

ABSTRACT

In the doctoral thesis entitled "Research on the parasite load of certain tributaries in the Dornelor basin and saprobiological correlations", we aimed to monitor water quality by concentrating parasitic elements of certain protozoa that can generate zoonotic diseases, as an effect of the discharge of organic matter originating from the animals raised in the micro farms or households in the mountain area of the Dornelor basin. This study was simultaneously correlated with the water quality analysis through multi-year physical-chemical, microbiological or algal content measurements, as well as with a study on the presence of benthic macroinvertebrates in the same 10 work stations on the studied watercourses, upstream and downstream from some micro farms or agglomerations of human communities that could influence water quality through different discharges. The streams from where samples were collected between 2017 and 2018 were Arinu, Bancu, Călimănel and Secu.

In the first part of the thesis, "Current state of knowledge", the Dornelor depression is characterized, taking into consideration the factors that influence parasitism in the water environment, and the hydrographic basin is described so as to highlight the correlations between animal breeding in the mountain area and its impact on the water environment. The workstations and the streams from which samples were taken for the entire spectrum of analyses during the study period are described in this subchapter.

The second chapter in the first part is dedicated to a bibliographic study on some parasitic agents that can contaminate water sources, as well as methods of isolation and identification of the same. **In the third chapter** water is characterized from a structural point of view and concerning its physical-chemical properties. **In the fourth chapter** a study is carried out on benthic macroinvertebrates, since a complete characterization of the trophic networks in running waters must also be achieved based on the information on these macroinvertebrates, because they represent an important link of certain trophic networks - starting from the organic matter existing in a watercourse - which are different at the source and at the river confluence.

The second part of the thesis includes my own research and is divided into five chapters. In the **fifth chapter**, the location of the experiments as well as the purpose and objectives of the research are described. While we previously referred to the aim of a holistic approach to the characterization of a water course, the objectives of this study are the phases through which this result is reached, namely:

- an analysis of the parasite load with species of zoonotic protozoa
- an analysis of the water physical-chemical indicators
- an analysis of microbiological parameters and the tracking of algal biomass
- an analysis of the macrozoobenthos, which is the synthetic expression of the general quality of running waters.

Each of these objectives constitutes a separate chapter of the own research part of the thesis, and the research results have been published in 3 scientific works in specialized journals. The thesis ends with a chapter on final conclusions, bibliography and appendices.

Chapter six is dedicated to the study of methods for the quantitative and qualitative detection of *Cryptosporidium spp.* oocysts and *Giardia spp.* cysts in surface waters, as well as their identification and molecular characterization. Among the 10 sampling stations, for *Giardia* cysts the highest load was recorded in Călimănel jos (Panaci), with a total of 33 cysts identified throughout 2017 - 2018, with an average of 2.8 cysts / month, followed by the Secu station (bottom) with a total of 23 cysts visualized in 2017-2018 and an average of 1.9 cysts / month. At the opposite end, the lowest load was recorded in Călimănel sus (stream), where the total number of cysts visualized was 3, with an average of 0.25 cysts / month, followed by the Bancu sus station with a total of 5 identified cysts and an average of 0.4 cysts

/ month. For *Cryptosporidium* oocysts, among the 10 sampling stations, the highest load was recorded in Bancu sus, with a total of 4 oocysts identified throughout 2017 - 2018, with an average of 0.33 oocysts / month, followed by the Secu stations with a total of 3 oocysts visualized in 2017-2018 and an average of 0.25 cysts / month. At the opposite end, is Călimănel sus (pârâru), where was not identified any oocyst.

The outcomes revealed the fact that the emissaries under study have a low parasite load and that upstream of the human settlements the water has a high degree of purity.

The higher load of *Giardia* spp and *Cryptosporidium* spp. corresponded to an existing animal husbandry activity in the area. The lower parasite load upstream compared to downstream proves the influence of human communities, and the exclusive PCR identification of the zoonotic species *Cryptosporidium parvum* may be an indicator of the effect that rural cattle farms as well as hunting stock have on mountain streams.

The study carried out for the characterization based on the physical-chemical parameters of the emissaries in the Dornelor basin is described in **chapter seven**, each in a separate sub-chapter: temperature, pH, organic substances, conductivity, dissolved oxygen, alkalinity, nitrates, nitrites, the content of cations, concluding the following aspects:

- water temperature varied depending on the season and the place of sampling
- the pH values are slightly alkaline, above 7.00, with only one exception (Călimănel jos, September 2017) which we consider a potential accidental contamination. The variations of this parameter are correlated with the geological substrate, but also with different economic activities. Thus, the lowest pH values are recorded by the stations on the Călimănel stream, where human activities are more intense.

- organic substances are an indicator closely related to the microbiological load and it is directly influenced by heavy summer rains, which wash organic matter off the slopes. moreover, a more intense downstream economic activity causes an increase in the values for this parameter. The highest values were obtained in samples taken from both stations on the Arinu stream after a heavy storm.

- dissolved oxygen is directly influenced by the speed of water flow and the activity of microorganisms in the water; nevertheless, all the monitored sources, being quick mountain water streams, have increased values for this parameter, and the determination was made within a maximum of one hour from sampling. The highest values were obtained from samples taken from the Călimănel jos station, where the water flow speed is very high, even if the influence of the human factor is significant.

- conductivity, bicarbonate and hardness are parameters closely related to the geomorphological structure specific to the source of each stream, being indicators of the total mineralization of the water, with the lowest values in the Călimănel stream - which has the lowest mineralization rate - and the highest values in the Secu stream, taking into account that mineralization is also supported by the highest calcium content.

- the content of anions (fluorides, bromides, chlorides, sulphates, phosphates, nitrates, nitrites) is very low, the values obtained falling within the norms imposed for natural mineral waters.

For nitrites, the maximum allowed value is 0.1 mg / L, and the maximum value read on the ion chromatograph was 0.08 mg / L, in July 2017 in the sample taken from the Călimănel jos station (in Panaci). For nitrates, the maximum allowed value is 50 mg / L, and the maximum value read on the ion chromatograph was 2.98 mg / L, in May 2017 in the sample taken from the Secu sus station.

- the content of cations (ammonium, lithium, sodium, potassium, magnesium, calcium) is within normal limits.

Chapter eight, entitled “Monitoring of certain microbiological parameters of water and algal biomass” is divided into subchapters regarding the determination of NTG, coliforms, enterococci, *Pseudomonas*, *Clostridium*, the algal biomass for green and blue algae, diatoms, cryptophytes and

cyanophytes. Following an analysis of the results for all the studied parameters, the specific conclusions are the following:

- all microbiological indicators showed significant variations between the sampling stations on the course of the same stream, thus upstream values are much lower than downstream values. For all parameters, the least contaminated samples were those upstream of the Călimănel stream.
- The *Pseudomonas aeruginosa* indicator appears in two of the stations, Călimănel sus (stream) and Secu sus, and spores of anaerobic sulphite-reducing bacteria (*Clostridium*) were absent in all the samples taken during the study in the Călimănel sus (stream) station.

The samples with the highest contamination were those taken from the station Arinu jos and those from Călimănel jos, the anthropic influence being obvious due to the economic activities carried out.

- every type of algae analysed had similar values, higher values for all parameters being recorded in the samples taken from the lower Arini station.

- the maximum values were recorded in the samples taken from the stations on the Arinu stream in August 2018, after a heavy rain, when large amounts of organic substances were washed off.

In the **ninth chapter** includes a study on the benthic macroinvertebrate fauna.

The quality of the tributaries of the Dornelor basin is also rendered by the presence of some macroinvertebrates belonging to various taxonomic groups. Thus, we tried to make this qualitative biological assessment, without resorting to establishing the NGBI (normalized global biological index), a method that requires a much more complex analysis of the taxa.

The optimal sampling conditions were observed in the summer and autumn periods, due to maximum pollution, high temperatures and relatively small disturbances in the water. In choosing the sampling periods, it is taken into account that certain taxa may be in the aerial phase, that is why we chose at least 2 sampling periods, in two stations comparable in terms of size, water flow speed, nature of the substrate. Sampling was done using the Surber type sampler on the faster watercourses or the limnological net for the slower watercourses.

Analysing the taxa identified during the periods when benthic macroinvertebrates were collected, we found increased abundance upstream compared to downstream, in all stations, with a clear detachment of taxa from the Arinu stream, with a remarkable number of taxa, most of which are Trichoptera (64) and Plecoptera (40), belonging to 8 faunal groups. Even in the Arinu-jos station (downstream), the 74 identified taxa, most of them Plecoptera, show us the ecological relevance of these biotopes. The following stream most abundant in taxa is Bancu-sus (upstream) with 45 taxa and respectively 96 taxa in the downstream station, with a predominance of Trichoptera (65), followed by Plecoptera (22) and Ephemeroptera (7). On the Călimănel stream, we report an abundance of taxa from the *Baetidae* group (35 upstream-23 downstream). The Călimănel stream in the upstream area is rich in taxa, with representatives of Ephemeroptera, (*Heptageniidae* 2, *Baetidae* 35), Plecoptera (*Perlidae* 1, *Perlodidae* 10, *Capniidae* 7), Trichoptera (*Rhyacophilidae* 1, Trichoptera with small house 3), Diptera (*Simuliidae* 1), Amphipods (*Gammarus* 3). Downstream, both taxa and families are missing from the collected zoobenthos, with a clear tendency to decrease the number of species sensitive to anthropogenic pressure (Plecopters are reduced, but also Trichoptera, there are no more amphipods - *Gammarus*). The most numerous group of taxa of the Secu stream was represented by *Gammaridae*.

Chapter ten summarizes in 4 pages the partial conclusions drawn for each study in my own research.

The thesis is completed with a bibliography and three appendices: list of figures, list of tables, list of published scientific works.