Project Update: March 2022

INTRODUCTION

Our nature and the living world contained in it are under threat! Unfortunately, in recent days we have witnessed the terrible destruction of nature in one of the most brutal ways, but also one of the largest migrations of the population in Europe. Although we as a civilisation have not recovered from the Covid 19 virus, these global pandemics of unfortunate war events have become relevant in Europe. Unfortunately, in war-torn Ukraine, in addition to the population, nature has also suffered, which is very unique and unrepeatable in this area. Vast plains with a large number of rare and endemic plant species have been destroyed, along with aquatic ecosystems and well preserved floodplain forests. The consequences of this degradation will be felt only in the future due to the loss of ecosystem services provided by these ecosystems. The aim of our project is, among other things, to provide information on the importance of freshwater oligotrophic habitat types and highlight the ecosystem services they provide. The protection of these habitats protects many rare and endemic species, but also permanently preserves nature for future generations.

Thanks to the grant, which was awarded by The Rufford Foundation, we started the implementation of the second project entitled: "*Conservation and Restoration of Freshwater Oligotrophic Habitat Types in the Area of the Dinaric Alps (Bosnia and Herzegovina)*".

Through our third project update, 15 realised activities were presented:

Activity 1. Literature analysis.
Activity 2. Assessment of student knowledge.
Activity 3. Preparation of presentation and tasks for the workshop.
Activity 4. Promotion of our project through social media.
Activity 5. Chemical analysis of water samples in the laboratory.
Activity 6. Analysis of collected samples of phytobenthos.
Activity 7. Preparation of algal species list (without diatoms).
Activity 8. Chemical processing samples of algae of phytobenthos.
Activity 9. Processing of permanent slides and determination of diatoms.
Activity 10. Establishment of a database of abiotic parameters.
Activity 11. Establishment of a database of biotic parameters.
Activity 12. Establishment of diatom collections.
Activity 13. Establishment of a complex matrix with abiotic and biotic data.
Activity 14. Preparation of original scientific papers.
Activity 15. Registration on different international conferences.

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. *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=DQSIkWdsW0yxEjajBLZtrQAAAAAAAAAAAAAYAALVGsZtUNVJJUzk0SllPSjVQNjRMV09PUVMxTjk3VC4u.

Activity 3. *Preparation of presentation and tasks for the workshop*
The motivation for realisation and the preliminary results of our project were presented during the period of 3 days of the workshop. Students from the University of Sarajevo were introduced into the plankton morphology and their role in the global ecological cycle. During the presentation, the main activity and outcomes of our project were presented. After the presentation we held a discussion with the students and realised some tasks related to our project.

Activity 4. *Promotion of our project through social media*
In order to raise ecological awareness about the importance of freshwater oligotrophic habitat types and role of phytoplankton in global ecological processes during current phases, continuous promotion of our project was carried out. For the promotion of results and current activities, we relied on social media.

Activity 5. *Chemical analysis of water samples in the laboratory*
Water samples collected on the field were analysed 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 (Cert Pur, 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 ionisation 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 6. *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 harmonised 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 7. *Preparation of algal species list (without diatoms)*
In the collected samples of phytoplancton and phytobenthos, different representatives of algae were determined. Quantitative and qualitative analysis of collected samples of phytoplancton and 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 and BS-2029 Inverted Biological Microscope) which we are purchased thanks to the grant of The Rufford Foundation.

Activity 8. *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 BHC3e-10805 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 sulphuric acid was added to the residue [5.2 EN_13946:2003 – cold concentrated sulphuric acid (H_2SO_4), potassium permanganate ($KMnO_4$) and oxalic acid ($COOH)_2xH_2O$)]. When handling with sulphuric acid it is very important to be very careful. Chemical process of samples takes place in the digester, and the sulphuric 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

7 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 9. *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 10. *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 Dinaric Alps, 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. Realised activity is marked with "X", while the activity which is not realised 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, recognisable algal taxa, recognisable aquatic macrophytes, anthropogenic disturbance and habitat types.

Activity 11. *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 Dinaric Alps, BIOTA 3 Biodiversity[1] Database Manager was used. More information on the structure and data management with BIOTA 3 Software, will be presented in our fourth project update.

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

Activity 12. *Establishment of diatom collections*
Research on diatoms in the wider area of Vranica Mountain (Dinaric Alps) 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 inventorisation of cyanobacteria and algae in the wider area of Vranica Mountain (Dinaric Alps). 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 organised in collection, which will be used as a reference and for generating future projects and publications.

Activity 13. *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 fourth project update.

Activity 14. *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 and glacial lake located on Dinaric Alps. 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 15. *Registration on different international conferences*
In order to present the results of our project, but also to promote and share our knowledge, we plannned to participate in several international conferences, which will be organised during 2022, as follows:

1. 14th Symposium on the Flora of Southeastern Serbia and Neighboring Regions, Kladovo, June 2022 (http://www.sfses.com/).
2. 14th European Diatom Meeting which will take place from 9-11 May 2022 in the town of Ohrid (https://edm2022.mk/).
3. Different Rufford Conference (https://www.rufford.org/conferences/).

Activity 9. Other additional activities
Various planned activities were realised within the project, however, thanks to good promotion and interest of students, various additional activities were realised, which include the following:

1. Preparation of promotive material of our project (fourth project update).

2. A documentary about the algae of Bosnia and Herzegovina was made and published. The complete content can be viewed at the following link: https://youtu.be/6cD-RU06Xhg .
3. Preparation of collection of permanent slides (cataloguing and digitisation).
4. Preparation of collection of cyanobacteria and algae (cataloguing and digitisation).
5. Preparation of a publication on silicate algae of Vranica mountain.
6. Preparation of a publication on Charophyceae algae of Bosnia and Herzegovina.
7. Completed and defended a master's thesis on the diversity of algae and ecological characteristics of the Kukavičko lake.
8. Preparation of diploma theses on the diversity of cyanobacteria and algae in the springs and streams of the Vranica mountain.
9. Establishment of a database on epiphytic and epizoic algae of Bosnia and Herzegovina.
10. Training of young researchers in the field of conservation and restoration ecology.
11. Preparations for a 3-day workshop called Phytoplankton - a role in global environmental processes.
12. Continuous promotion of the project through the social networks of IG, FB and YouTube Channels.

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[2] and Youtube[3].

More information about progress dealing with planned activities will be presented in our additional and fifth 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 fourth part of fieldwork.
5. Preparation for final project update.

Progress about our project is also available on ResearchGate.

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

[2] https://www.inaturalist.org/projects/conservation-of-freshwater-oligotrophic-habitats-on-vranica-mountain?tab=about

[3] https://www.youtube.com/channel/UCSZBVEUVQG04oETRFVyGieA?view_as=subscriber


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Avantura
doc. dr. Ermin MAŠIĆ
Odsjek za biologiju, PMF UNSA
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