Project Update: February 2019

Mountain biodiversity faces a number of serious and growing challenges. Habitat degradation caused by unsustainable clearing of land results in erosion of fertile soil and increases the threat of avalanches, landslides and flooding. With this change in habitat, rare species of plants and animals can face extinction.

There are many challenges to overcome in order to successfully and sustainably use mountain biodiversity. The growing demand for water, the consequences of global climate change, the growth in tourism and the pressures of industry and agriculture in a world of increased globalization are just some of these challenges.

Mountain species and habitats are subject to many stresses and vulnerabilities due to anthropogenic factors, including land-use practices and changes, freshwater abstraction, tourism and recreation, infrastructure development, the introduction and expansion of invasive species, and also air and water pollution.

Species endemism, in particular, often increases with altitude within mountain regions, partly due to the isolation of populations and speciation processes over geological timescales. Mountain ecosystems are fragile and vulnerable to changes due to their particular and extreme climatic and biogeographic conditions[1'2].

Thanks to the grant, which was awarded by the Rufford Foundation, we started the implementation of the project entitled as: "*Conservation of Freshwater Oligotrophic Habitats on Vranica Mountain and Establishment of Long-Term Monitoring of Biodiversity*" which is primarily focused on different freshwater oligotrophic habitat types on this mountain and on rich algal biodiversity, especially diatoms.

During the months of October, November, December and January, various project activities were realized, followed by the dynamic plan of our project.

In the continuation of the current Fourth Project Update, all realized activities will be described in details, but also those that are still in the process of realization.

In addition to the above activities, the preliminary results of the previous research (Appendix I) and future planned activities will be presented.

It is important to note that all project activities were realized according to the dynamic plan and until now we have not encountered any problems that would prevent the implementation of the proposed project.

Further in the Fourth Project Update, description of various project activities and those which are still in progress are presented.

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[1]https://biodiversity.europa.eu/topics/ecosystems-and-habitats/mountains

[2]https://www.eea.europa.eu/data-and-maps/figures/mountain-massifs

OVERVIEW OF REALIZED ACTIVITY

Through our Fourth project update, 20 realized activities were presented as follows:

Activity 1. Literature analysis
Activity 2. Detailed fieldwork
Activity 3. Education and work with students on the field
Activity 4. Assessment of students' knowledge
Activity 5. Preparation of official logo for our Rufford project
Activity 6. Preparation of presentation and tasks for the workshop
Activity 7. Workshop at the Faculty of Science, University of Sarajevo (3 days)
Activity 8. Promotion of our project through social media
Activity 9. Chemical analysis of water samples in the laboratory
Activity 10. Analysis of collected samples of phytobenthos
Activity 11. Preparation of algal species list (without diatoms)
Activity 12. Chemical processing of collected samples of algae of phytobenthos
Activity 13. Processing of permanent slides and determination of diatoms
Activity 14. Establishment of a database of abiotic parameters
Activity 15. Establishment of a database of biotic parameters (with diatoms)
Activity 16. Establishment of diatom collections
Activity 17. Establishment of a complex matrix with abiotic and biotic data
Activity 18. Preparation of Android App for entering data on the field
Activity 19. Preparation of original scientific papers
Activity 20. Registration on different international conferences

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Activity 1. *Literature analysis*
In order to understand the structure and dynamics of selected habitat types, a large number of original scientific papers have been collected and analysed. These papers will serve as a basis for the establishment of a large database of reference, which will be expanded and analysed in the next period of realization of our project. Scientific papers were downloaded from the official Journal pages, through the ResearchGate platform, and through Google search engine. Our base of thematic scientific papers, has more than 500 references so far. The above mentioned scientific papers will be used for preparation of promotive materials, future congress and original scientific papers. Mendeley reference software has been selected to manage the collected original scientific papers (http://www.mendeley.com/download-desktop).

Activity 2. *Detailed fieldwork*
During the month of October, the last two days of planned fieldwork in 2018 were realized (20.10.2018/27.10.2018). In addition to sampling phytobenthos, sampling of water for chemical analysis was performed. As with the previous fieldwork, special attention in this project is focused on the following habitat types: a) mountain springs, b) mountain creeks, c) mountain rivers, d) mountain lake and e) mountain peatlands. In order to obtain comparable results, for each studied habitat types, a

robust field protocol was used, which was previously prepared[3] and introduced in our 1st project update. Field protocols are stored in the Dropbox database and it can be accessed by scanning a QR code that is unique for each location, but also for each sample. During the first part of the field work realized in 2018, 84 field protocols were filled and 168 samples of phytobenthos were collected. For each studied site, two phytobenthos samples were collected. One sample is for diatoms and the second sample is for other algal groups. Samples of phytobenthos were fixed with formalin 4% and stored until laboratory analysis. Each collected sample contains a unique QR code which is related to same field protocol. By scanning unique QR code with QR scanner (Android application) it is possible to access the database and reach general information about habitat characteristics from which the sample was collected. On each investigated site sampling of aquatic macrophytes (bryophyte and vascular plants) was carried out. During the fieldwork, plant materials were collected in plastic bags and in laboratory conditions they were dried. Collected samples of phytobenthos and aquatic macrophytes were stored in the Laboratory for Systematics of Algae and Fungi at the Faculty of Science, University of Sarajevo.

Activity 3. *Education and work with students on the field*
During the month of October, as a part of our planned field research as a project coordinator, I had the opportunity to introduce our project to the high school students on Vranica Mountain near Prokosko lake. I talked with the students about the freshwater oligotrophic habitat types and about their conservation potential and also about the current ecological state. The current threats to the freshwater oligotrophic habitat types are also highlighted, as well as the possible measures that should to be taken in the future in order to prevent further degradation. Finally, students were also acquainted with algae as indicator organisms that will be used as a tool for assessing the ecological status of present freshwater oligotrophic habitat types on Vranica Mountain.

Activity 4. *Assessment of students' knowledge*
In order to assess the level of knowledge about freshwater oligotrophic habitat types, we conducted an online questionnaire. This way of gathering information is a good indicator for determining the future direction dealing with restoration, conservation and establishment of long-term monitoring. The questionnaire contained 16 questions which are in the form of multiple-choice, choice of the correct assertions and the form of expressing one's own opinion. More information about the results of the questionnaire will be presented in our Last project update. The above questionnaire can be accessed via the following link:
https://forms.office.com/Pages/ResponsePage.aspx?id=DQSIkWdsW0yxEjajBLZtrQAAAAAAAAAAAAYAALVGsZtUNVJJUzk0SlIPSjVQNjRMV09PUVMxTjk3VC4u.

[3] https://www.rufford.org/files/24578-1%20June%202018.pdf

Activity 5. *Preparation of official logo for our Rufford project*
In order to promote the importance of freshwater oligotrophic habitat types, an official logo for our project has been designed. The logo will be displayed on all our promotional materials. Basically, the logo presents a view of the Prokosko Lake as one of the studied habitat types. Some characteristic mountain landscapes are also visible on the logo. Characteristic diatom species which inhabit freshwater oligotrophic habitat types and indicate their state are present on the logo as follows: *Diatoma ehrenbergii* (Mountain springs), *Hannea arcus* (Mountain creeks and stream) and *Eunotia tetraodon* (Mountain peatlands). A complete picture is surrounded by *Sphagnum* sp., bryophyte which is the main constituent of very rare and sensitive mountain peatlands.

Activity 6. *Preparation of presentation and tasks for the workshop*
The motivation for realization and the preliminary results of our project were presented during the period of three days of the workshop. Students from the Faculty of Science were introduced into the ecology of freshwater oligotrophic habitat types in general with special regard to the Vranica Mountain. During the presentation, the main activity and outcomes of our project were presented. We also presented the official logo and a draft version of promotive materials. After the presentation we held a discussion with the students and realized some tasks related to our project.

Activity 7. *Workshop at the Faculty of Science, University of Sarajevo* During the period of three days a workshop was held at the Faculty of Science, University of Sarajevo. Students from the first and fourth year of study (Biology department) were participants. During the workshop, students were introduced to the basic goals and outcomes of our project with special emphasis on freshwater oligotrophic habitat types which are distributed on the Vranica Mountain. During the workshop a conservation potential of this habitat types was highlighted. The main reason for that potential comes from the fact that they represent a *hot spot* for biodiversity. These habitat types are under high anthropogenic influences. This workshop is important from the aspect of raising of the ecological awareness, and also for dissemination of knowledge. The students who were involved in this activity have shown a great interest in the workshop, both by their presence and by active participation through the setting of certain questions and the realization of prepared tasks. One group of students (First year of biology) observed characteristic representatives of cyanobacteria and algae through a microscope. They analyzed samples from the Prokosko Lake and from mountain peatlands distributed near the mountain creeks. The second group of students (Fourth year of biology/ecology) addressed some issues which are related to the conservation and restoration ecology.

Activity 8. *Promotion of our project through social media*
In order to raise ecological awareness about the importance of freshwater oligotrophic habitat types, during current phases, continuous promotion of our project was carried out. For the promotion of results and current activities, we relied on social media. All photo albums and other information about current project activities are available at the following links:

Link 1. https://sway.office.com/DyFZNyCLbFUD0F3w?ref=Link
Link 2. https://sway.office.com/imFxPDaGFpyixR5V?ref=Link
Link 3. https://sway.office.com/dOdIJ3phBkw6gdit?ref=Link
Link 4. https://sway.office.com/Or9lb9lvSUQU2xOV?ref=Link
Link 5. https://sway.office.com/iz2pCCFLaxmrqmKg?ref=Link
Link 6. https://sway.office.com/CTRE0FC3rFnTQMlP?ref=Link
Link 7. https://sway.office.com/TElTYS14Y4FK2zlh?ref=Link
Link 8. https://sway.office.com/aN9noO8Y1ioLOAWF?ref=Link
Link 9. https://sway.office.com/H9wMSxepZ3piAMzL?ref=Link
Link 10. https://sway.office.com/sY3BmH3OspgBbIQB?ref=Link

Activity 9. *Chemical analysis of water samples in the laboratory*
Water samples collected on the field were analyzed at Department of Chemistry at Faculty of Science, University of Sarajevo. All samples were collected in plastic bottles. The plastic bottles have been soaked in 10% (v/v) HNO_3 and then washed with double distillate water. The bottles have been rinsed out three times with the sample water prior to taking the sample. The analysis of water samples has been performed in the following 24 hours after the sampling. All reagents used for the preparation of calibration standard solutions were of analytical grade. Single standard stock solutions (CertPur, Merck, Darmstadt, Germany) containing 1000 mg/L of K, Na, Ca, Mg, Cr, Cu, Mn, Fe, Ni, Cd, Pb and Zn were used. Caesium chloride and potassium nitrate (Merck, Darmstadt, Germany) were used as ionization suppressor. The measurements were performed on an atomic absorption spectrometer model AA240FS, Varian, Australia. The concentration of Na and K in water samples was determined by flame atomic emission spectrometry (FAES). The concentration of Ca, Mg, Cr, Cu. Mn, Fe, Ni, Cd, Pb and Zn in water samples was determined by flame atomic absorption spectrometry (FAAS).

Activity 10. *Analysis of collected samples of phytobenthos*
The algae analysis included several phases as follows: determination of algae which are sensitive to the chemical processing, chemical processing of samples, preparation of permanent slides, determination of diatoms and preparation of the synthetic table. Determination of cyanobacteria and algae was performed using the following keys and the guides for determination: Cantonati et al. 2017., John, 2015., Hofman et al. 2013., Bey & Ector, 2013a,b,c,d,e,f., John et al. 2003., Krammer, 1997a,b., 2000., 2001., 2003., Lange-Bertalot, 1993., 2001., Reichardt, 1999., Lange-Bertalot & Metzeltin, 1996., Cvijan & Blaženčić, 1996., Krammer & Lange-Bertalot, 1981-1991., and Hustedt et al. 1930., and relevant monograph papers: Pavlov et al. 2016., Lange-Bertalot & Wojtal, 2014., Bucko et al. 2013., Pavlov & Levkov, 2013., Pavlov et al. 2013., Levkov & Williams, 2012., Cantonati et al., 2010., Levkov & Ector, 2010., Levkov et al., 2010., Wojtal et al. 2011., Wojtal, 2009., Wojtal & Kwandrans, 2006., Schmidt et al., 2004., Witkowski et al. 2004., Wojtal, 2003. The nomenclature of cyanobacteria and algae is harmonized according to the following Internet base: Guiry, M.D. & Guiry, G.M. 2019 (AlgaeBase). In order to further verify the name of the taxon they were used in the addition to the following database: Jahn, R. & Kusber, W.-H., 2019 (AlgaTerra Information System)., Spaulding, S.A., Lubinski, D.J. and Potapova, M. 2019 (Diatoms of the United States) and Jüttner, I., Bennion, H., Carter, C., Cox, E.J.,

Ector, L., Flower, R., Jones, V., Kelly, M.G., Mann, D.G., Sayer, C., Turner, J.A., Williams, D.M., 2019 (Freshwater Diatoms Flora of Britain and Ireland).

Activity 11. *Preparation of algal species list (without diatoms)*
In the collected samples of phytobenthos, different representatives of algae were determined. Quantitative and qualitative analysis of collected samples of phytobenthos was performed using a binocular magnifier (BS-3300 Zoom Stereo Microscope). At the same time, large inorganic particles, such as silt, sand, clay and detritus, have been removed. The rest of the material was analysed using a light microscope (BestScope BS2020B).

Activity 12. *Chemical processing samples of algae of phytobenthos*
In order to determine diatom species, it was necessary to chemically process collected samples and after that prepare permanent slides. Relative abundance was estimated after enumeration 300 to 400 diatom frustules. Selected representatives of cyanobacteria and algae were photographed using MD-130 Best Scope digital camera. Further in this report, the method of chemical processing of sample will be described as follows: In laboratory beaker, 50 ml of sample was prepared. After that, the beaker with a sample was filled up with water and the contents were well-mixed. After 30 minutes, precipitate accumulated on the top of the beaker was carefully poured off, and then the suspension with the algae was deposited for 1 to 2 hours. After precipitation, about 2/3 of the mixed sample were poured off and the same amount of 96% concentrated sulfuric acid was added to the residue [5.2 EN_13946:2003 – Cold concentrated sulfuric acid (H_2SO_4), potassium permanganate ($KMnO_4$) and oxalic acid (COOH) 2xH_2O)]. When handling with sulfuric acid it is very important to be very careful. Chemical process of samples takes place in the digester, and the sulfuric acid is poured into the sample over a glass rod or at the edge of the glass, and never directly on the sample. After adding potassium permanganate and oxalic acid prepared aliqout with diatoms the settling lasts for about seven days or to the moment when the sample reaches pH 7. After obtaining a white suspension, 96% ethanol was added to conserve material, which will be used to prepare permanent slides, but also as a permanent reference.

Activity 13. *Processing of permanent slides and determination of diatoms* Permanent slides of diatoms were prepared as follows: on a pure glass slide, two drops of diluted suspension are placed and heated. When the suspension is dry, one or two drops of Canada balsam are added on slides and covered with a cover glass. After that, the suspension is heated up until first bubbles appear. Permanent slides are then dried in a dryer for 24 h at 50 °C. After drying, permanent slide are completely prepared for analysis.

Activity 14. *Establishment of a database of abiotic parameters*
In order to establish long-term biomonitoring of freshwater oligotrophic habitat types in the wider area of the Vranica Mountain, a database of abiotic parameters was established. The database of abiotic parameters was established in excel table and divided into seven parts as follows: sample header, related sample activities, physical site conditions, water measurements, sampling information (QTHP & QMH) and data analysis. In the header of each table there is an analysed parameter, and within the

first column, there is an ID number of each individual sample. Sample header includes the following components: Station ID, Sample Code, Date, Latitude, Longitude, Altitude and Habitat type. Related sample activities include the following components: Station ID, Sample Code, Water chemistry, Habitat survey, Fish survey, Invertebrate survey, Environmental DNA, Bed sediment and Habitat type. Realized activity is marked with "X", while the activity which is not realized is marked with "0". Physical site conditions include the following components: Station ID, Sample Code, Air temperature, Air humidity, Light intensity, Clouds, Wind, Precipitation, Precipitation intensity, Shading, Water color and Habitat type. Water measurement includes the following components: Station ID, Sample Code, Time, Water temperature, pH, Dissolved oxygen, Specific conductance, Tubidity, TDS and Habitat type. Sampling information (QTHP) includes the following components: Station ID, Sample Code, Primary sample, Sample component, PHS and Habitat type, while Sampling information (QMH) includes a slightly higher number of parameters than the previous table. Sampling information (QMH) includes the following components: Station ID, Sample Code, Primary sample, Repeated sample, Sample component, type of substrate (epilithic, epiphytic, epipelic, epidendric, epipsamic), Dominant substrate, Periphyton abundance, Recognizable algal taxa, Recognizable aquatic macrophytes, Anthropogenic disturbance and Habitat types. A complete database with abiotic parameters is in the appendix of this report (APPENDIX I).

Activity 15. *Establishment of a database of biotic parameters*
In addition to the prepared database of abiotic parameters which contains the basic characteristics of habitats from which sampling was carried out, a long database for diatoms and other algae groups was established. This database will be updated with the species and their ecological guilds in the next period. The database was prepared in the form of an excel table, but for the easier and simpler inventory and possible mapping of cyanobacteria and algae in the wider area of Vranica Mountain, BIOTA 3 Biodiversity[4] Database Manager was used. More information on the structure and data management with BIOTA 3 Software, will be presented in our 5th project Update.

Activity 16. *Establishment of diatom collections*
Research on diatoms in the wider area of Vranica Mountain has not been carried out so far. First data about the biodiversity of cyanobacteria and algae in some specific habitat types are described by Protić (1926), Kapetanović & Hafner (2007) and Barudanović et al. (2017). It is very important to highlight that this comprehensive study is first in regards to inventarization of cyanobacteria and algae in the wider area of Vranica Mountain. All collected samples are stored in the Laboratory for Systematics of Algae and Fungi on the Faculty of Science, University of Sarajevo, and also aliquot with diatoms and permanent slides. It is very important to note that from each sample (aliquot), we prepared five permanent slides which are associated with unique field protocol. All protocols and permanent slides are organized in collection, which will be used as a reference and for generating future projects and publications.

[4] http://viceroy.eeb.uconn.edu/biota/

Activity 17. *Establishment of a complex matrix with abiotic and biotic data* In order to carry out statistical analysis, a complex matrix with abiotic and biotic data which were collected during laboratory and fieldwork will be prepared. Based on the outcomes, certain original scientific and conference papers will be prepared. More information about data matrix with abiotic and biotic parameters will be presented in our 5th Project update.

Activity 18. *Preparation of Android App for entering data on the field* entering a data into this long-database of abiotic factors in the future will be possible through our newly prepared Android application. Access to the application and entering data will be possible through a tablet or smartphone. More information about the process of entering data in the form via the Android application and management of the database will be available in our 5th project update. Measurements of basic parameters of water in the field were carried out using equipment purchased through the Rufford Foundation grant and additional equipment owned by the Laboratory for Systematics of Algae and Fungi at the Department of Biology. The following parameters of water were measured: water temperature, pH, dissolved oxygen, specific conductance, turbidity and TDS.

Activity 19. *Preparation of original scientific papers*
After synthesis of all obtained results, original scientific papers will be prepared. In our papers, we will indicate the presence of rare and endangered species of diatoms, and also highlight the state of freshwater oligotrophic habitat types on Vranica Mountain. As we mentioned in our project proposal the main goal of this project is protection and monitoring these very unique and sensitive habitat types. We will also prepare other specific papers and participate in different Conferences which deal with this topics.

Activity 20. *Registration on different international conferences*
In order to present the results of our project, but also to promote and share our knowledge, we planned to participate in several International Conference, which will be organized during 2019, as follows:

- EPC 7 – *7 European Phycological Congress* (Zagreb, 25.08. – 30.08)[5].
- CBS – 6 *Croatian Botanical Symposium* (Zagreb, 30.08. – 31.08)[6].

Plans for the future
We continued also with the promotion of our work to the broad range of public through very popular media as follows: iNaturalist[7] and Youtube[8].

More information about progress dealing with planned activities will be presented in our Additional and 5th project update as follows:

1. Establishment of a database of abiotic and biotic parameters,
2. Establishment of a complex matrix with abiotic and biotic parameters,
3. Statistical data analysis
4. Preparation for the second part of Fieldwork and
5. Preparation for Final Project Update.

Progress about our project is also available on ResearchGate.

On the next pages of our 4th project update, we present our activities through some very interesting pictures.

Official logo for project entitled as:
"Conservation of Freshwater Oligotrophic Habitats on Vranica Mountain and Establishment of Long-Term Monitoring of Biodiversity"

[5] http://epcseven.biol.pmf.hr/
[6] http://www.imp-du.com/sesti-hrvatski-botanicki-simpozij/invitation
[7] https://www.inaturalist.org/projects/conservation-of-freshwater-oligotrophic-habitats-on-vranica-mountain?tab=about
[8] https://www.youtube.com/channel/UCSZBVEUVQG04oETRFVyGieA?view_as=subscriber

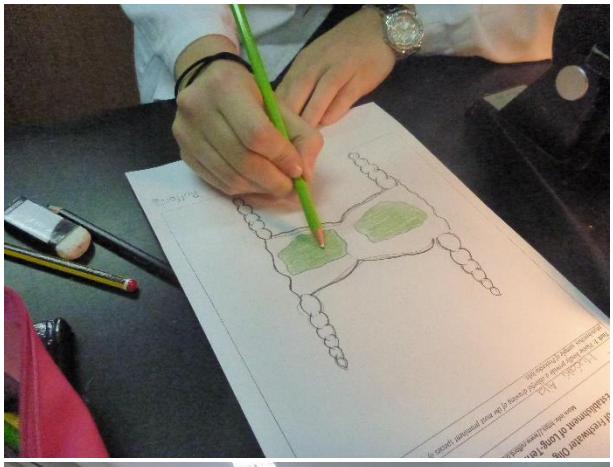




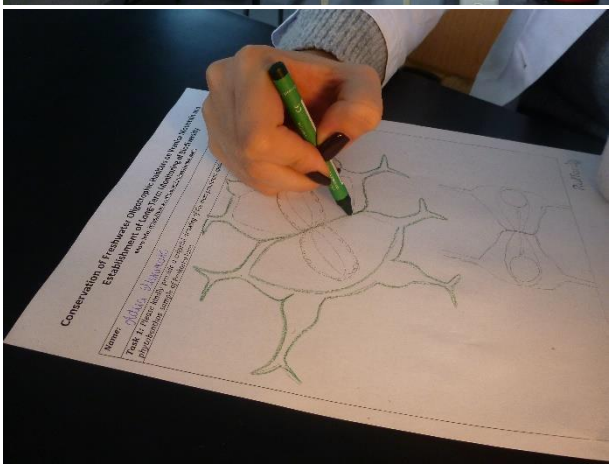

Education and work with students on the field: Day 8 - 20.10.2018.

Water and phytobenthos sampling: Day 9 - 27.10.2018.

Workshop in Faculty of Science: *Day 1 - 17.12.2018. / Day 2 - 18.12.2018.*





Workshop in Faculty of Science: Day 3 - 27.12.2018.

DISTRIBUTION OF FRESHWATER OLIGOTROPHIC HABITAT TYPES ON VRANICA MOUNTAIN

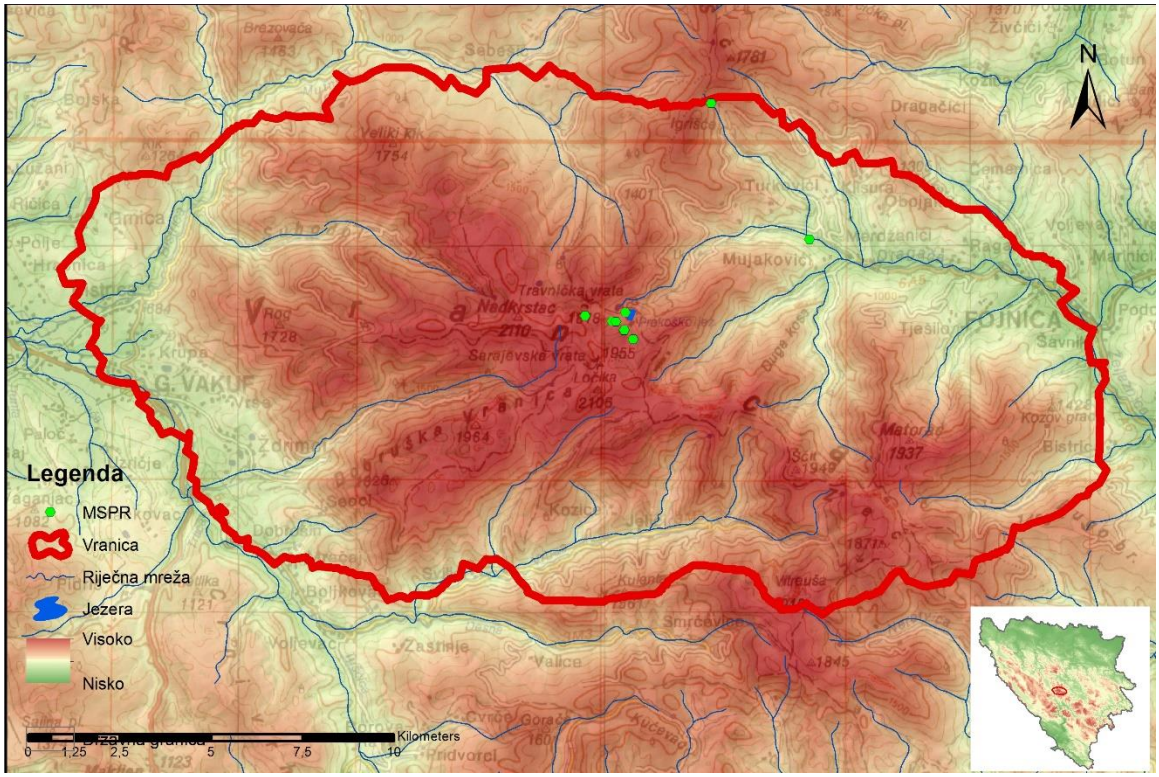


Fig. 1. *Mountain Springs*

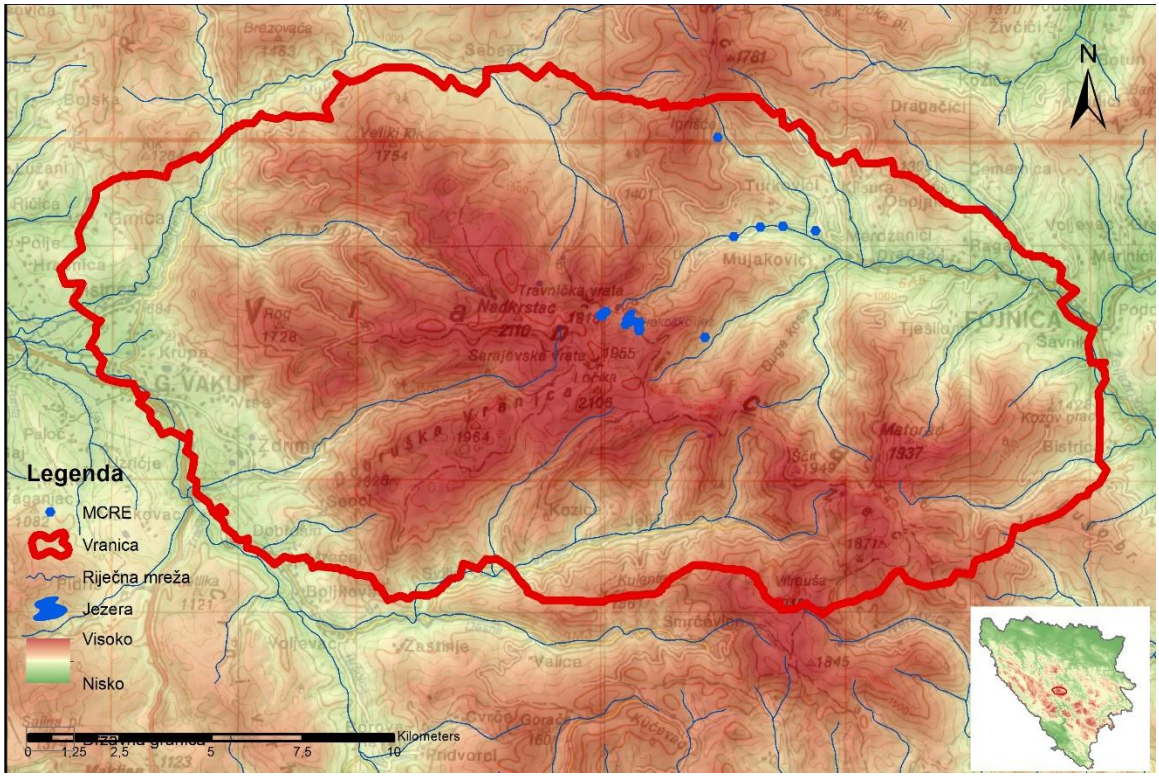


Fig. 2. *Mountain Creeks*

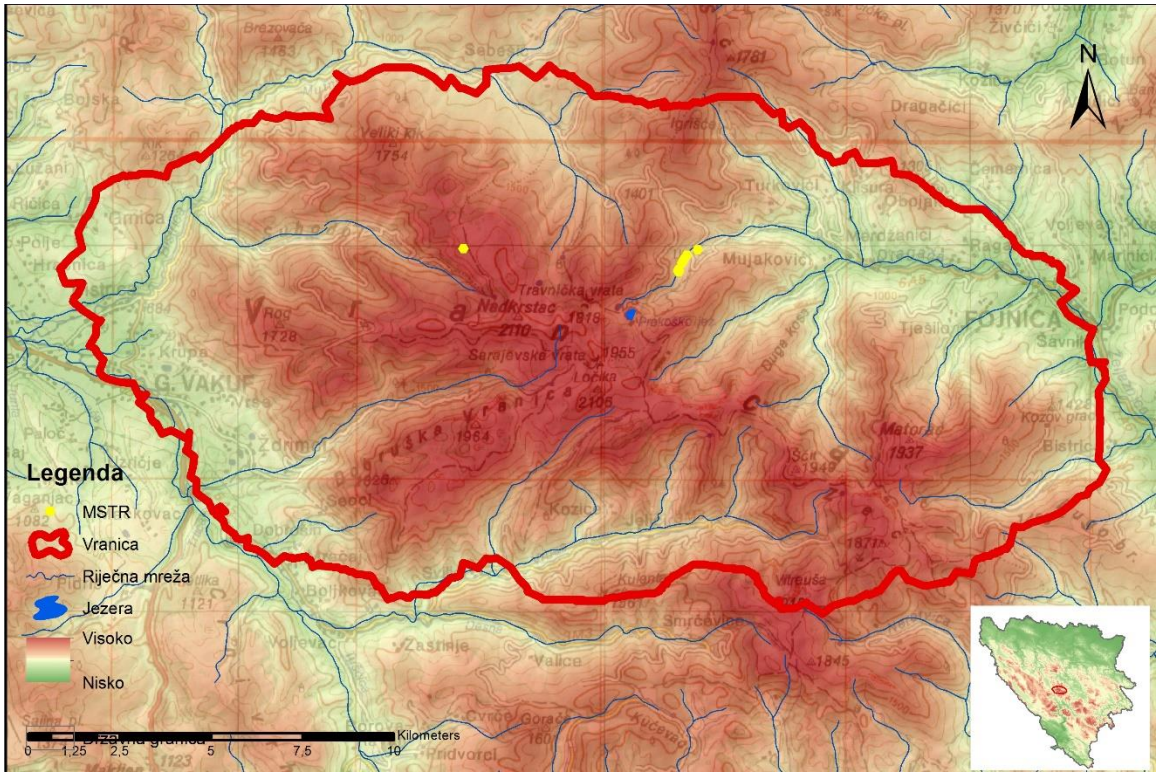


Fig. 3. *Mountain Stream*

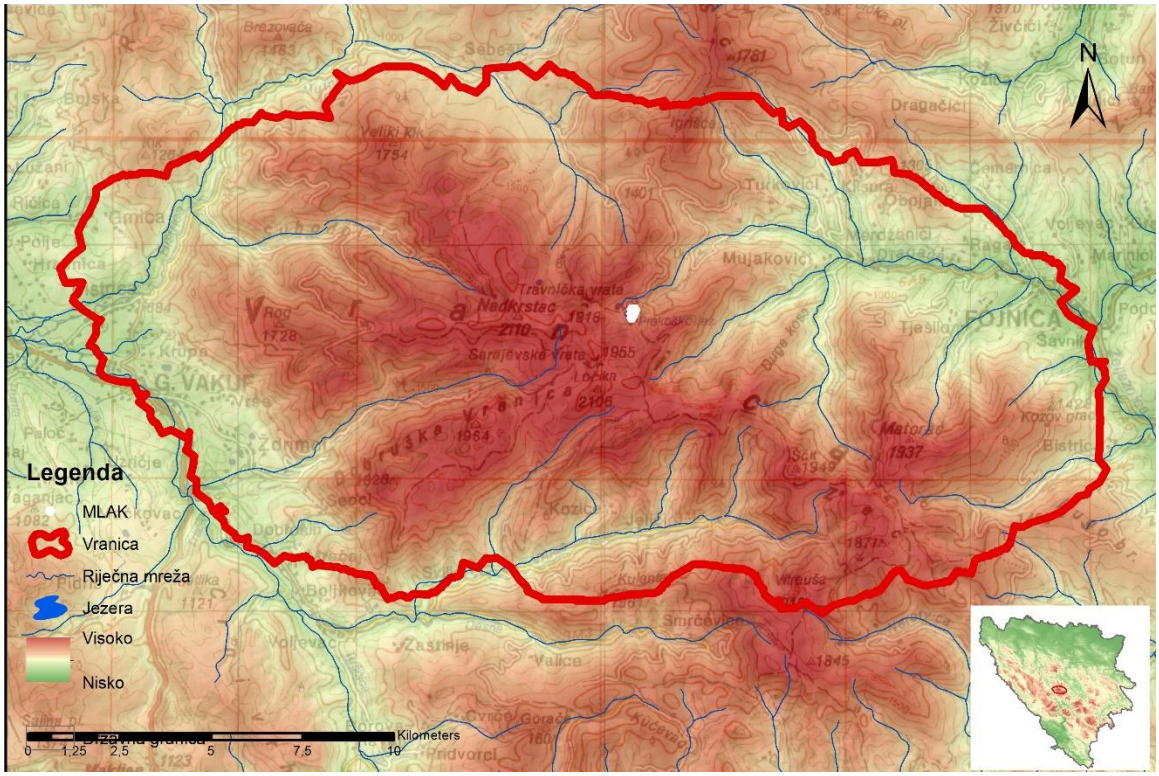


Fig. 4. *Mountain Lake*

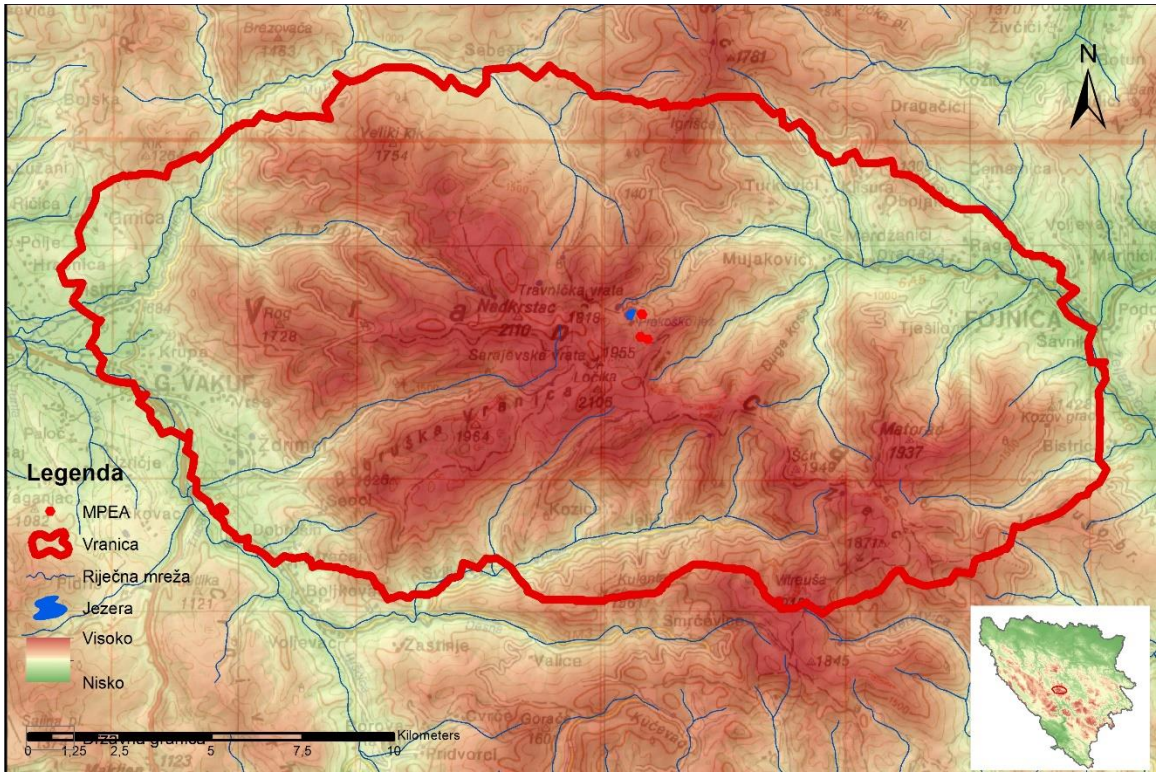


Fig. 5. *Mountain Peatlands*

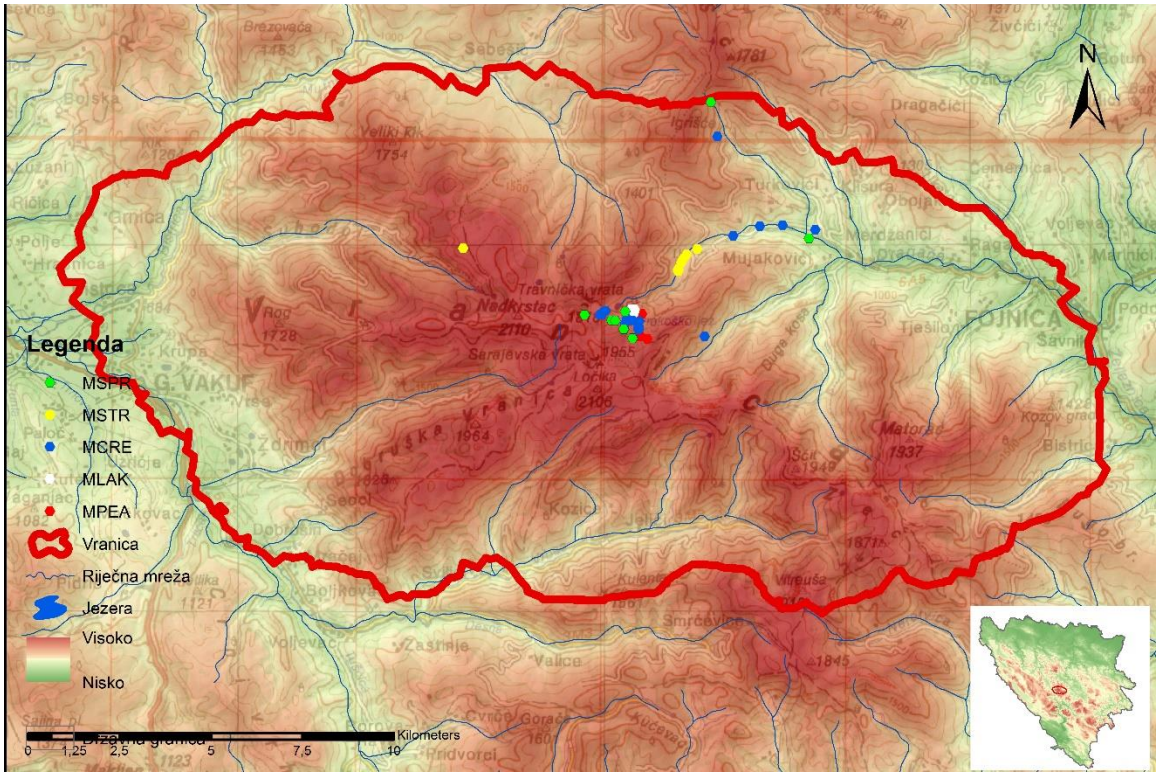


Fig. 6. *Distribution of freshwater oligotrophic habitat types on Vranica Mountain*