STUDY OF THE BACTERIOLOGICAL INDICATORS OF POLLUTION IN THE BASIN OF THE CIRIC RIVER

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Key words: water, pollution, fecal coliforms, fecal streptococci, sulphite-reducing clostridia*, Pseudomonas, Salmonella*

Abstract To the purpose of assessing the level of bacteriological polllution of the water in the basin of the Ciric river, the study had the following objectives: determination of the probable number of fecal coliforms, fecal streptococci, sulphite-reducing clostridia, and identification of the bacteria belonging to the Pseudomonas aeruginosa species and Salmonella genus. The results of the research studies showed fluctuations in terms of number in the hygienic-sanitary groups in all the points examined, even from one harvest period to the other (July and September 2006). The PNFS index exhibited low or even undetectable values in almost all the sampling points examined, while the PNFC/PNFS ratio higher than 4 in most of the points indicated the presence of a human source of water pollution. The water of the Ciric river, in most of the sampling areas and for both sample collection periods, was classified into quality classes III and IV both in terms of total coliforms and fecal coliforms.

INTRODUCTION

Pollution of the surface waters, the same as that of the ground ones, seriously affects the biosphere, with negative impact on the aquatic life forms, from microorganisms to insects, birds, fish, and, at the same time, the health of the terrestrial animals and plants. In addition, pollution affects people’s capability of using the water. Depending on the nature and intensity of pollution, water availability for any purpose (e.g. physiologic, hygienic, industrial, or recreative) may be diminished or annulled (Madigan, 2000).

Pollution with pathogens may cause both in human beings and animals numerous waterborne diseases, either as a result of ingestion or direct contact, or inhalation of aerosols generated by contaminated water (Zarnea, 1994). The percentage increase in the number of viral diseases over the last decades is not real; the explanation lies in the increase in the number of people being diagnosed, due to technological development. Many bacterial species have pathogenic strains and non-pathogenic ones (or just opportunistic), causing diseases in organisms with low immunity.

Taking into consideration that Romania is among the European countries with relatively limited water sources, the management and usage of the waters in our country has become a priority of extreme importance, which resulted, in the last decades, in the expansion of hydro-technical works aiming at increasing the water reserves and decreasing their dependence on the environmental factors. Due to the more and more numerous and higher pressures onto the water supplies, the creation of legal instruments addressing specifically the new issues and contributing to the provision of water resources for the following generations has become crucial.

Romania’s accession to the European Union, a national priority, implies the harmonization of national legislation to the legislation of the European Union, which includes the EU Water Framework Directive (2000/60/EC) and other 17 different directives in the field of water policy.

This study is a necessity in order to fully assess the level of bacteriological pollution of the water in the basin of the Ciric river and to know the risk it poses to people’s health.

MATERIAL AND METHOD

The sanitary quality of water is assessed based on the presence or absence of pathogenic microorganisms or of microorganisms indicating their potential presence. A potential carrier of pathogenic microorganisms, water may pose a threat to health and life. The main bacteriological indicators for pollution contained by water are the group of total and fecal coliform bacteria (considered as primary indicator of fecal contamination of water) and fecal enterococci (Dunca, 2004). Based on whether these bacteria are present isolated or in association as well as on their seasonal and annual quantitative variation, the hygienic-sanitary condition of water can be determined.

Determining the probable number of coliform bacteria (*total coliform count*)

The coliform germs are the microorganisms most commonly found in the feces and have been recommended as index of fecal pollution. Their presence in natural environments indicate recent contamination with faecal matter (An, 2000). Coliform bacteria (*total coliforms*) are motile, Gram negative, non-sporulated bacilli fermenting lactose at 37 ± 0.5^0C. They are characterized by acid and gas generation in bouillon – lactose and identified by the characteristic appearance of the colonies formed on selective media.The presence of coliform bacteria (*total coliforms*) is determined by the presumptive test, inoculating the water and/or ten-fold dilutions into several flasks containing enriching liquid medium. The positive reaction was shown by a confirmation test on a solid medium at 37 ± 0.5^0C, after 24 hours. Starting from the number of positive tubes confirm, the probable number of coliform bacteria is calculated (*total coliforms*).

Determining the probable number of thermotolerant coliform bacteria (fecal coliforms)

Thermotolerant coliform bacteria (*fecal coliforms*) produce acid and gas by metabolic fermentation of lactose in liquid media containing lactose at 44 ± 0.5^{0}C (Madigan, 2000). The presence of thermotolerant coliform bacteria (fecal coliforms) is determined starting from the flasks and vials found positive for coliform bacteria (total coliform count) in the presumptive test and by confirmation on selective liquid medium, at 44 ± 0.5^{0}C in 24 hours. Taking into consideration the number of positive tubes at 44 ± 0.5^{0}C, the probable number of thermotolerant coliform bacteria (fecal coliforms) is calculated.

Determining the probable number of enterococci

Enterococci or fecal streptococci are naturally present in the digestive tract of humans and animals. Their presence, however, in aquatic basins, usually indicates recent contamination (Bernhard, 2003). They are capable of growing and multiplying at 45°C in the presence of a 40% concentration of bile salts and sodium azide (coliform inhibiting) (Nicolescu, C., 2002). The presence of fecal streptococci is determined by the presumptive test with the inoculation of the sample and/or ten-fold dilutions in a number of flasks and test tubes containing enriching liquid medium, at 37 ± 0.5^{0}C. The positive reaction is shown by conducting a confirmation test on a selective liquid medium, at 44 ± 0.5^{0}C for 24 hours, or on a selective solid medium at 44 ± 0.5^{0}C for 48 hours. Starting from the number of positive tubes confirmed, the probable number of fecal streptococci is calculated.

Determining the probable number of anaerobe sulphite-reducing germs

The third group of bacteria of sanitary importance, which can indicate the level of fecal pollution of water besides the fecal coliforms and fecal streptococci, consists of the anaerobe, sporulated, sulphite-reducing germs, the most relevant representative of which is the species *Clostridium perfringens*. This indicator is extremely important in determining remote or intermittent pollution. *Clostridia* are sporulated, anaerobe, Gram positive bacilli, which reduce sulphite to sulphide and produce black coloured ferrous sulphide in the presence of divalent iron. The presence of sulphite-reducing clostridia is determined by inoculation of the sample and/or ten-fold dilutions into a number of test tubes containing enriching liquid medium and incubation at 37 ± 0.5^0 C for 48 hours in anaerobic conditions. The bacteria belonging to the *Clostridium perfringens* species are identified by confirmation of the tubes with positive reaction due to the presence of sulphite-reducing clostridia in milk-containing medium, at 37 ± 0.5^0 C for 48 hours.

Detection of the bacteria belonging to the Pseudomonas aeruginosa species

This is a bacterial species widely spread in nature and, at the same time, very often isolated from mucous membranes, skin, and gastrointestinal tract of healthy people and animals. *Pseudomonas aeruginosa* is the most important conditional pathogenic bacterium causing serious infections in individuals with immunodeficiency. It belongs to the *Pseudomonaceae* family (group of fluorescent species), which includes non-sporulated Gram negative bacilli producing two characteristic pigments, pyocyanin and fluorescein. The species can be differentiated from other fluorescent pseudomonads by: synthesis of pyocyanin, growth at 42^0 C, casein hydrolysis, and metabolization of triphenyltetrazolium chloride (Boenigk, 2004). The presence of *Pseudomonas aeruginosa* can be detected by a presumptive test inoculating the water and/or ten-fold dilutions into a number of flasks and test tubes containing enriching liquid medium and incubating them at 37 ± 0.5^0 C for 48 hours. The positive reaction is shown by means of a confirmation test on solid media, which promotes the production of pigments characteristic to the species, and additional tests, if required. Starting from the number of positive flasks and test tubes confirmed and using statistical tables the probable number of *Pseudomonas aeruginosa* bacteria is calculated.

Detection of the bacteria belonging to the *Salmonella* genus

The *Salmonella* genus belongs to the *Enterobacteriaceae* family, and includes Gram negative, facultative anaerobe bacteria capable to produce hydrogen sulphide, decarboxylate L-lysine, and ferment glucose, but not lactose (as a rule). The bacteria belonging to the *Salmonella* genus are identified by concentrating them in large sample sizes using filtration through gauze pads followed by enriching, dispersion, isolation on selective media, and biochemical and serological confirmation.

RESULTS AND DISCUSSIONS

The research studies were conducted in July and September 2006, and were aimed at determining the level of bacteriological pollution of the water in the basin of the Ciric river and assessing the risk potential for human communities. I order to achieve such objectives, the points for collecting water samples were determined (Photo 1-6): 1. Spring; 2. Nautical Base Dorobanț; 3. Dorobanț Dam; 4. Aroneanu Dam; 5. Lake Ciric 1; 6. Lake Ciric 2. To evaluate the hygienic level of the basin of the Ciric river, a number of 7 hygienic-sanitary indicators were monitored, as follows: total coliforms - *PNTC*, fecal coliforms - *PNFC*, fecal streptococci - *PNFS,* sulphite-reducing clostridia - *PNRSC*, bacteria belonging to the *Pseudomonas aeruginosa* genus – *PNPA* and *Salmonella* genus.

An overview of the ***results of the study of bacteriological indicators for pollution*** (PNTC, PNFC, PNFS, PNPA, PNRSC) conducted in July (see Table I) shows that the spring exhibits a high bacteriological content indicated by the large probable number of total coliforms (PNTC) and fecal streptococci (PNFS), as compared to the values reported for the Aroneanu Dam area. Thus, while in the Spring area were reported 23000 total coliforms /100 ml, in the Aroneanu Dam area, only 790 total coliforms /100 ml were found. The same results were reported for the probable number of fecal streptococci (PNFS), which is by 6.3% higher in the former area (i.e. the Spring) than the value determined in the latter sampling point (i.e. Aroneanu Dam). In terms of the content of sulphite-reducing clostridia, the difference between the two sampling points was 12.2%.

Table I. Results of the study of bacteriological indicators of pollution (July 2006)

Sample no.	Sampling point	Germs examined				
		PNTC	PNFC	PNFS	PNPA	PNRSC
1.	Spring	23000	7900	790	2400	27000
2.	Nautical Base Dorobanţ	1410	7900	460	221	1100
3.	Dorobanţ Dam	4900	50	170	40	1720
4.	Aroneanu Dam	790	790	50	790	3300
5.	Lake Ciric 1	23000	3300	220	170	17200
6.	Lake Ciric 2	2300	2300	70	2400	1400

Table I contains the data on the bacterial content (July 2006) found in the different areas of the Ciric river. In terms of quantity, the levels in the Dorobanţ Dam area reported for probable fecal coliforms were lower (50 germs/ml) than the levels found in the Nautical Base Dorobanţ area (i.e. 7900 germs/ml). Moreover, in the Spring area, the level of fecal coliforms equaled the content found in the Nautical Base Dorobanţ area (i.e. 7900 germs/100 ml). While in the Spring area the total coliform content was similar to that found in the Lake Ciric 1 (i.e. 23000 germs / 100 ml), in the Lake Ciric 2 area, the bacterial content of clostridia was significantly lower (i.e. 1400 germs/ml) than the content found in Lake Ciric 1 area (i.e. 17200 germs / ml).

The ***results of the study of bacteriological indicators of pollution*** (PNTC, PNFC, PNFS, PNPA, PNRSC) conducted in ***September 2006*** (see Table II) showed that the water in the sampling points Spring and Lake Ciric 1 exhibited high bacterial content (high levels of total coliforms, fecal coliforms and fecal streptococci), as compared to the water in the sampling points Dorobanţ Dam and Nautical Base Dorobanţ. In the Dorobanţ Dam area, the probable number of coliform bacteria decreased (by 14.8%) when compared to the values found in the Aroneanu Dam area; in the sampling points Spring and Lake Ciric 1 the number of total coliform bacteria found was similar. In terms of quantity, the levels for the probable number of fecal streptococci found in the sampling point Nautical Base Dorobanţ were much lower (i.e. 20 germs/100 ml) than in the Spring sampling point (3400 germs/100 ml.) As for the sampling points Aroneanu Dam and Lake Ciric 1, the levels recorded for this indicator were similar (80 germs/ml). In the Lake Ciric area, the levels reported for the probable number of fecal coliforms were high (i.e. 79000 germs/100 ml) as compared to the Dorobanț Dam area (i.e. 70 germs/100 ml). High values of bacterial content of fecal coliforms were reported also in the sampling points Spring (4900 germs/100 ml) and Aroneanu Dam (3300 germs/100 ml), while the levels of clostridia content at the Nautical Base Dorobanț and Aroneanu Dam were similar (790 germs/100 ml).

Table II. Results of the study of bacteriological indicators of pollution (September, 2006)

Sample no.	Sampling point	Germs examined				
		PNTC	PNFC	PNFS	PNPA	PNRSC
1.	Spring	79000	4900	3400	940	17200
2.	Nautical Base Dorobanț	1300	110	20	130	790
3.	Dorobanț Dam	490	70	0	0	1300
4.	Aroneanu Dam	3300	3300	80	90	790
5.	Lake Ciric 1	79000	79000	80	340	700
6.	Lake Ciric 2	1750	460	50	141	70

A comparative analysis of the graphical representations below shows the differences in the results obtained for the water samples collected in July and September 2006.

The total number of fecal coliforms exhibited an increase at the sampling points Spring and Lake Ciric I in July and September (Fig. 1), while the level of fecal coliform germs did not exhibit considerable differences from one month to the other, except at the Lake Ciric 2 sampling point, where high values were reported. (Fig. 2). The number of fecal streptococci exhibited low levels in most sampling points, both in July and September, except the Spring area, where the highest values were reported in September (Fig. 3).

Determining the nature of fecal pollution of water

In order to establish the source of fecal pollution of the water of lake or rivers, an appropriate indicator (index) is used. The index is the ratio of fecal coliform bacteria (PNFC) to fecal streptococci (PNFS). A ratio higher that 4 (PNFC/PNFS>4) shows human pollution. When the ratio is between 2 and 4 (2>PNFC/PNFS<4), the pollution has a mixed source, but human pollution prevails; when the ratio is between 0.7 and 1 (0.7<PNFC/PNFS<1), the pollution is again mixed, but animal pollution prevails. Still to identify and discriminate the nature of fecal pollution source (either human or animal), recent studies have demonstrated the availability of a new PCR technique using specific genetic markers (Bernhard, A.E. et al., 2003). Table III indicates the values of the NPCF/NPSF ratio reported for July and September 2006.

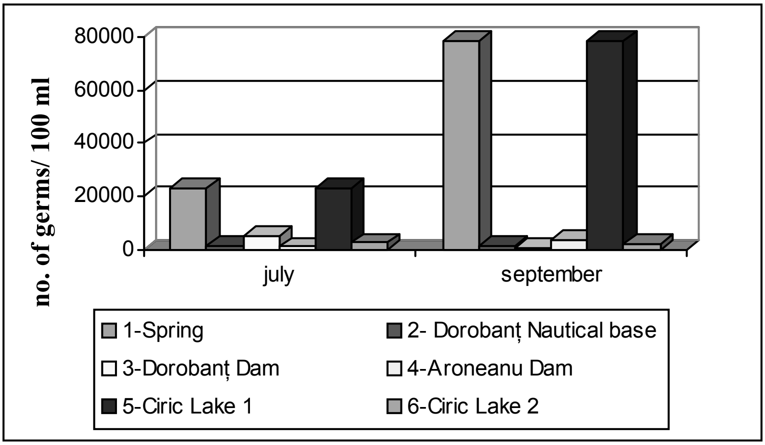

Fig. 1. Variation in total coliform germs level in the water samples examined (July, September 2006)

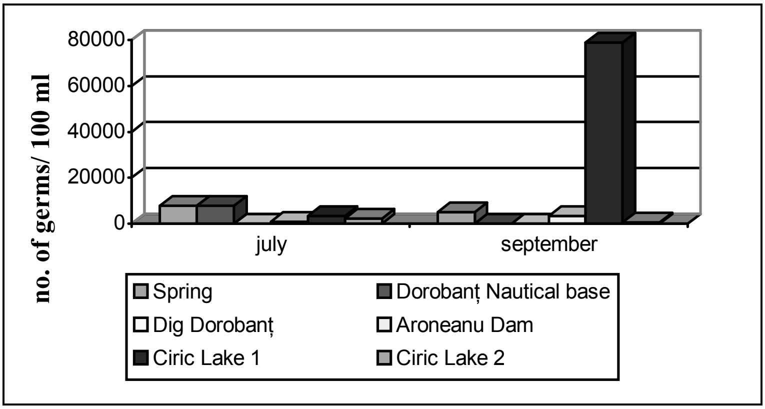

Fig. 2. Variation in fecal coliform germs level in the water samples examined (July, September 2006)

Since both in July and September 2006 the PNFC/PNFS ratio exceeded 4 in most sampling points, it was concluded that the water pollution source is of human origin. The Dorobanţ Dam area was not considered because the PNFS levels could not be properly detected in September. Extremely high values were reported particularly at the Aroneanu Dam and Lake Ciric 1 (in September); as an exception, we can point out the sampling point Dorobanţ Dam (in July) which exhibited levels <0.7 indicating pollution with animal dejections.

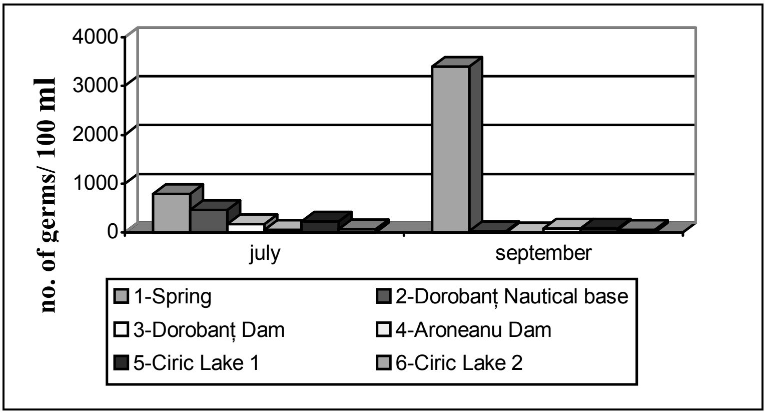

Fig. 3. Variation in fecal streptococci level in the water samples examined (July, September 2006)

Table III. PNFC/PNFS ratio (July, September 2006)

Sample no.	Sampling point	PNFC/PNFS ratio	PNFC/PNFS ratio
1.	Spring	10	1,44
2.	Nautical Base Dorobanț	17	5,5
3.	Dorobanț Dam	0,3	-
4.	Aroneanu Dam	16	41,25
5.	Lake Ciric 1	15	987,5
6.	Lake Ciric 2	33	9,2

The number of sulphite-reducing clostridia varied from one point to the other. Thus, in July 2006, high levels were reported for the Spring and Lake Ciric 1 areas. In September, the clostridia loads in the water recorded at the Spring remained high, while those reported for the other sampling points were considerably lower (Fig. 4).

The load with *Pseudomonas aeruginosa* bacteria was significant in July 2006, particularly in the sampling points Spring and Lake Ciric 2. In September, the levels decreased (Fig. 5).

A comparative analysis of the results of the tests conducted to detect the presence of the bacteria belonging to the *Salmonella* genus shows that in July 2006 (Fig. 6), only 34 % of the samples examined produced positive results (particularly the samples collected from the Spring and Lake Ciric 1 areas), while in September 2006 (Fig. 7), *Salmonella* bacteria were identified in 66% of the samples subjected to examination.

The results of the bacteriological tests conducted on samples collected from the basin of the Ciric river were reported to the levels specified in the *Regulations on the reference objectives for the classification of the quality of surface waters*, developed in 2002 (replacing the STAS 4706-88) (*** 2002). The data obtained show that the water of the Ciric river, in most sampling points, meets the classification requirements for classes III and IV, both in terms of total coliforms and fecal coliforms. The water sampled from the Aroneanu Dam point (in July) and Dorobanţ Dam point (in September) can be assigned to quality class II in terms of total coliforms, while in terms of fecal coliforms, the water sampled from the latter point (both in July and September 2006) falls into the quality class II.

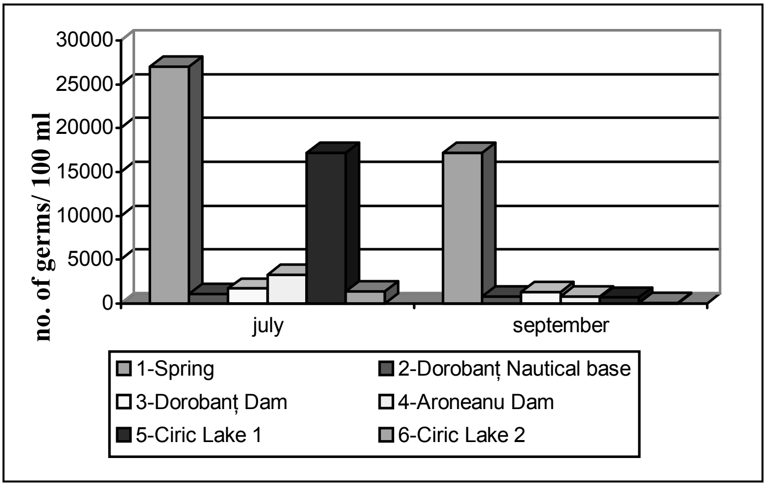

Fig. 4. Variation in the level of sulphite-reducing anaerobe germs in the water samples examined (July, September 2006)

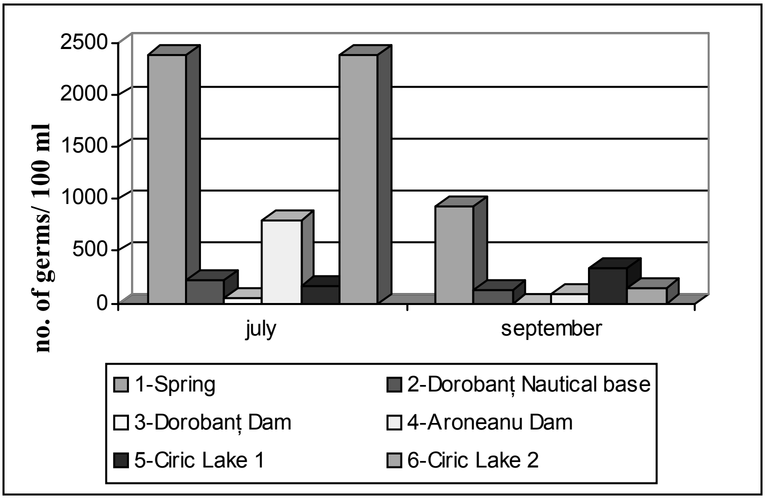

Fig. 5. Variation in the level of bacteria belonging to the *Pseudomonas aeruginosa* species in the water samples examined (July, September 2006)

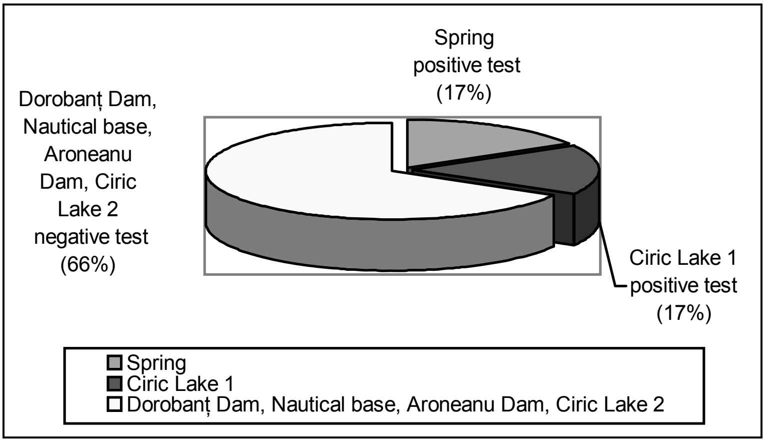

Fig. 6. Percentage representation of sampling points according to the presence/absence of bacteria belonging to the *Salmonella* genus (July, 2006)

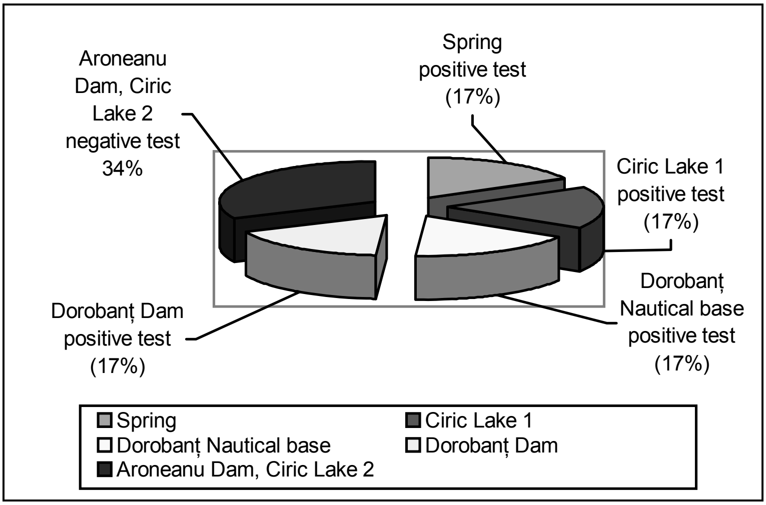

Fig. 7. Percentage representation of sampling points according to the presence/absence of bacteria belonging to the *Salmonella* genus (September, 2006)

CONCLUSIONS

The analytical tests performed to the purpose of evaluating the level of bacteriological pollution of the water in the Ciric basin had the following findings and conclusions:
Numerical fluctuations were detected in the hygienic-sanitary groups in all the points subjected to examination, even from one sampling period to the other (i.e. July and September 2006).
Of the hygienic-sanitary bacteria examined, the PNSF index exhibited low or untraceable levels in almost all the sampling points subjected to examination.

The test for fecal coliforms showed that the PNFC levels in the water samples examined in July and September 2006 meet the limits indicated in the STAS 3001/91 in most the sampling points (below 10.000 germs/100 ml), except the Lake Ciric 1, where the level recorded in September was 79.000 germs/ml.
The PNFC/PNFS ratio, both in July and September 2006, exceeded 4 in most sampling points, which indicates the presence of a human source of water contamination.
The results reported for September showed a decrease in the number of microorganisms per ml (in the case of fecal streptococci, sulphite-reducing clostridia, and bacteria belonging to the *Pseudomonas aeruginosa* species), due to the low temperatures during sample collection.
The bacteria belonging to the *Salmonella* genus were detected in almost all the sampling points, except Aroneanu Dam and Lake Ciric 1 in September 2006.
The comparative analysis of the results of the bacteriological tests to the maximum levels indicated in the “Regulations on the reference objectives for the classification of the quality of surface waters” drafted in 2002 shows that, in terms of both total coliforms and fecal coliforms, the water in the Ciric river falls into the quality categories III and IV, in most sampling points and for both sampling periods.
The quality of the waters in the Ciric basin should be monitored to maintain their ecological potential to the highest level, as well as to determine and remove any possible pollution sources.

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