Revisiting the interrelation between nitrate and microbiological pollution of spring water

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Abstract. The article provides statistical grounds for the formation of a data array to be used at estimating the interrelation between the chemical and microbiological groundwater quality indices substantially exceeding sanitation and hygiene standards. This has provided relevant data on low linear dependence between the nitrate content and total bacterial count, and absence of linear dependence between the nitrate content and coliform index in the groundwater of 29 springs in Kaluga Region for the period from 2011 to 2016.

1. Introduction
Numerous studies on spring water pollution generally prove high content of nitrates and microorganisms in the groundwater [1-3]. Considering the fact that nitrogen is one of the principal biogenous elements, we developed the hypothesis on a certain interrelation between the nitrate content and the number of microorganisms in spring water.

The hypothesis investigation requires a considerable data array on chemical and bacteriological analysis of groundwater. The majority of studies, including the above studies [1-3], are based on the data collected from several springs in different regions and during a short period of time, which limits the possibility to include these results in the general experimental data array.

The studies made in Kaluga Urban District in the period from 2001 to 2016 are free from these limitations. The microbiological pollution of groundwater was estimated through the total bacterial count in the water (CFU – the number of bacterial colony-forming units present in 1 cm³) and amount of coliform bacteria (CB), which is indicated by a coliform index (CI) and an amount of CB per 1 dm³ in spring water. Quantitative characteristics of nitrate and microbiological groundwater pollution for 34 springs are partially reflected in works [4, 5], but interrelation between the said indices has not been studied.

The objective of this work is to identify regularities between the nitrate content and microbiological pollution (CFU and CI) in the groundwater of 29 springs in Kaluga Urban District on the basis of multi-year research.

2. Methods
The subject of this study is groundwater from 34 springs. Due to the fact that 5 springs ran dry during the period of our research, we processed the statistical data collected from chemical and biological
analysis of water from 29 springs. The objects of this study are the concentration of nitrates, total bacterial count in the water (CFU – the number of bacterial colony-forming units present in 1 cm$^3$) and amount of coliform bacteria (CB), which is indicated by coliform index (CI) and amount of CB per 1 dm$^3$ in spring water.

Point water samples were taken in the spring-summer season in the period from 2011 to 2016. To determine the concentrations of nitrates, CFU and CI in the studied water, standard methods were used [6-11].

3. Results and discussion
Table 1 presents the nitrate content, total bacterial count and coliform index in spring water collected in the period from 2011 to 2013; table 2 shows the results in the period from 2014 to 2016.

Table 1. The analysis results for nitrates (mg/dm$^3$), total bacterial count (CFU per 1 cm$^3$) and coliform index (amount of coliform bacteria in 1 dm$^3$) in spring water collected in the period from 2011 to 2013.

| Spring | 2011 | 2012 | 2013 |
|--------|------|------|------|
|        | NO$_3^-$ | CFU | CI | NO$_3^-$ | CFU | CI | NO$_3^-$ | CFU | CI |
| 1      | 67    | 100  | 15  | 60    | 105  | 3  | 46    | 105  | 3  |
| 4      | 65    | 2    | 30  | 58    | 140  | 7  | 52    | 140  | 7  |
| 5      | 145   | 10   | <3  | 37    | 10   | <3 | 37    | 10   | <3 |
| 6      | 39    | <1   | <3  | 140   | 335  | 23 | 124   | 335  | 23 |
| 8      | 166   | 100  | 30  | 169   | 310  | 21 | 201   | 310  | 21 |
| 9      | 0,1   | 20   | <3  | 0,1   | 10   | <3 | 7,2   | 10   | <3 |
| 10     | 92    | 50   | <3  | 87    | 130  | <3 | 85    | 30   | <3 |
| 11     | 41    | <1   | <3  | 43    | 20   | <3 | 46    | 20   | <3 |
| 12     | 25    | 10   | 45  | 27    | 15   | <3 | 31    | 15   | <3 |
| 13     | 63    | 50   | 15  | 53    | 15   | <3 | 56    | 15   | <3 |
| 14     | 21    | <1   | <3  | 32    | 220  | 16 | 44    | 220  | 16 |
| 15     | 88    | 20   | 120 | 56    | 125  | 3  | 62    | 125  | 3  |
| 16     | 52    | 5    | 15  | 52    | 210  | 10 | 63    | 210  | 10 |
| 17     | 80    | 300  | 60  | 53    | 120  | 4  | 55    | 120  | 4  |
| 18     | 24    | <1   | 30  | 38    | 120  | 16 | 27    | 120  | 16 |
| 19     | 18    | <1   | 30  | 1,2   | 30   | <3 | 22    | 30   | <3 |
| 20     | 18    | 25   | 30  | 32    | 240  | 16 | 66    | 240  | 16 |
| 21     | 45    | 5    | <3  | 51    | 25   | <3 | 45    | 25   | <3 |
| 22     | 59    | 22   | <3  | 65    | <10  | <3 | 58    | <10  | <3 |
| 23     | 32    | 5    | 23  | 41    | 260  | 50 | 23    | 260  | 50 |
| 24     | 15    | 10   | 15  | 34    | 200  | 33 | 22    | 245  | 33 |
| 25     | 13    | <1   | <3  | 14    | <10  | 133 | 19    | 20   | <3 |
| 26     | 20    | 50   | 15  | 18    | 220  | 66 | 12    | 40   | <3 |
| 27     | 33    | 22   | <3  | 42    | 10   | 50 | 39    | 50   | <3 |
| 28     | 9,1   | 5    | <3  | 9,9   | 25   | 33 | 12    | 25   | <3 |
| 30     | 79    | 100  | <3  | 57    | 50   | 133 | 79    | 135  | 3  |
| 32     | 155   | 150  | 90  | 108   | 50   | 83 | 32    | 240  | 40 |
| 33     | 58    | 120  | 30  | 50    | 250  | 200 | 35    | 255  | 50 |
| 34     | 22    | 50   | <3  | 84    | 70   | 66 | 54    | 140  | 3  |
| TLV    | ≤45   | ≤100 | ≤10 | ≤45   | ≤100 | ≤10 | ≤45   | ≤100 | ≤10 |
| C$_{max}$ | 166 | 300  | 120 | 169   | 335  | 200 | 201   | 335  | 50 |
| C$_{min}$ | 0,1 | 1    | 3   | 0,1   | 10   | 3  | 7,2   | 10   | 3  |
| Average | 53,2  | 42,7 | 21,8 | 52,1  | 115  | 34,2 | 50,1  | 121  | 11,6 |
| Exceeding | 45  | 50   | 52  | 50    | 48   | 55 | 31    |
With CFU and CI microbiological indices, the values of which experimental data received for 2015 and the data received for other years. This difference is associated index (amount of coliform bacteria in 1 dm$^3$) in spring water collected in the period from 2014 to 2016.

The analysis results for nitrates (mg/dm$^3$), total bacterial count (CFU per 1 cm$^3$) and coliform index (amount of coliform bacteria in 1 dm$^3$) in spring water collected in the period from 2014 to 2016.

| Spring | 2014 | 2015 | 2016 |
|--------|------|------|------|
|        | NO$_3^-$ | CFU | CI  | NO$_3^-$ | CFU | CI  | NO$_3^-$ | CFU | CI  |
| 1      | 14    | 35   | 3   | 39      | 5    | <3  | 39      | 145  | 100 |
| 4      | 48    | 150  | 7   | 39      | 10   | <3  | 39      | 125  | 33  |
| 5      | 49    | 35   | <3  | 35      | 12   | <3  | 40      | 180  | 100 |
| 6      | 187   | 140  | 5   | 115     | 20   | <3  | $10^1$  | 145  | 35  |
| 8      | 116   | 310  | 33  | 88      | 20   | <3  | $130$  | 320  | 57  |
| 9      | 4,3   | 10   | <3  | 6,7     | 40   | <3  | 0,1    | 80   | 10  |
| 10     | 71    | 150  | 3   | 57      | 15   | <3  | 54      | 150  | 66  |
| 11     | 38    | 115  | 16  | 32      | 25   | <3  | 40      | 110  | 50  |
| 12     | 36    | 120  | 6   | 32      | 20   | <3  | 28      | 10   | <3  |
| 13     | 62    | 100  | 3   | 51      | 25   | <3  | 57      | 20   | 10  |
| 14     | 56    | 10   | <3  | 52      | 10   | <3  | 47      | 10   | <3  |
| 15     | 67    | 300  | 33  | 55      | 30   | <3  | 56      | 10   | <3  |
| 16     | 63    | 200  | 10  | 60      | 15   | <3  | 65      | 200  | 16  |
| 17     | 51    | 190  | 25  | 44      | 17   | <3  | 55      | 170  | 43  |
| 18     | 27    | 10   | <3  | 21      | 10   | <3  | 29      | 13   | <3  |
| 19     | 22    | 15   | <3  | 17      | 25   | <3  | 14      | 10   | <3  |
| 20     | 47    | 10   | <3  | 14      | 50   | <3  | 20      | 45   | 50  |
| 21     | 47    | 30   | 16  | 29      | 10   | <3  | 38      | 10   | <3  |
| 22     | 58    | 60   | <3  | 44      | 15   | <3  | 50      | 10   | <3  |
| 23     | 49    | 15   | <3  | 27      | 10   | <3  | 27      | 25   | 16  |
| 24     | 20    | 65   | 16  | 20      | 65   | <3  | 20      | 110  | 13  |
| 25     | 101   | 20   | <3  | 68      | 20   | <3  | 9       | 20   | 16  |
| 26     | 104   | 35   | 16  | 32      | 10   | <3  | 10      | 35   | 33  |
| 27     | 20    | 35   | 16  | 25      | 15   | <3  | 19      | 23   | 33  |
| 28     | 15    | 15   | <3  | 13      | 25   | <3  | 7       | 220  | 23  |
| 30     | 81    | 130  | 22  | 68      | 30   | <3  | 56      | 50   | 15  |
| 32     | 41    | 40   | 33  | 37      | 40   | <3  | 37      | 120  | 16  |
| 33     | 40    | 60   | 26  | 38      | 60   | <3  | 38      | 140  | 33  |
| 34     | 3     | 10   | <3  | 39      | 15   | <3  | $79$    | 155  | 50  |
| TLV    | $\leq 45$ | $\leq 100$ | $\leq 10$ | $\leq 45$ | $\leq 100$ | $\leq 10$ | $\leq 45$ | $\leq 100$ | $\leq 10$ |
| $C_{\text{max}}$ | 187 | 310 | 33 | 115 | 65 | 3 | 130 | 320 | 100 |
| $C_{\text{min}}$ | 3 | 10 | 3 | 6,7 | 5 | 3 | 0,1 | 10 | 3 |
| Average | 53,0 | 83,3 | 11,1 | 41,3 | 22,9 | 3,0 | 41,5 | 91,8 | 30,0 |
| Exceeding TLV, % | 57 | 31 | 34 | 31 | 0 | 0 | 38 | 48 | 72 |

*a* The results exceeding the TLV are right-aligned.

*b* The results within the TLV by all three indices are left-aligned and in bold.

*c* The results exceeding the TLV by all three indices are right-aligned and in bold italics.

An analysis of the foregoing data array demonstrates a considerable difference between the experimental data received for 2015 and the data received for other years. This difference is associated with CFU and CI microbiological indices, the values of which did not exceed the stated norms in all studied spring water, while the nitrate concentration exceeded the threshold limit value (TLV) in every
third sample. Thus, there are grounds to exclude the data for 2015 from the total data array comprising of n=145 results of chemical and biological analysis.

If we observe the number of springs which complied and did not comply with the stated norms in terms of the three studied indices, we can find out the following percentage: 25% and 14% respectively, which means that on the average for the five years in 4 springs out of 10, the groundwater complied with the sanitation and hygiene standards; in the water of 2 springs all the three indices exceeded the norm; in 4 springs the water did not comply with the norm in terms of one of the indices.

On the average, the nitrate amount exceeded the threshold limit value by 40%-50%. The total bacterial count and coliform index exceeding sanitation and hygiene standards are observed in the same range (table 1 and table 2), which provides the grounds for studying the data for the five years in order to determine certain dependences.

The dependence between the nitrate content and the total bacterial count in the spring water studied in the period from 2011 to 2014 and in 2016 is demonstrated in figure 1. Correlation index value (0.18) indicates very low linear dependence between the nitrate concentration and total bacterial count in the studied ranges. The positive value of the linear dependence angle points out to the increased nitrate concentration in the spring water along with the increased total bacterial count. On the average, the nitrate concentration increases by 50% with the increase in CFU by 100 units.

![Figure 1. The dependence of the nitrate content on the total bacterial count in the studied spring water (n=145).](image)

Although the linear dependence between the nitrate concentration and the total bacterial count in the spring water is slightly probable, the linear dependence between the nitrate content and the coliform index value was not confirmed during our study (figure 2). We made such a conclusion on the grounds of two criteria: First, the correlation index value is less than 0.1 (0.014). Second, the point of intersection between the average nitrate content (50.1 mg/dm$^3$) and the average CI value (21.7 per 1 dm$^3$) is outside the trend line (figure 2), as compared to the point of intersection between the average nitrate content (50.1 mg/dm$^3$) and the average CFU value (90.7 per 1 dm$^3$), which is on the trend line (figure 1).
4. Conclusion
The determined low linear dependence between the nitrate content and total bacterial count points out that nitrates in spring water may have an effect on an increase in the amount of mesophilic, mesotrophic aerobes and facultative anaerobes.

The absence of linear dependence between the nitrate content and coliform index in the groundwater proves that the amount of coliform bacteria as an index of water faecal pollution is not associated with the increased groundwater nitrate content and is a random variable.

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