied spring waters in some settlements of Mostyska district was determined based on the indicators of the mitotic index in the roots of the bulbs. The results of the studies showed little cytogetic effect.

The highest proliferative activity of the root meristem cells was observed in the drinking water sample from the village Husakiv (MI = 79%, p<0.05). The lowest cell proliferation was observed in the water sample from Mostyska (MI = 48%, p<0.01) and Volytsia (MI = 40%, p<0.05), which leads to disturbance of cell division of the meristem. Water samples from the village Nagirne (MI = 51%, p<0.05), village Pidgat (MI = 60% , p<0.05), village Malniv (MI = 64%, p<0.05) and village Krukenychi (MI = 70%, p<0.05) have similar indicators of mitotic index compared to control (MI = 74%) and meet the optimal conditions for plant germination (fig 2).

**Figure 2** Phase index comparison in Allium cepa root meristem

Decreased mitotic activity under the influence of xenobiotics is a nonspecific response of meristematic cells in response to any stressor, but pathologies of mitosis and trends in the proliferation of cells depending on the phases of the mitotic cycle are specific changes in the action of the stress factor. A high prophase index was detected in all water samples, indicating that the cells are at the beginning of mitotic division. Compared to the control of water samples in the village Malniv, Mostyska, villages Pidgat and Husakiv have high metaphase index scores. According to the values of the anaphase index, the lowest rates are in the villages Volytsia and Nagirne. The low indexes of the telophase index indicate the suppression of division in the root meristem, in the water sample from the village Volytsia telophase index is 0%.

**Physico-chemical indicators of spring waters quality in Mostyska district**

The content and dynamics of individual nitrogen compounds are important indicators for the chemical composition of water used in environmental assessment and regulation of the natural waters quality [8]. The presence of various forms of nitrogen compounds in natural waters may depend on the factors of biotic and abiotic genesis: the rate of organic matter ingestion, the
activity and abundance of various forms of microorganisms regulating the stages of transformation, temperature, the presence of antibiotics and dissolved oxygen, etc.

The increased content of nitrates - intermediates of biochemical oxidation of ammonium ions - can indicate fecal contamination of water. The content of NO₂⁻ ions in the drinking water of the study area ranged from 0.0124 to 0.07788 mg/dm³ (p<0.05) and the annual average did not exceed the MPC (3.3 mg/dm³).

**Figure 3** Nitrite content in the studied waters

Nitrites are unstable compounds and are found in sources during severe "fresh" contamination. In undisturbed ecosystems, nitrate formation processes are balanced, and nitrite ion does not accumulate in large quantities. The NO₃⁻ ions content in the drinking water of the study area ranged from 10.3 to 56.7 mg/dm³ (p<0.05) and the annual average exceeded the MPC (45 mg/dm³). Exceedance of the MPC is most likely due to the incorrect placement of wells and surface runoff, i.e. surface water pollution. Significant increase of nitrates in spring waters was recorded in the spring-summer period in Mostyska and in the villages Malniv, Husakiv, Volytsia, and Krukenychi. Samples of spring water in the villages of Pidgat and Nagirne did not exceed the norms.

**Figure 4** Nitrate content in the investigated waters

Elevated levels of nitrates are the result of penetration of mineral and organic fertilizers into aquifers, as well as sewage from places storage of solid waste (garbage from the household, garden waste, etc.), liquid effluents generated in the livestock holding and at increased amount of liquid manure in the soil. High nitrate content can be the evidence of previous pollution with fecal waters. Not absorbed by the soil, nitrates are easily washed off by rainwater, migrate to the depths of the soil profile to groundwater.

In aquatic ecosystems, bacteria-nitrificators are either a part of the periphyton, or they are components of the bottom silt [11, 12]. Representatives of the first phase of nitrification are developing, usually in periphyton, along with bacteria amonificators. Therefore, in a balanced aquatic ecosystem, ammonia, which is formed during the decomposition of organic matter, is instantly consumed on the spot, and therefore the appearance of ammonia in water is often associated with the development of planktonic ammonifiers.

The concentration of ammonium ions in the water of the studied territories ranged from 1.5 - 7.9 mg/l (p>0.05-0.01) and the average annual values exceeded the MPC (2.6 mg/l) in the wells of Mostyska, villages Malniv, Husakiv, Volytsia and Nagirne. This is explained by the specifics of the area where the wells are located, where a large number of private plots, long-term cesspools, cattle holdings are located, and the accumulation of manure and household organic waste takes place.

Samples of spring water from the villages of Krukenychi and Pidgat did not exceed the norms.

**Figure 5** Ammonium ion content in the studied waters

The phosphates content in the water of the study area fluctuated within 0.00031-0.06 mg/l (p<0.05-0.01) and does not exceed the MPC (1.5 mg/l) of the sanitary norms of Ukraine DSanPiN 2.2.4-171-10. In all investigated villages and cities of Mostyska district, there was an increase in the phosphates concentration in the summer-autumn period, which is three times more than at the beginning of the study. In winter and spring, the value of this indicator was minimal. The mineralization of the investigated waters did not exceed the limits allowed for drinking table water - 1000 mg/l (p<0.05).

Even in case of the low toxicity of pollutants that do not cause acute poisoning, long-term consumption of water containing such compounds causes chronic intoxication and, consequently, the development of pathological changes in the human body [5, 8].

**Analysis of bacteriological indicators of spring water quality**

Water quality is analyzed according bacteriological indicators for drinking water: total microbial count and coli index (Table 2).
The number of microorganisms in water depends on many abiotic and biogenic factors: the organic matter content, the water flow rate, the temperature of the environment, the seasons, the location and contamination of the reservoir [1, 9, 10]. As a result of our research, it was established that the average annual number of microorganisms in the spring waters of Mostyska district exceeds the norm (no more than 100 CFU). Only the samples of the spring waters from the villages of Husakiv, Volytsia and Pidgat meet the norms of TMC. Analyzing the seasonal dynamics of the changes in the TMC in spring water, it should be noted that this indicator sharply decreases with decreasing air temperature. In the spring-summer and summer-autumn period, the value of the TMC is 2.2-5.7 times (p<0.05-0.01) higher than the allowable norm of 100 CFU. During the winter-spring period, this figure is much lower, but it also does not meet the norm. It is necessary to clean the wells regularly and also to put bacteriological filters on the water.

The analysis of the results showed that the annual average index of the coli index does not meet the norm (no more than 10 CFU for spring water) in the samples of spring waters of the villages of Krukenychi and Maliv. In other settlements, the dynamics of the coli-index fluctuates within 3-11 CFUs (p=0.05-0.01) and does not exceed the norm (Table II).

One of the main causes of pollution for spring water is poor sanitary condition of springs (absence or damage of scaffolding, roofs, lids, public buckets), close location at less than 20 meters distance to sources of pollution (outside gutters, cesspools and compost manure reservoirs), as well as not carrying out of work on the repair, cleaning and disinfection of wells for more than one year, as required by sanitary rules. In addition, most wells are shallow, rarely cleaned and located near outbuildings [15, 17].

### Table 2 Dynamics of bacteriological indicators for spring waters of Mostyska district (M±M, N = 7)

<table>
<thead>
<tr>
<th>Settlement</th>
<th>Autumn</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMC</td>
<td>Coli-index</td>
<td>TMC</td>
<td>Coli-index</td>
<td>TMC</td>
</tr>
<tr>
<td>Krukenychi</td>
<td>554±47</td>
<td>4±1</td>
<td>416±59</td>
<td>7±2</td>
</tr>
<tr>
<td>Volytsia</td>
<td>31±5</td>
<td>0</td>
<td>38±1</td>
<td>0</td>
</tr>
<tr>
<td>Mostyska</td>
<td>119±26</td>
<td>7±2</td>
<td>102±21</td>
<td>7±2</td>
</tr>
<tr>
<td>Husakiv</td>
<td>42±5</td>
<td>7±2</td>
<td>35±7</td>
<td>4±1</td>
</tr>
<tr>
<td>Nagirne</td>
<td>123±15</td>
<td>240±33</td>
<td>94±21</td>
<td>210±20</td>
</tr>
<tr>
<td>Pidgat</td>
<td>52±6</td>
<td>15±2</td>
<td>47±12</td>
<td>7±2</td>
</tr>
<tr>
<td>Maliv</td>
<td>480±75</td>
<td>320±55</td>
<td>228±36</td>
<td>240±33</td>
</tr>
</tbody>
</table>

### 5. DISCUSSION

During 2018-2019, integrated monitoring of source water quality of some settlements of Mostyska district of Lviv region was carried out. The assessment of water quality was carried out by biological and chemical methods, which made it possible to see the problem systematically. The results of biotesting and cytogenesis showed that the most problematic settlements in terms of the spring water quality are the district center – Mostyska, and the village Volytsya. This is probably due to significant anthropogenic pressure on Mostyska and the village Volytsya, which are borderline with EU and close to checkpoint “Shehy”, where the landfill is located. The decrease in the percentage of telophase cells of the test object is the result of partial elimination by the plant of possible genetic disorders due to the delay of the cell cycle, which increases the time for repair of DNA damage. In the literature, there is a decrease in the number of recorded anaphases as a consequence of the toxic effect of low heavy metals concentrations on the cell [19].

Investigating the factors, causing the toxicity, the physico-chemical and bacteriological parameters were determined. The analysis of the results showed that in these settlements of Mostyska district the maximum permissible concentrations of nitrates are significantly exceeded, which can cause a cytotoxic effect. The presence of ammonium ions in underground waters is a result of the microorganisms’ activity. In some cases, ammonium ions can be formed due to anaerobic processes of the of nitrites and nitrates reduction. The high content of ammonium ions indicates deterioration in the sanitary state of water sources. This increase in concentration is due to the inflow of household wastewater, sewage from food and chemical industry, nitrogen and organic fertilizers in underground waters [11]. Increased nitrate levels also inhibit the microflora of water in the village of Volytsya, since the coli index is zero. Monitoring of spring water quality in some settlements of Mostyska district shows that water supply problem is brewing.
6. CONCLUSION

Water quality of local water sources is regulated by the sanitary norms of Ukraine. Biotesting was carried out, the source waters of the Mostyskyi district were analyzed by physical, chemical and bacteriological parameters. Monitoring of the phytotoxicity index of settlements in Mostysky district during 2018-2019 shows that the average annual phytotoxicity of spring water in the settlements of Nagirne and Pidgat is weak. In the villages Kruekenychi, Husakiv, Volysia and Malnih, toxicity is above average and in Mostyska there is a high level of toxicity. The highest cytotoxicity index is in Mostyska and Volysia. In general, during the study period, only spring water samples from Mostyska exceed the permissible toxicity level for drinking water. The content of nitrites and phosphates did not exceed the MPC in all studied sources. The content of nitrites and ammonia in the spring waters of the study area exceeded the MPC in most settlements, except for the samples of spring waters in the villages of Pidgat and Nagirne. The vast majority of the studied waters of the Mostyska district do not meet the bacteriological indicators of drinking water quality. In Ukraine, it is necessary to develop a comprehensive system for monitoring the water quality in individual sources, which will be able to control their quality and ensure the population health.

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