

# GRANT WRITING IN Environmental studies

Institute of Environmental Sciences Jagiellonian University

COMPENDIUM OF STUDENTS' PROJECTS 2021/2022

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#### About the course

The aim of the course is to give students practical experience with writing project in the field of environmental sciences. The course consists of 10 meetings, each lasting 3 hours. During the meetings, two teachers give conservatory lectures and guide participant in the process of writing project. Students come up with their own ideas, form groups and write proposals on the topic of their own interests. The proposal can either be hypothesis-driven research project or project aiming at nature conservation. The projects are written according to the British Ecological Society grant scheme (https://www.britishecologicalsociety.org/funding/research-grants/), with some modifications. Proposals are evaluated by external reviewers and by the fellow students. They are corrected and defended in front of the audience. Proposal included in the current compendium are the final versions of projects (excluding CVs of the applicants).

The final score is based on timeliness and formal and substantive correctness of the assignments. Scale of final grade (5 to 2 scale and corresponding A - F scale):

- 5.0 (A): All written assignments completed according to the guidelines and deadlines, active participation in the discussions.
- 4.5 (B): All written assignments completed according to the guidelines and deadlines.
- 4.0 (C): Written assignments completed according to the deadlines, with some errors in substantive correctness
- 3.5 (D): Written assignments completed according to the deadlines, with major errors in substantive correctness
- 3.0 (E): Written assignments competed past the deadlines
- 2.0 (F): Failed to meet the minimal requirements, i.e. attendance in at least 24 h of classes and completed assignments.

The course is facultative, has limit of 15 participants, and thus is recommend only for highly motivated students. Good command of English is required. Team work is fun!

#### Teachers, Reviewers & Students

#### Teachers

Dr hab. Joanna Rutkowska, prof. UJ – Institute of Environmental Sciences, Jagiellonian University Dr hab. Taduesz Zając, prof. IO PAN- Institute of Nature Conservation, Polish Academy of Sciences

#### **Reviewers**

Dr hab. Paweł Adamski, prof. IO PAN - Institute of Nature Conservation, Polish Academy of Sciences Dr hab. Agnieszka Bednarska - Institute of Nature Conservation, Polish Academy of Sciences Dr hab. Paweł Olejniczak - Institute of Nature Conservation, Polish Academy of Sciences Dr Agnieszka Olszańska - Institute of Nature Conservation, Polish Academy of Sciences

#### Students

Toyeeb Atanda, 1st year of 'Environmental Protection and Management', Jagiellonian University Ruihua Cui, 1st year of 'Environmental Protection and Management', Jagiellonian University Maja Gajdek, 2nd year of 'Environmental Protection and Management', Jagiellonian University Kseniia Khmelevska, 2nd year of 'Environmental Protection and Management', Jagiellonian University Agatha Mwangemi, 1st year of 'Environmental Protection and Management', Jagiellonian University Barbara Papiż, 1st year of 'Environmental Protection and Management', Jagiellonian University Katarzyna Potera, 1st year of 'Environmental Protection and Management', Jagiellonian University Allison Proszowska, 2nd year of 'Environmental Protection and Management', Jagiellonian University Tine Roelant, Erasums + student from Catholic University of Leuven in Belgium Ekaterina Rostovskaya, 2nd year of 'Environmental Protection and Management', Jagiellonian University Barış Tanrıverdi, Erasums + student from Istinye University in Turkey Agata Tarczyńska, 2nd year of 'Environmental Protection and Management', Jagiellonian University

#### Topics for projects suggested by the course participants

#### Voting carried out during the class identified the most popular topics (in bold)

- 1. Coexistence of people and wolves in Poland-the new reality. Resolving the conflict through a large-scale education (HW)
- 2. Genetic analysis combined with camera traps in assessing the scale of hybridization between wolves and dogs in Poland (HW)
- **3.** Should we limit the number of hikers? An evaluation of environmental threats of tourism in Tatra National Park (AP)
- 4. Pollution mitigation strategies for smog in major Polish cities: economic and environmental perspectives (AP)
- 5. Restoration of green vegetation in Western China by "Ant Forest" project (RC)
- 6. "Sesame Credit" project for the protection of Panda National Park in Anzihe reserve, Si Chuan Province, China (RC)
- 7. "The Ark" Project. Determination of terrestrial species appropriate for Mars colonization (KK)
- 8. Unity system. Keystone species identification and protection software (KK)
- 9. Assessment of actual pros and cons of enlarging the protected water terrains of The Great Arctic Reserve (MG)
- 10. The impact of feed/food use in fjord (arctic) aquaculture on environmental responses, biodevelopment and environmental quality (MG)
- 11. Efficient classification and description of tropical insect communities (TR)
- 12. Updated survey of current yak populations (Bos grunniers) (TR)
- 13. Air pollution sources in Turkey, how they affect the public health and solutions to improve air quality (BT)
- 14. Death of Flamingos: Reasons of water level decrease in the Lake Tuz and developing prevention mechanisms against it (BT)
- 15. The potential application of the halophyte salt-cedar (Tamarix) in phytoremediation of Leadcontaminated water sources (AM)
- 16. The pertinence of bacterial nanocellulose in curbing the plastic waste footprint due to covid-19 single-use disposable facemasks (AM)
- 17. Social dynamics of Human-wildlife Conflict: Understanding Resident Perception towards Red Fox in Krakow (TA)
- 18. Managing Epidemics in Wildlife with Acquired Resistance (TA)
- 19. Spatial-temporal distribution analysis of Eurasian lynx (Lynx lynx L.) in Gorce National Park (ER)
- 20. Interactions between large and middle-sized carnivoras within the territories of Gorce National Park (ER)
- 21. Restoration of Masuria, Pomerania and the Biebrza River protection of the Widgeon species against extinction in Poland (BP)
- 22. Protection of the European and Eastern hedgehogs in the Kraków town (BP)
- 23. Scuba diving and its impact on bottom in protected marine areas in Tortugerro National Park, Costa Rica (AT)
- 24. Host- parasite interaction starving as a non-immunological method of defense of Gryllodes sigillatus to Heterohabditis bacteriophora (AT)
- 25. Impact of prolonged high-stress temperature on molecular response in ectotherms (KP)
- 26. Enzymatic adaptation to high temperature the role of missense mutations and biophysical properties of amino acids (KP)

## 1. The potential application of the halophyte salt-cedar (*Tamarix smyrnensis*) in phytoremediation of lead-contaminated water sources

#### Applicants names

#### Agatha Mwangemi, Katarzyna Potera

#### Abstract

Heavy metal pollution is a global concern that has imposed extreme health conditions and environmental degradation. For many decades, industrial wastes constitute heavy metals that have been ending up in landfills or water sources. Lead is the most abundant and toxic element, causing irreversible and harmful impacts on the ecosystem, plants, animals, and humans. Phytoremediation has shown promising opportunities in cleaning up the environment polluted by heavy metals in a more environmental-friendly and affordable manner. Salt cedar is a metal hyperaccumulator, which has shown desirable characteristics in phytoextraction of heavy metals, more so, lead metal ions. The aims of this research will be to overexpress the HMA3 and P1B-ATPase genes responsible for metal transportation in the salt cedar plant, lead acquisition from Flint River, and riverbank soil in Michigan-USA by transgenic salt cedar plants, and finally the phytoextraction of lead metals from the salt cedar plant. The research will have three hypotheses: First, overexpression of HMA3 and P1B-ATPase genes enhances the hyperaccumulation of lead ions in salt cedar plants. Second, lead concentration in water and soil samples introduced with transgenic salt cedar will be lower than water and soil samples obtained before the experiment, and third, lead concentration from salt cedar glands was higher in leaves from plants uprooted from the water environment than those from riverbank soil. The expected prediction in the research is that lead ions are expected to accumulate and secreted in the salt glands of the plants as salt crystals.

Total project cost:	£ 234,808.40
Project title:	The potential application of the halophyte salt-cedar (Tamarix smyrnensis) in phytoremediation of lead-contaminated water sources.
Project start date:	01.09.2022
Project end date:	01.07.2024
Project country:	Poland, United States of America
Up to 6 key-words:	phytoremediation, heavy metal, lead pollution, phytoextraction, salt cedar, genetically modified plant
Project lay summary:	Heavy metals contamination is a serious threat for organisms in polluted ecosystems. Lead is the most common pollutant and its exposure results in harmful effects on every part of the food chain. An effective way to remove lead from the environment i via phytoremediation. Salt cedar is a metal hyper accumulator that is able to secrete heavy metals through the glands on the surface of its leaves. This project aims to genetically modify this plant to enhance the phytoremediation mechanism and to prevent the uncontrolled spreading of the species in an ecosystem. Our goal is to test modified plants in the field, a highly lead-polluted, Flint River in Michigan, US. Plants will be cultivated in the soil and pods in the river. Finally, a procedure of lead extraction from salt cedar glands will be executed. The developed technique will be applicable to other lead- contaminated environments.

#### Summary Project Details

#### Project description

#### Core project description

#### A) Background and Rationale

Water sources become increasingly polluted by heavy metals, mainly due to anthropogenic impacts (Zhou et al., 2020). Heavy metal pollution poses a serious threat to people's health and other living organisms. Lead metal has been demonstrated to be the most common pollutant that affects all living organisms. Exposure to lead metal results in harmful effects on plants, animals as well as humans such as damage to the endocrine system, impact on immunity, neurological disorders, and in some cases cancer. To remove lead from soil and water in environmental and affordable manner phytoremediation may be used.

Hyperaccumulator salt cedar (*Tamarix smyrnensis*) has a set of unique features such as high evapotranspiration rate and secreting ions in salt glands on leaves surfaces (Kaducova et al., 2008). Enhancing the efficiency of lead ions hyperaccumulation and secretion in plants will be possible due to an overexpression of genes involved in ion transport (Yan et al., 2020). The salt cedar plant is invasive, and its rapid growth of biomass is beneficial for phytoremediation but dangerous to the environment. To decrease the invasive species from spreading in the environment and the risk of hybridisation, another genetic change will be introduced to suppress pollen production.

Phytoremediation by salt cedar will be applied on the Flint River (Michigan, USA), this is because the Flint River is heavily polluted with lead. More than 100,000 residents have been exposed to elevated lead poisoning as a result of aging pipes leaching into the water supply (Ray, 2020). Our approach allows for a method of cleaning up a polluted river and recovering lead. The effective strategy will become a convenient solution to lead pollution problems in water sources.

#### B) The question or hypothesis to be tested/applied problem to be solved

<u>Aim 1.</u> Enhancing lead acquisition in salt cedar by overexpression of HMA3 and P1B-ATPase genes. <u>Hypothesis 1.</u> Overexpression of HMA3 and P1B-ATPase genes enhances the hyperaccumulation of lead ions in salt cedar plants.

<u>Aim 2.</u> In-field measurement of lead acquisition from Flint River and riverbank soil by transgenic salt cedar plants.

<u>Hypothesis 2.</u> Lead concentration in water and soil samples introduced with transgenic salt cedar will be lower than water and soil samples obtained before the experiment.

Aim 3. Extraction of accumulated lead from salt cedar plant.

<u>Hypothesis 3.</u> Lead concentration from salt cedar glands will be higher in leaves from plants uprooted from water environment than those from riverbank soil.

#### C) An outline of the methods to be used

#### Aim 1. Enhancing lead acquisition in salt cedar by overexpression of HMA3 and P1B-ATPase genes

Salt cedar seedlings will be obtained from a reliable supplier. Transformation with Agrobacterium tumefaciens will be used to insert HMA3 and P1B-ATPase genes that will allow for their high expression

and enhance lead acquisition. siRNA, will also be inserted using A. tumefaciens, this will suppress TKPR1 gene expression that prevents pollen development. Efficiency in the lead acquisition of modified plants will be tested in different concentrations of  $Pb(NO_3)_2$  as demonstrated in the table below. Lead ion concentration from the water and soil samples from all groups will be analysed. Salt cedar plants will be uprooted, their leaves washed in weak HNO<sub>3</sub> acid, and the solution analysed for lead ion concentration by the use of Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

lead	0 m	g/kg	200 n	ng/kg	800 n	ng/kg	<b>1600</b> 1	mg/kg
modification	water	soil	water	soil	water	soil	water	soil
HMA3 and P1B-ATPase genes overexpressed	10	10	10	10	10	10	10	10
siRNA-mediated TKPR1 gene suppressed	10	10	10	10	10	10	10	10
HMA3 and P1B-ATPase genes overexpressed and TKPR1 gene suppressed	10	10	10	10	10	10	10	10
no modification	10	10	10	10	10	10	10	10

**Table 1.** Number of genetically modified salt cedar plants that will be tested in different lead concentrations is provided below. The same concentration will be applied in water and soil.

After the initial experiment, 72 more seedlings will be acquired from the same supplier and will gain the genetic modifications similar from the seedlings in the first experiment, the overexpression of both HMA3 and P1B-ATPase genes, and the suppression of the TKPR1 gene and transformation with *Agrobacterium tumefaciens* and siRNA will be used. The seedlings will be cultivated for six weeks in controlled laboratory conditions in Michigan University Laboratory for the field experiment.

### Aim 2. In-field measurement of lead acquisition from Flint River and riverbank soil by transgenic salt cedar plants

72 transgenic seedlings prepared in the previous step will be transplanted in the natural setting -12 plants in 6 sites of the Flint River and riverbank soils. Pods will be built to grow in the transgenic plants in the river. These pods will allow input and output of water, as well as prevention of any plants or plant pieces from escaping into the river (Jones et al., 2018).

Water and soil samples will be collected before the implantation of the transgenic plants. Water and soil samples will undergo analysis such as temperature, PH, salinity, conductivity, and metal concentration. Metal concentration will be analysed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Standard A. Annual book of ASTM standards, 2004). Water and soil samples will be collected and analysed for metal ions concentration two times a day. The experiment will be repeated for ten consecutive weeks.

#### Aim 3. Extraction of accumulated lead from salt cedar plant

On the last day of the experiment, the plants will be uprooted and the leaves plucked and washed immediately and thoroughly by a weak acid solution of 0.1% HNO<sub>3</sub> for approximately three minutes. Washing the leaves in weak acid will dissolve all the crystals on the surface of the leaves as well as remove any compounds in the crypts of the glands. The solution will then be analysed by ICP-MS in the University of Michigan laboratory (Standard A. Annual book of ASTM standards, 2004).

The plants will be uprooted in the morning hours because previous studies reveal the salt absorption by the salt cedar, transported to the shoots and leaves in the transpiration stream during the day (Kaducova, 2008), and eventually, salt crystals secreted during the night.

#### **D) Expected Outputs**

The leaf surface of the salt cedar has salt glands that will excrete the lead ions. Lead ions might be accumulated and excreted in salt crystals on the leaves of the salt cedar plants as a detoxification mechanism (Nedjimi, 2021). Overexpression of genes encoded ion transporters in salt cedar leaves will allow to accumulate lead ions from water and soil faster and more effective then in case of unmodified plants. This property will be used to detoxify water from the heavily lead-polluted Flint river (Michigan, USA) and river bank soils. Lead ions will be extracted from the salt glands and leaves of the plant easily. Phytoremediation in field with *Tamarix smyrnensis* carries a risk of spreading this invasive species. Therefore, specific measures will be taken to prevent uncontrolled spread of these plants – supressing pollen development and cultivating in the pods. This will provide an affordable, universal and cheap method in the removal of different heavy metals in polluted environments.

#### E) Expected timescales

- September 2022: Gene modification of salt cedar plants (Jagiellonian University)
- April 2023: Growing gene modified salt cedar plants (Michigan University)
- July 2023: Transplanting transgenic plants in Flint river and river bank soils
- September 2023: Phytoextraction of lead ions and lead ions analysis
- January 2024-June 2024: Analysis of results and writing manuscripts

#### F) Practical Implementation

The salt cedar, due to its characteristics of tolerability of heavy metals and detoxification mechanisms by the salt glands, poses promising opportunities for the phytoremediation of heavy metals such as lead. The plant has also shown positive approaches to the biomining of heavy metals. This can be applied on a large scale, both in water and soil sources heavily polluted by lead. Overexpression of different metal transporter genes in one plant can be used in the phytoremediation of other and numerous heavy metals such as zinc, mercury, copper, or cadmium. Enhanced acquisition of a few heavy metals in one single plant might be a promising solution in detoxifying polluted water and soils.

#### Additional questions:

### What are the risks to the health and safety of those involved in the project and how are these risks to be minimised?

Individuals taking part in the field research will be required to be under a health insurance that will be applied in the U.S.

Salt cedar plant is highly invasive, but in this case, careful approaches will be put in place. The TKPR1 gene will be suppressed to prevent propagation of the transgenic invasive plant in the river or environment.

### Please provide details of agreed collaborations and project partners that will facilitate the proposed project:

Collaboration with Dr. Haether Dawson from University of Michigan-Flint, USA.

### Please provide details of the suitability of the institution where the work will be carried out and the availability of equipment and facilities required for the work:

Jagiellonian University, Kraków, Poland - Laboratory – the access of the Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

University of Michigan-Flint, USA – the access of the Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

#### Please provide details of necessary permits/licences obtained, if applicable:

The National Invasive Species Act (NISA).

Scientific Research and Collecting Permits.

### Please indicate how you will assess the impact of the project and the benefits for non-academic stakeholders:

The research study will create a more affordable and biological friendly approach in the removal of lead ions in contaminated environments. The study will regain the hope of local communities that have been exposed and suffered from lead pollution effects. The possibility of removal of lead in soils will regain the fertility in soils, hence higher yields in farm produce. The project will have the potential of utilisation in government bodies such as environmental departments, as it provides an affordable and efficient solution to the enormous issue of heavy metal pollution.

#### How do you intend to make your research data publically available?

Our goal is to publish at least one high-impact paper and at least one will be open access. Team members will present results at conferences and seminars. Popular science paper will be published in the local media. All data will be collected in repository of our university.

#### Please provide details of any published papers relevant to this project:

Jones, J.L., Jenkins, R.O. and Haris, P.I. (2018). Extending the geographic reach of the water hyacinth plant in removal of heavy metals from a temperate Northern Hemisphere river. *Sci Rep* 8, 11071. https://doi.org/10.1038/s41598-018-29387-6

Kadukova J, Manousaki E, Kalogerakis N. (2008). Pb and Cd accumulation and phyto- excretion by salt cedar (Tamarix smyrnensis Bunge). *Int J Phytoremediation*. doi: 10.1080/15226510701827051. PMID: 18709930.

Nedjimi, B. (2021). Phytoremediation: A sustainable environmental technology for heavy metals decontamination. *SN Appl. Sci. 3*, 286. https://doi.org/10.1007/s42452-021-04301-4

Ray, Michael. (20 Jan. 2021) "Flint water crisis". Encyclopedia Britannica, Retrieved from https://www.britannica.com/event/Flint-water-crisis, on 01 December 2021.

5<sup>th</sup> Standard A. (2004). Annual book of ASTM standards, Philadelphia: *American Society for Testing and Materials Annual*.

Yan, A., Wang, Y., Tan, S. N., Mohd Yusof, M. L., Ghosh, S., & Chen, Z. (2020). Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land. *Frontiers in Plant Science*, 11. https://doi.org/10.3389/fpls.2020.00359

Zhou, Q., Yang, N., Li, Y., Ren, B., Ding, X., Bian, H., & Yao, X. (2020). Total concentrations and sources of heavy metal pollution in global river and lake water bodies from 1972 to 2017. *Global Ecology and Conservation*, 22, e00925. https://doi.org/10.1016/j.gecco.2020.e00925

#### Budget

Equipment

Item	Quantity	Description	Total Cost
Planting pods	6 Pods: each @ GBP	2 x 2 m. Materials to	£ 1,041
	173.5	build one cost ca. £	
		173.5	
Salt cedar seedlings	392 seedlings	70 cm salt cedar	£1,960
	5 GBP each	seedlings	
Laboratory equipment	1	Laboratory materials:	£ 3,000
		beakers, pippetes,	
		tips, Petri dishes,	
		autoclaves, cylinders,	
		reverse pipettes	
Field work equipment	1	Materials for field	£ 800
		work: tape measure,	
		PH meters, containers,	
		thermometers, etc.	
Overall Equipment Cost:			£ 6,801

Consumables

Item	Quantity	Description	Total Cost
ElectroMAX™ A.	2	5 x 40 μL each 463 £	£ 926
tumefaciens LBA4404			
Cells			
TransStart Tip Green	2	Quantitative real-time	£ 966
qPCR SuperMix		PCR @483	
Plasmids	2	Individual Plasmid (	£ 143.40
		pSIM24-GUS) each	
		71.7	
cDNA Synthesis Kit	2	(K1621, Thermo,	£ 792
		Waltham, MA, USA)	
		@393 GPB	
Plant Genomic DNA	2	DP305, TIANGEN	£ 260
Extraction Kit			
Overall Consumables Co	ost:		£ 3,087.4

Personal and Field Travel/Accommodation/Subsistence

Description	Total Cost
Flight tickets to US: £780x2 pp	£ 3,120
Accommodation (fieldwork, personal): 70 nights (average) x £20 pp	£ 2,800
Per diem	£ 216 000
Car travel to field work	£ 900
Overall Travel Cost:	£ 222,820

Employment (note only casual, short term assistance will be considered)

Position	Description of role	Rate & Duration of employment	Total Cost		
3 field work assistants	Planting plants cuttings, analysing lead ions both in water and soil	10 weeks	£ 2,100		
Overall Employment Cos	Overall Employment Cost:				

Total project cost	£ 234,808.40

#### Applicants names

Hanna Witerek, Ruihua Cui, Agata Tarczyńska

#### Abstract

Lake Tuz is the second largest lake in Turkey, and it is used as a breeding site for Greater Flamingo. Due to current mismanagement on a local scale, breeding success is lower compared to previous years. In 2021 the Lake Tuz dried out completely contributing to death of thousands of flamingo's fledglings, creating an exigent need to restore the water in the Lake and bring back the possibility of breeding for flamingos in this area. The first objective of the project is to develop a land-use strategy, which will act as a sound framework for optimal land use. Our main goal here is to encourage the government to subsidize crops that are less water demanding which will make them more attainable for farmers. Our next objective is to educate citizens, farmers, and local government. We hypothesize that through the large-scale educational campaign, increased number of people will become aware of disappearing of the flamingo's breeding site and that the local decision makers will strive to stop this process. Another objective is to evaluate the hydrological systems around the Lake Tuz using multi-influencing factors analysis with the use of GIS mapping technics. Final objective is to present it to the government along with the strategies on sustainable use of the land and the water sources. We hypothesize that these guidelines on managing the land and protecting the underground waters of the area are going to mobilise government to undertake actions. Outcomes of our educational campaign are going to be measured by comparing the results of the surveys conducted before and after. The aim of our project is to create a basis with strong scientific reasoning and background for Turkish government to start implementing ready solutions developed by us and to make public sector aware of the impact of their actions.

Sammary of Frojest Details	
Total project cost:	272 505€
Project title:	Conservation of (Phoenicopterus roseus) in Turkey - mitigation strategies for preventing the Lake Tuz from drying out
Project start date:	January 2023
Project end date:	December 2025
Project country:	Turkey
Up to 6 key-words:	Phoenicopterus roseus, Lake Tuz, conservation, mitigating strategies

#### Summary of Project Details

We aim to develop strategies to mitigate the problem of the **Project lay summary:** Lake Tuz drying out, which this year has led to death of thousands of flamingos. We will create the land quality assessment and a map of groundwater potential zones as the basis for developing the strategies serving as sound frameworks for optimal land and water use with detailed guidelines for decision makers. We will run an education campaign to promote sustainable use of water among farmers and to educate them about the results of their past actions. We are going to measure the outcome and effectiveness of educational campaign by conducting surveys during the first year of the project duration, before the educational campaign after the campaign is finished. We hypothesise that the government presented with this plan and the strategies based on our largescale scientific research will start the process of setting new agricultural policies.

#### **Project description**

#### Core project description

#### **Background**

Lake Tuz is the second largest lake in Turkey. It is used by the Greater Flamingo *(Phoenicopterus roseus)* as the breeding site [1]. Among 69 000 flamingo chicks hatched in Mediterranean region, almost 20 000 of them were in Lake Tuz basin [2]. Due to current mismanagement of water on a local scale and uncontrolled watering methods on agricultural lands, problems with water shortage started to occur. Reckless and illegal activities influencing hydrology (digging wells and diverting stream courses) in Anatolian Basin caused a rapid decrease of underground water levels. Moreover, there are large increases in the cultivation of water demanding crops such as clover, sugar beet, and corn. As a result of it, in 2007 breeding success of flamingos was lower comparing to earlier years [3]. In 2021 Lake Tuz dried out completely contributing to death of thousands of flamingo's fledglings. Flamingos remain classified as "endangered" despite over 15 000 breeding pairs and almost 40 000 wintering individuals in Turkey. According to IUCN criteria from years 2001, 2004 and 2006, >80% decline in population is suspected to happen in the next 10 years [4]. Any event contributing to a significant decline number of species in this area can affect the breeding fitness and whole population of these birds. There is an exigent need to restore the water in The Lake Tuz and bring back the possibility of breeding for flamingos in this area.

#### The hypotheses to be tested/applied problem to be solved

**Objective 1** (O1) is to create a land quality assessment, which is a powerful tool helping in taking decisions on sustainable land use.

**Hypothesis 1 (H1)** The government provided with a sound framework and detailed guidelines on how to use the land in the basin of the Lake Tuz in a more sustainable way and what type of crops to grow there so the farmers can still benefit from them, will change their agricultural policies, e.g., will subsidize the plants that are not so water demanding.

**Objective 2 (O2)** is the evaluation of the hydrological systems around the Lake Tuz and delineation and assessment of the groundwater recharge potential zones in order to develop a strategy of managing and protecting the underground waters of the area and creating a plan for a modern irrigation system.

**Hypothesis 2 (H2)** Developing a strategy of managing and protecting the underground waters of the area will help decision makers to implement the mitigation strategies in the form of subsidizing setting new irrigation systems and implying high fines on farmers who will still use illegal irrigation technics (diverting streams, digging the wells).

**Objective 3** (O3) is to run an educational campaign in Konya Region to teach farmers about more efficient watering techniques and crops requiring less water and to raise awareness of local citizens on existing problem. Objective 3 includes and uses work done in objective 2. The land quality assessment will act as a guideline for local education campaign and will help pit-point aspects of water management and sustainable land use needing most attention. Objective 2 includes surveying people before the campaign and after to measure if opinions has changed or not and creating a website related to our project, which will be used to inform the public about the current situation, about our project and its progress. We will put here all the results of our research and toward the end of the project, we will make public our strategies and the plan of the new irrigation system. Website will be a space for sharing opinions, comments and for an open discussion.

**Hypothesis 3 (H3)** Running an educational campaign will bring the attention to the drying lake and following consequences of it. Effectiveness of this campaign will be evaluated by surveying people before and after it. The website will help us develop and adjust our educational campaign as it will progress in time, as well as our proposals to the Turkish government.

**Objective 4** (O4) is to present the government with all the data about the land and the hydrological system of the basin of the Lake Tuz, which we will obtain during our study, with the detailed guidelines and strategies on sustainable use of these resources and with the plan for setting the new irrigation system.

**Hypothesis 4 (H4)** presenting the government with scientifically developed strategies that will enable to use the land and the waters in the way which will be beneficial both for the environment and the farmers and which will allow to implement the changes and to keep the government's political power at the same time, will encourage decision makers to implement the solutions proposed by us.

#### **Methods**

Creating a land quality assessment is a crucial step which will help undertake decisions on sustainable land use and conservation of ecosystem. This systematic process will follow three stages: 1. generating a comprehensive database for the area (**Task 1**), including land use and environmental factors (soil, climate, water quality, fertilizing status, heavy metal, and pesticide pollution); to do this we will analyze digital soil maps, digital elevation models, satellite images and climatic data using remote sensing and geographic information system technics. In a laboratory we will test samples of the underground waters and soils from the area surrounding the Lake Tuz. In addition, in this stage we will create a preliminary land management strategy (**Task 2**) and preliminary environmental risk assessment (**Task 3**). In stage 2 we will identify some feasible sustainable land use options which will not be harmful to the hydrology of the area (e.g., proposing

new crops which are not so water demanding) (Task 4) and we will develop the land use strategy (Task 5). Finally in stage 3 we will prepare the implementation plan (Task 6), design the possible ways of verification of works (Task 7) and the plan for a long-term monitoring (Task 8), which will give the government the detailed guidelines for the future actions. The evaluation of the hydrological systems around the Lake Tuz and delineation and assessment of a groundwater recharge potential zones will be done using geographic information system (GIS), remote sensing (RS) and multi-influencing factors (MIF) approaches. We will consider many factors, such as lithology, drainage density, slope, rainfall, and soil texture. These thematic layers will be established in the GIS environment for the purpose of identifying the groundwater recharge potential zones (Task 9) and creating the map of the Lake Tuz basin area using weighing analysis in ArcGIS software (Task 10). Then we will use MIF technique, where ranks and weights assigned to each factor, are computed statistically. On this basis we will categorize the groundwater recharge potential zones into three classes: good, moderate, and low (depending on their recharge potential) (Task 11) [5].

Education campaign will cover reasons of underground water shortage in Lake Tuz Basin and possible following incidents if no actions are going to be applied in the future. Education will also cover topics of climate change and extreme weather events, which have also contributed to shrinking of the Lake Tuz. Gaining attention of local authorities and societies is the first step to implementing a successful intervention. We aim to conduct a campaign in form of meetings with specialists (hydrologists, geographers, geologists, meteorologists) to ensure that crucial topics are covered (Task 13). Meetings with specialists will be prepared and held with education methodologist and teachers. Along with meetings we also plan to run informative channels on social media (Facebook, YouTube, Instagram) to maximize the scope of people informed about the Lake Tuz problems (Task 14). Social media channels are going to be led by marketing specialist and volunteers. Videos, posts, and photos are going to be created by graphic designers along with editors and volunteers willing to take part in the project. To check if the educational campaign was effective, surveys with authorities, farmers and citizens will be conducted. First part of surveys will be held at the beginning of the project, before campaign (Task 12). Second part of surveys will be held after campaign. Outcomes will be evaluated by a statistician (Task 15). All applicants will be taking active part in the campaign. All people taking part in the education campaign are going to be trained and informed about the ongoing problem in Lake Tuz at first. Presenting government with data and guidelines (Task 16).

#### **Expected outputs**

The expected future measurable outcome of **Objective 1** is an implementation of our strategy for sustainable use of the land by Turkish government, which means starting the process of changing the crops into less water demanding and subsidizing them, so it is beneficial for the farmers. To assess it is outside of the scope of our project.

The expected output of **Objective 2** is greater awareness among government members, farmers, and citizens. We will measure it by comparing the results of the survey conducted before and after the campaign.

The expected measurable output of **Objective 3** is that the government will use our detailed strategy and methodology for sustainable use of the underground waters in the basin of the Lake Tuz, but to assess it is outside the scope of our project.

The expected outcome of presenting the government (**Objective 4**) with scientifically developed strategies the government will start the process of implementing the solutions proposed by us (assessing it is outside the timeframe of our project).

#### **Expected timescales**

<u>Objectives</u>	Tasks	Year		
I		2023	2024	<u>2025</u>
O1	Task 1			
	Task 2			
	<u>Task 3</u>			
	Task 4			
	<u>Task 5</u>			
	<u>Task 6</u>			
	<u>Task 7</u>			
	<u>Task 8</u>			
O2	<u>Task 9</u>			
	<u>Task 10</u>			
	<u>Task 11</u>			
03	<u>Task 12</u>			
	Task 13			
	<u>Task 14</u>			
	<u>Task 15</u>			
O4	<u>Task 16</u>			

#### Tab1. Timetable for the implementation of consecutive tasks.

#### **Practical implementation**

Applicants, along with the local government, are organizers of the educational campaign. Employed advertisement companies are professional, they are responsible for making videos, writing posts and taking photos. Volunteers will be recruited from the internet or from universities and need to be trained at first. After training, they will help the applicants to promote the videos, posts and phots through Facebook, YouTube and Instagram and survey the educated famers/citizens. Teachers and methodologist are recruited and trained at first, they responsible for teaching the farmers and citizens. We will present the Turkish government with our strategies for sustainable use of the land and underground waters and the plan of the modern irrigation system towards the end of our project.

#### Additional questions:

### What are the risks to the health and safety of those involved in the project and how are these risks to be minimised?

The applicants and the specialists working in the field will be insured for any possible accidents and injuries that may happen.

### Please provide details of agreed collaborations and project partners that will facilitate the proposed project:

For development of the strategies on sustainable use of the land and on sustainable use of the underground waters we will collaborate with GIS specialist, hydrologist, geologist, climatologist, agriculture scientist and economist. For the education campaign applicants along with the local government will be organisers of the campaign. We will employ professional advertisement companies, teachers, and methodologist. Volunteers will be recruited from the internet or from universities, they will be trained for the purpose of the campaign.

### Please provide details of the suitability of the institution where the work will be carried out and the availability of equipment and facilities required for the work:

#### Developing the strategy and methodology for land use and sustainable water use:

Laboratory work will be carried out in the laboratory of the Environmental Institute of Konya, which has all necessary equipment which will be provided to us for the purpose of the project. Work involving GIS, RS, MIF technics will be carried in the same Institute. The Institute has good quality computers, hence there is no need to include them in budget proposal.

#### Education campaign:

Advertisement companies are professional and do not need equipment or fixed office space, they have their own offices. Volunteers need to be trained to carry out relevant work, all communication can be maintained via the internet, they also do not need the fixed office space. Teachers also need to be trained to carry out related work and they need to rent fixed office space. In addition, multimedia equipment will be used to support their teaching.

#### Please provide details of necessary permits/licences obtained, if applicable:

We will obtain necessary permits from the government for carrying the field works in the protected area around the Lake Tuz and on private lands. Collaboration with schools to run the workshops in their buildings, but no permits or licenses will be needed.

### Please indicate how you will assess the impact of the project and the benefits for non-academic stakeholders:

We will carry a large-scale survey among farmers, citizens, and local government at the beginning of the project and toward the end of the third year, thanks to it we will be able to assess the impact of the educational campaign. Comments and opinions from the public put on our website will also give us a good insight into the impact of our actions on people. Assessing the benefits for farmers is outside of the timeframe of our project as the process of implementing the new agricultural policies and setting up the new irrigation system will last for some years to come – this is not included in our project scope and its duration but is mentioned to give an idea for further actions and research.

#### How do you intend to make your research data publicly available?

All results of our research and the strategies that we will develop will be made public on our website. We will publish the research data in scientific papers. Data regarding usage of water will be added to public records.

#### Please provide details of any published papers relevant to this project:

[1] Balkız, Özge; Onmuş, Ortaç; Sıkı, Mehmet; Döndürenc, Ömer; Gül, Orhan; Arnaud, Antoine; Germain, Christophe; İsfendiyaroğlu, Süreyya; Özbek, Melih; Çağlayan, Eray; Araç, Nilüfer; Parmak, Burcu; Özesmi, Uygar; Béchet, Arnaud, 2015. *Turkey as a crossroad for Greater Flamingos (Phoenicopterus roseus): evidence from population trends and ring-resighting (Aves: Phoenicopteridae). Zoology in the Middle East, 61(3), 201–214. doi:10.1080/09397140.2015.1058452* 

[2] Kuzucuoğlu, Catherine; Çiner, Attila; Kazancı, Nizamettin, 2019. [World Geomorphological Landscapes] Landscapes and Landforms of Turkey // Salted Landscapes in the Tuz Gölü (Central Anatolia): The End Stage of a Tertiary Basin., 10.1007/978-3-030-03515-0(Chapter 16), 339–351. doi:10.1007/978-3-030-03515-0\_16

[3] Ruedi G. Nager; Alan R. Johnson; Vincent Boy; Manuel Rendon-Martos; Juan Calderon; Frank Cézilly, 1996. *Temporal and spatial variation in dispersal in the greater flamingo (Phoenicopterus ruber roseus)*., 107(2), 204–211. doi:10.1007/bf00327904

[4] Andrew T. Knight, Robert J. Smith, Richard M. Cowling, Philip G. Desmet, Daniel P. Faith, Simon Ferrier, Caroline M. Gelderblom, Hedley Grantham, Amanda T. Lombard, Kristal Maze, 2007. *Improving the key biodiversity areas approach for effective conservation planning*. Bioscience, Vol. 57(3), pp 256-261.

[5] Ahmed, A.; Alrajhi, A.; Alquwaizany, A.S., 2021. *Identification of Groundwater Potential Recharge Zones in Flinders Ranges, South Australia Using Remote Sensing, GIS, and MIF Techniques.* Water 2021, 13, 2571. https://doi.org/10.3390/ w131825

#### Budget

Equipment

Item	Quantity	Description	Total Cost
Computer	2	Teaching equipment	660€
Projectors	2	Teaching equipment	220€
Printed surveys	5 000		1000€
Pens	4 000		2000€
Tablet digitizer (Wacom Intuos Pro pen drawing tablet)	1	GIS mapping	334€
GPS receiver (SI TEX GPS-915 receiver)	1	Data will be transferred from this unit to GIS software	583€
External mounted tripod antennae (Trimble R10)	1	To help increase location accuracy	56€
GIS hardware for the office: Samsung LU28E570 28" monitor UHD 4K - Ultra HD 3840x2160, 1ms, 2 x HDMI, DisplayPort,	1	Large display monitor	278€
HP DesignJet T830 Large Format Multifunction Wireless Plotter Printer - 36", with Mobile Printing	1	Printer for large scale GIS maps	5628€
ArcGIS desktop advanced (annual subscription)	1	GIS software	3670€
Laboratory equipment		Water and soil quality testing lab equipment	259€
Overall Equipment Cost:			11248€

#### Consumables

Item	Quantity	Description	Total Cost
The insurance for the applicants		Travel plus medical	552€
		assistance insurance for	
		3 years	
The insurance for specialists	3	GIS specialist (1 year)	88.50€
working in the field		agricultural scientist	
		(6 months)	44.25€
		hydrologist (6 months)	44.25€
The insurance for the teachers	8	Two teachers and six	3677€
and volunteers		volunteers for a year	
Promotion	2	Promotion of the Lake	4400€
fee(advertisement)/per2200€		Tuz environment	
Overall Consumables Cost:			8 806€

Personal and Field Travel/Accommodation/Subsistence

Description	Total Cost
Accommodation for the applicants for 3 years	5475€
Plain tickets from Warsaw to Ankara and return	618€
Bus tickets for applicants from Ankara to Kulu and return	18€
Overall Travel Cost:	6111€

Employment (note only casual, short term assistance will be considered)

Position	Description of role	Rate & Duration of	Total Cost
		employment	
Teacher	Teaching citizens and tourist	For 1 year (275€ euros per 1 week)	14300€
Training teacher	Train the volunteers, teachers.	For1 year	12000€
Education Methodologist	Present during meetings, developing plan for effective learning and best possible campaign outcome	For 1 year (275€ euros per 1 week)	14300€
Geologist	Present during meetings, developing topics for education campaign, involved in developing the lad use strategy and the map of underground water recharge zones.	For 6 months (300€ euros per 1 week)	7200€
Geographer	Present during meetings, developing topics for education campaign.	For 6 months (300€ euros per 1 week)	7200€
Hydrologist	Present during meetings, developing topics for education campaign, involved in developing the lad use strategy and the map of underground water recharge zones.	For 6 months (300€ per 1 week)	7200€
Graphic designer	Developing posts and visual communication	For 1 year (320€ per 1 week)	16640€
Editor	Developing videos	For 1 year (320€ per 1 week)	16640€
Marketing specialist	Developing plan and running social media channels	For 1 year (330€ per 1 week)	17160€
GIS specialist		For 1 year	14000€
Climatologist		For 3 months	3500€
Agricultural		For 6 months	7000€
scientist			
Economist		For 3 months	4000€
Overall Employm	ent Cost:	·	141140€

Total project cost	268105€

#### 3. Determination of terrestrial species appropriate for Mars colonization

#### Applicants names

Kseniia Khmelevska, Ekaterina Rostovskaya, Toyeeb Atanda, Maja Gajdek

#### Abstract

Mars colonization is one of the challenges in the XXI century, as this process requires highly developed technology and a deeply scientific approach. To make Mars a suitable place to live, humanity needs to terraform the planet, create sustainable ecosystems adapted to its severe conditions: dry climate, acidic land, and different gas content. To determine which species are able to survive Mars and are appropriate for its colonization, the ability of species to adapt should be checked and assessed. Here we show the plan for research aiming to create a list of potential species to colonize Mars. Results include a list of species of 4 categories – microorganisms, invertebrates, vertebrates, and Spermatophyta plants sorted according to their ability to adjust to Mars conditions. Additionally, results include description of functions that organisms can perform on Mars, helpful for humanity. Our results show which species can be transported to Mars, as a part of the planet population process. Created database can be used as a basis for choosing species for terraforming Mars. Further research in this area with updates on the chosen species will be done.

#### Summary of Project Details

Total project cost:	\$1.521.618		
Project title:	Determination of terrestrial species appropriate for Mars colonization		
Project start date:	01/02/2022		
Project end date:	01/02/2025		
Project country:	United States		
Up to 6 key-words:	colonization, Mars, terraformation, life, habitat, survival		
Project lay summary:	The capability of life to adapt to extreme martian conditions is one of the challenges delaying the colonization of Mars in recent years. To determine which species are able to survive on Mars and are appropriate for its colonization, species adaptability to its conditions should be assessed. Therefore, this project aims to create a list of potential species that can help in Mars colonization. By identifying list of species based on their ability to adjust to the Mars conditions, this research will serve as a database that can be used as a basis for choosing species appropriate for terraformation.		

#### Project description

#### Core project description

#### a) background and rationale

Colonization of Mars – one of the greatest challenges of the 21-st century. To make humanity the first interplanetary species, not only technological aspects should be taken into consideration, but conditions for adaptation itself. The question as to whether Mars is a habitable planet for future human colonization has been explored for many years, with the first models of microbial communities suggested in the previous century (Rothschild, 1990). However, for now, the evidence that Mars is a habitable planet that can support terrestrial organisms remains elusive, even though there are some signs of some life presence in the past (Schulze-Makuch *et al.*, 2008).

Successful colonization of new land or even planets depend not only on conditions of a place, but resources humanity will transport there, in the case of Mars – the ecosystem that will be created on this currently unpopulated planet. The process of terraforming Mars is as challenging as transporting life to this planet, first of all, because of harsh conditions of cold temperature, low oxygen level and high radiation, that make Mars an unavailable place to survive for many Earth species (Edwards, 2012). To ensure that terrestrial species will survive and adjust on the fourth planet from the Sun, the ability of species to adapt should be assessed from the point of conditions existing on the planet. In order to develop the process of Mars colonization, assessed species should be formed in the list of species appropriate for Mars colonization and sorted by levels of adaptability. Our project aims to create such a list of species to form the base for further research.

#### b) the question or hypothesis to be tested/applied problem to be solved

<u>Which species are appropriate to Mars conditions?</u> Four groups of life-beings will be checked: burrowing vertebrates, invertebrates, Spermatophyta plants and microorganisms – to find the ones able to adapt to the environmental conditions of Mars and be helpful in their functionality.

The four above-mentioned groups were marked due to their usefulness for the first Mars greenhouses and hypothetical possibility to adapt outside cold and dry conditions. The applied problem is to choose among them species which have the highest probability to suit the goal of Mars primary colonization.

#### c) an outline of the methods to be used

Overall, the research will contain three following one-by-one steps. The first one is a literature review to choose the species able to live in Mars-like conditions and having functions helpful to colonize this planet. The second step is to check these species on their ability to adapt to conditions close to Mars (with lower pressure and dry and cold climate), including species survival, growth, and reproduction rates in 1/30 of Mars-like conditions, as well as species ability to survive transporting by a spaceship. Finally, the list of species most suitable to Mars colonization will be written. Later, this list is expected to become a base for other research genetical projects.

#### Systematic literature review

Systematic literature review will be conducted as the first process to know type of terrestrial species that has the capability to survive in Mars conditions following our research questions. Scientific literature will be identified using Web of Science and Scopus, which are still considered the main sources of citation data for the period between 1998 and 2020. The literature search will be conducted between January and June 2022 using the following search terms: (Terrestrial\* OR species\* OR Animal\*) and (Appropriate\* Adapted\* OR Survival\*) and (Mars\*) and ("Colonization") screening titles and abstracts. Our systematic reviews will be limited to scientific peer-reviewed publications (journal articles, reviews and book chapters) and articles published in English. The bibliography will then be verified manually to eliminate any false positives, which are more detrimental than false negatives in a systematic review (Kwon *et al.*, 2015). After excluding the duplicate articles, we will examine again each article's title, abstract and keywords. All these articles will be further screened by examining full texts to determine final inclusion.

As for the literature content analysis, four groups of living organisms are expected to have necessary functionality, naming burrowing vertebrates and soil invertebrates, anemochorous Spermatophyta plants and soil microorganisms. These groups are matching the following conditions:

- 1. Survival in environment closed to martial one, including low humidity and low temperatures it excludes hygrophilous groups of organisms, such as water invertebrates or algae.
- 2. Functional role should be related to terraformation, and that makes soil organisms a focus group of the project.
- 3. The groups chosen should be able to form at least a simplified, but sustainable ecosystem in predicted martian greenhouses. With the development of the Mars colonization project various pollinators and higher trophic level organisms may be added, but for the first steps they are expected to be excluded. In the simplified model soil microorganisms and invertebrates, as well as the plants, present the first level of a grazing food chain, with the soil vertebrates being consumers and a source of energy for redescent microorganisms and invertebrates after death.

#### Experimental part

To achieve conditions as close as possible to those on Mars, we plan to subject certain organisms to altered pressure, temperature changes and artificial light in adapted laboratories – testing chambers. The aim is to check whether the organism can cope with the conditions that we are able to create on another planet inside the greenhouses.

#### 1. Experiments on lower temperature and humidity

According to NASA Science Mars Exploration Program, average martial temperature is about  $-63^{\circ}$ C – almost 77 degrees lower than on the Earth (13.9°C). That means lack of non-frozen water and extremely cold conditions corresponding to our Antarctica. There are known mostly exclusively microbial communities

living inside the stones there, so for terraforming purposes milder conditions will be taken, as if there would be greenhouses with heating.

The organisms chosen after the literature review will be put in a several sets of conditions similar for all the organism groups. The characteristic of water availability is directly depending on the freezing temperature, so the above-zero temperatures and soil moisture above 0.05 volumetric water content in the top 5 cm of the soil (i.e. 0.05 cm<sup>3</sup>/cm<sup>3</sup>, which correspond to the arid zones) are taken (Unninayar & Olsen, 2015). Noteworthy, the temperature of the soil and underground ecosystems is higher and more stable than the air one.

If Mars year length is 1.88 times longer than Earth one, 2 cycles can be planned for plants. In the northern regions of the Earth belonging to tundra zone the minimal growing season can be about a month, and in the deserts it can be about several months. That means at least 200 Earth days of stable above-zero temperatures in the greenhouses for Spermatophyta plants development and growth.

Literature review will let to clarify the conditions, but it can be already predicted, that experimental conditions will be close to the following ones. 300 individuals of each selected species (and colonies for microorganisms) will be participating in the experiments, in such a way that all of them will be participating in all the experiments.

Plants will be grown up from the seeds in the laboratory, while other organism groups will be bought in a ready-to-reproduce condition. All of them will be preliminary placed into soil of standardized content, each species is planned to be analysed for 2-4 months; in such a way during the 26 months of experimental part from 6 to 7 "sessions" can be provided. That means by 50 microorganism and 50 invertebrate species will be conducted during 2 month, and then next 50, and that is repeated 7 times – in such a way overall 350 species of each group can be checked. As for Spermatophyta plants and vertebrates, their conduction time is 4 month and 6 sessions (cycles) can be repeated, with 300 species of each one to be analyzed.

Overall, 300 of individuals will be places under "temperature & humidity" conditions, divided into 6 groups by these variables. Average above-zero seasonal air temperatures are +3, +5 and  $+7^{\circ}$ C, while the soil moisture for aridic and semi aridic zones (0.05 and 0.10 cm<sup>3</sup>/cm<sup>3</sup>) are taken. Each organism's mortality, reproduction, and growth rates will be accessed. After this each group (50 individuals, or colonies) will be affected within the conditions of the nest experiments, to both check the synergetic effects and decrease the statistical error.

#### 2. Experiments on lower pressure and overloads, as well as gravitation, simulated by NASA equipment.

To check adaptability of the species, chambers with artificially created pressure will be used. Potentially, chamber of low-pressure from NASA Manned Spacecraft Center (Chamber A) can be used for experiment with low pressure (artificial pressure up to  $10^{-8}$  torr, Martian conditions = 6 to 10 torr). Vacuum conditions for considering transportation needs are not included in the analysis. High pressure effect can be assessed with the help of hyperbaric pressure chamber (1520 – 19000 torr).

To check influence of gravitation Zero Gravity Research Facility from Glenn Research Center, NASA, is planned to be used.

Containers and cages with soil and the species will be put into modelled conditions starting with the last one month for microorganisms and invertebrates and the last month and a half for Spermatophyta plants and vertebrates. Each group of 50 individuals (or 50 colonies of microorganisms) from the first experiment will be placed into the same variety of conditions, with one gravity chamber settings and two types of pressure – lower Mars-like one (6 torr) and spaceship-like higher one (2000 torr) which is used for cosmonauts. Species growth rates will be accessed along a day for microorganisms and invertebrates and along a week for Spermatophyta plants and vertebrates.

#### 3. Decreased oxygen concentration and Mars-like atmosphere (O2 content chambers) and soils

After the "pressure & gravity" experiment organisms will be affected by the decreased oxygen concentration and changed soil content, details to this experiment require deeper literature review before the detailed description, as long as they will be combined with low temperatures and lack of liquid water.

Overall, the plan of one session is shown in the table 1.

Table 1. Experiment time scale, where MO means microorganisms, IV – invertebrates, SP – Spermatophyta plants, SV – soil vertebrates.

MO	1 month of "temperature &	1 week of "pressure &	3 weeks of combined "temperature &
IV	water" experiment	gravitation"	water" and soil content
SP	1,5 month of "temperature &	2 weeks of "pressure	1 month of combined "temperature &
SV	water" experiment	& gravitation"	water" and soil content

#### Comparative analysis with conclusions

The selection of organisms will be preceded by a deep meta-analysis of the articles with which we will create the terraforming plan, and of the organisms that could pass for further tests and genetic modifications. Each creature will come from certified cultures.

After a suitable period (12 months) of observation, we will be able to answer the question of which animals adapt the fastest, which had the greatest physical problems, etc. We will assess within animal groups their mortality, reproduction, and growth rates.

#### d) Expected outputs

The main assumption and goal of our experiment are to answer the question of whether there are organisms in the world now, which with their currently existing traits are suitable for the mission of 'greening' Mars and which possess some number of traits that could be developed in further genetic studies.

After three years of study, we would like to have a list of animal species with a meaningful assessment of the rate of change of their evolutionary traits.

#### e) Expected timescales

Literature	Experiment	Results (Analysis)
1/12/21 - 1/1/22 - problem	1/6/22 Start of experiments on	2/8/24 - 1/12/24

determination (1 month)	the base of an existing NASA scientific institution's	Analysis of obtained data through 4 main categories of
	greenhouse and apparatuses.	organisms: microorganisms,
2/1/22 - 1/6/22 - deep systematic search in the		invertebrates, vertebrates, higher plants.
determined questions	2/6/22 - 1/8/24 - experiments	ingher plants.
(hypotheses) with the help of professional virtual assistant - 5	based on literature review and with additional search.	
month	with additional search.	
Experimental period - providing additional analysis in order to correct the mistakes and find better options (plus checking the new papers)		
Results period - discussion, conclusions - literature		
overview.		

#### f) practical implementation

Creating the database of species appropriate for Mars colonization. Further research can be done based on this data. We hope that our research will be useful in further studies seeking answers about new evolution on another planet. A database for willing researchers will always be made available, as well as a final list of species with an assessment of their adaptations.

Mars colonisation will become possible in decades, and we consider this time period long enough for geneticists to provide all the changes needed to make the species ready to it.

#### Additional questions:

### What are the risks to the health and safety of those involved in the project and how are these risks to be minimised?

Safety during the experiment (i.e., at each stage of the study) will be ensured by following the health and safety rules of each workstation. Each research participant will first be trained on "good-work" practices in terms of the use of laboratory, office, and associated equipment, around animals of each test species and accompanying animals. Procedure of "do's and don'ts" will be established for correct behaviour within the team to avoid sexual assault. First aid training will also be mandatory for each team member, will be monitored, and implemented to ensure a risk-free research activity.

### Please provide details of agreed collaborations and project partners that will facilitate the proposed project:

Within the scope of the collaboration are entities such as The National Aeronautics and Space Administration (NASA) and European Space Agency (ESA). Partners will provide us with the necessary apparatus and appropriate laboratories, and access to their literature collections. These two entities were considered because of the sophisticated equipment essential for the project that are available at their disposal.

### Please provide details of the suitability of the institution where the work will be carried out and the availability of equipment and facilities required for the work:

Because this project requires some distinct equipment and tools such as gravitation training ones, which are expensive, the experiment practical part of the work is planned to be provided in the above-mentioned partner laboratories. Consequently, no purchase will be made and only renting of the required and available equipment will be needed.

#### Please provide details of necessary permits/licences obtained, if applicable:

To trap animals partially protected by the Wildlife and Countryside Act 1981, like shrews, the licence is needed - "General licence 017 – Licence to take shrews for scientific or educational purpose". Nevertheless, for ecological experiments with bought animals no licences are needed, while permission is still required.

To do research in animals you must obtain a special permission from the animal experimentation administration – it is for free but will take not less than 2-3 months to get it. Moreover, a minimum training program must be completed by each participant and this documentation attached to your application.

### Please indicate how you will assess the scientific impact of the project and the benefits for non-academic stakeholders: we could add here the not scientific impact

The scientific impact of the project will be evaluated based on the number of citations in high impact factor journals and its whole contribution to knowledge as this is the general metric used by scientists to track the impact and spread of their research. The benefit to non-academic stakeholders will be assessed by envisaging forwards from when the research is concluded to see where and how it is communicated, and the level of acceptance among non-academic communities.

#### How do you intend to make your research data publicly available?

We will make data recorded/obtained from this research accessible and available by depositing it in appropriate global data depositories which will be available for download through a digital object identifier (DOI) or on request from a specific author. We can also make our data available to the public by providing it as a supplementary file when submitting our research for publication based on acceptable standards. A social media group across some social media platforms will also be created to enable more people have access to the result of our project.

#### Please provide details of any published papers relevant to this project:

There are no certain articles on the topic developed in project application, as the idea is scientifically new. However, some idea of organisms, mostly Halophiles, Nanobacteria and lichens, which are likely to survive in martian conditions of cold, salty environments with elevated levels of radiation, has already been highlighted (Edwards, 2012). In addition to this, previous possible life on Mars was studied, and methane and water presence were crucial in there (Schulze-Makuch *et al.*, 2008), even though now there is no liquid water on the Red Planet – and that keeps scientists optimistic on its possible colonization. For example, both various extremophilic terra communities are linked, such as rocks, ice caps and volcanic "oases", and evaporites as possible "endoevaporitic" ecosystems, meaning salt crystals, that occur on Mars and were shown to decrease the radiation effect (Rothschild, 1990). In such a way, there is quite a wide field for future exobiological experiments on the planet (Coustenis & Encrenaz, 2013) within the whole range of models suitable for implementation.

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#### Budget

Equipment

Item	Quantity	Description	Total Cost, \$
		Literature review	
Computers	4	Powerful stationary computers, processor cache and RAM more than 12 GB, 8 cores or more (\$3,000 per device)	12.000
Printers	1	Colourful business printer	500
Computer software (machine learning)	4	Windows (4x\$130), Microsoft Office (4x\$40)	680
Hard disk drive	4	HDD 8 TB	3000
Experiment			

Living organisms for	50.000	300 individuals of each species	50.000
the experiment		selected will be bought from reliable	
		suppliers, for microorganisms the	
		calculation in colonies is used	
Animal cages	50	cages of different sizes for keeping	35.000
~ .	1.50	animals	40.000
Containers	150	containers for keeping plants, lichens,	40.000
	-	microorganisms	
Eq for creating the	5	heaters and coolers, sustaining later	150.000
temperature		particular temperature of model	
		environment	
Eq for creating the		NASA equipment rent	10.000
pressure			
Eq for creating of the		NASA equipment rent	100.000
gravitation			
Eq for creating the O2		NASA equipment rent	10.000
concentration			
pH-meter	4	2 stationary pH-meters with the 0.01	1500
		pH accuracy (1000); 2 ExSticks (500)	
barometer	2	stationary barometer	500
Chemicals		agar for microorganism cultivation,	100.000
		acids and metals to create a Mars soil	
		model	
Gas-burner	1	To prevent environment contamination	200
		(microorganism cultivation)	
Gas (air) analyzer	2	gas combustion analyzer (for most	1250
· · ·		frequent gases)	
		Analysis	
software	2	Stata/MP for the largest datasets, for	2270
		multiple users (business network)	-
Overall Equipment Cos	t:		\$516.900
• •			

#### Consumables

Item	Quantity	Description	Total Cost, \$		
	Literature review				
Internet bandwidth	100gb/month x 12 months	For efficient and effective literature search.	5000		
	Experiment				
Tubes	10.000	50 mL plastic tubes, 25 mL tubes, 10 mL glass tubes, eppendorfs	36.000		
Field record book	1	For taking records of our experiment	20		
Back-up field record book	1	For taking records of our experiment	20		
Gloves	500 packs	For handling equipment	2500		

Labels and markers	1000 pieces	For labelling and sorting	3500	
Pens	50 pieces	For taking notes	500	
Paper	10 packs	Printing	1000	
Ink for printer	5	For printing documents	1000	
	Analysis			
Data analyst/scientist	2	5000\$ per expert, to ensure all the data are analyzed appropriately	10000\$	
Overall Consumables C	ost:		59, 540\$	

Personal and Field Travel/Accommodation/Subsistence

Description	Total Cost, \$
Office (1 room x 3 years)	36.000
Laboratory rooms (1500\$/month x 4 rooms x 3 years)	216.000
Greenhouse (200\$/month x 3 years)	7.200
Accommodation (4 rooms x 3 years)	144.000
Tickets (Houston - California (SpaceX Central)) (269\$/1 two-way flight x	1076
4 team member)	
Tickets (Poland - Houston) (5,050\$/1 flight + back x 4 team member)	40.400
Healthcare insurance (7470\$/ year x 3 years x 4 team member)	89.640
'Per diem' subsistence allowance (35\$/day x 3 years x 4 team members)	153.300
Visa (190\$ x 4 team members)	760
Overall Travel Cost:	\$558.778

#### Employment (note only casual, short term assistance will be considered)

Position	Description of role	Rate & Duration of employment	Total Cost, \$
	Litera	ture review	
Virtual	Collecting of metadata	2/1/22 - 1/6/22	4000
Assistant		5\$/h, 40h/week, 5 months	
	Exp	periment	
Laboratory	Performing lab activities	2/6/22 - 1/8/24	83.200
workers		20\$/h, 40h/week, 26 months	
Safety	Safety of labourers and	2/6/22 - 1/8/24	83.200
Engineer	laboratory space.	20\$/h, 40h/week, 26 months	
Maintenance	Sanitization of working and	2/6/22 - 1/8/24	41.600
workers	laboratory space.	10\$/h, 40h/week, 26 months	
Technician	Basic technical support for	2/6/22 - 1/8/24	83.200
	all the processes of	20\$/h, 40h/week, 26 months	
	experiment, fixing broken		
	elements.		

Greenhouse Manager	Control of the main experiment processes and time management of project stages.	2/6/22 - 1/8/24, 20\$/h, 40h/week, 26 months	83.200	
Analysis				
Data Analyst	Performing of statistical data2/8/24 - 1/12/248000analysis.10\$/h, 40h/week, 5 months			
Total cost:		386 400\$		

Total project cost	\$1.521.618
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## 4. Should we limit the number of hikers? An evaluation of environmental threats of tourism in Tatra Mountain national parks

#### Applicants names

Barbara Papiż, Tine Roelant, Allison Proszowska, Barış Tanrıverdi

#### Abstract

The two national parks in Tatra Mountains, in Poland and Slovakia, have both seen drastic increases in tourism in recent years, with the annual number of visitors nearing 7 million, as of 2019. This poses a severe threat to the wildlife in the park, including several endemic and endangered plant and animal species. Masses of tourists can contribute to destruction of habitats, disturbance of animals, and pollution of soil and water. To date, no in-depth environmental impact study has been done across the entirety of the two parks, with the monitoring of multiple biological and abiotic factors. Our proposal involves precisely such a study: by closing several sections of the national parks for a period of three years, and comparing to sections with regular human usage, we can effectively analyse the precise impact humans have on the alpine ecosystems. Variables observed will include the appearance and behaviour of two large animal species, namely the black bear (Ursus arctos) and Tatra chamois (Rupicapra rupicapra tatrica), the appearance of rare plant species, (Saxifranga retusa, Chamorchis alpina, Ranunculus glacialis), examination of width of trails and root exposure and the chemical composition and features of the soil near the trails. This detailed analysis will allow us to identify what are some of the biggest threats that humans inflict on the ecosystem, and if reducing the number of hikers will provide any improvements in preserving the nature in this protected area. The results of this project can potentially be used to inform public policies for local governments, or to introduce caps on the number of visitor tickets which can be sold each day. Although these national parks are strictly protected areas, it is still necessary to determine to what extent the high number of tourists may have adverse effects on the natural world, and if this number needs to be reduced to further preserve the area.

Total project cost:	400,000 PLN (84,000 €)	
Project title:	Should we limit the number of hikers? An evaluation of environmental threats of tourism in Tatra Mountain national parks	
Project start date:	May 1, 2022	
Project end date:	December 30, 2024	
Project country:	Poland, Slovakia	
Up to 6 key-words:	National parks, environmental impact, ecotourism, nature preservation, human impact, hiking	

#### Summary of Project Details

#### The two national parks in Tatra Mountains, in Poland and **Project lay summary:** Slovakia, have seen drastic increases in tourism in recent years. The question arises if the parks are effectively protecting the habitat and the endangered species because masses of tourists can contribute to destruction of habitats and disturbance of animals. To our knowledge, no in-depth study on the environmental impact of tourism in the Tatra Mountains across both parks has been conducted. Our proposal involves monitoring black bear and Tatra chamois populations, monitoring three rare plant species examining the width of trails, root exposure and soil features in closed sections of the parks, and comparing them to sections with regular touristic use. This will allow us to identify some of the main threats of human usage on the ecosystem, and evaluate whether reducing the number of hikers is advantageous for the protection of the alpine ecosystem.

#### **Project description**

#### Core project description

#### 1. Background

The Tatra mountains are a large, protected alpine area on the border between Poland and Slovakia, containing the highest peaks of the Carpathian Mountain range. This region can be divided into two sections, the Tatra National Park in Poland (TPN), established in 1954, with an area of 211 km<sup>2</sup>, and Tatra National Park in Slovakia (TANAP), established in 1949 with 738 km<sup>2</sup>. Historically, many of these lowland regions were cultivated for crops and livestock but have been in the process of restoration in the last century or so. However, there is a new threat to the environment in these national parks: tourism. There are nearly 7 million visitors annually, of which over 3 million are on the Polish side, and 3.5 million on the Slovakian side. Currently, there exist no limits to the number of people allowed to enter the parks each day, and during peak tourist season in the summer, there are frequently over 40,000 total visitors every day, in both TANAP and TPN. Both national parks list anthropogenic pressure as a major threat on their information websites. This includes both direct threats such as habitat destruction, disruption of animals' natural movements, and pollution of soil and water in the park, as well as indirect threats such as air pollution from all the traffic in the region, and expansion of tourist industries close to the park boundaries.

#### 2. Aims of the Project

There has not yet been a comprehensive environmental impact analysis done in this region to determine if there is a maximum capacity of visitors that should be enforced, in order to minimize damages done to the parks' natural environments. The number of visitors continues to rise every year, and it may be difficult to predict the long-term impact of humans on the ecosystem. Many small-scale studies have shown the risk to ecosystems, including reduction of vegetation and topsoil degradation near trails, disturbances to animals resulting in behavioral changes, water pollution of mountain lakes, and many other negative impacts (Kołodziejczyk 2020). One animal of high interest in this study is the Tatra chamois (*Rupicapra tatrica*), an endangered, endemic subspecies that has less than 1400 individuals, which appear only in the area of both

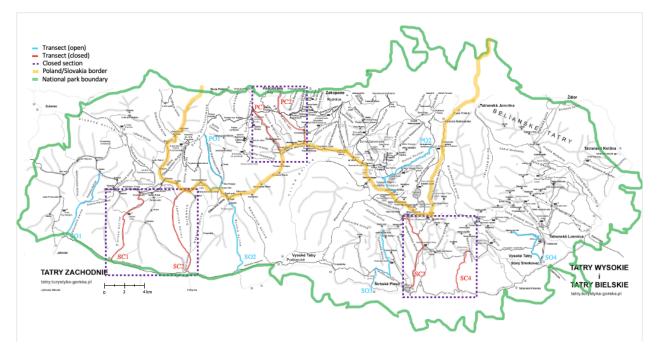
national parks. The brown bear (*Ursus arcotus*) population is also extremely critical, with only about 50-60 individuals in both parks (according to tpn.pl). The purpose of our study is to look at a combination of various factors in the environment and determine how the presence of humans may affect the entire ecosystem. This will be carried out by closing several sections of the park to tourists for a period of three years and monitoring both the closed and open sections. The key variables that will be evaluated are the distribution and behaviors of brown bears and mountain chamois, and assessments of rare plant species and soil factors near trails (trail widening, root exposure and soil composition).

#### 3. Methods

The study site will consist of three sections of the Tatra Mountains, one within the Polish borders and two in the Slovakian park, which will be completely closed off for hikers and backcountry skiers for the three year study period. Two hiking trails will be selected in each section to be used as transects for data collection, and an additional six trails will be selected in the open sections for study, as illustrated in Figure 1. These trails will pass through the entire altitude zonation (from lowland forest region (~900 m.a.s.l.) to the upper alpine region (~2000 m.a.s.l.), over a distance ranging from 6-9 km. Within all open and closed trails, the brown bear, mountain chamois populations and selected plant species will be monitored, and the changes in trail widths, root exposure and soil composition will be assessed. Additionally, the daily number of visitors to both TPN and TANAP will be recorded, based on ticket sales. We anticipate situations when individuals will intentionally or unintentionally enter closed areas despite the ban, however strict fines will be implemented to deter such behaviour.

#### 3.1 Assessment of occurrence and behaviour of large mammals

Brown bear (*Ursus arctos*) and mountain chamois (*Rupicapra rupicapra tatrica*) populations will be observed along the 12 transects, once a week for four months (June-September), following procedure from a similar study performed by Pęksa and Ciach (2015) on chamois. TPN and TANAP already conduct a biannual simultaneous count of all the chamois individuals in the park, so this data can be easily obtained through cooperation with the parks. Additionally, the data from national parks related to bear tracking will be obtained to help with identification. The occurrence and behaviour of both of these mammals will be checked on the entire surface of previously designated areas, particularly taking note of the following characteristics: ages and sexes of individuals (if possible to discern), herd size (chamois), and approximate distance from trail. In addition to field observation, bear records from the GPS will also be monitored, with data from the national parks, and any recorded human-bear conflicts during this study will be noted. The aim of this is to determine how the behaviour and occurrence of large mammals will change in the open and closed areas of the park.



**Figure 1.** Map of national parks in Poland and Slovakia (green area) with planned sections for closure (purple boxes) and 12 selected trails for study: 6 open (blue) and 6 closed (red).

#### 3.2 Assessment of rare plant species

Three rare plant species (*Saxifranga retusa, Chamorchis alpina and Ranunculus glacials*) will be monitored alongside the chosen closed and open trails. These plants are chosen because of their endemicity to the habitat, rarity, and because they are representative of the region. The assessment consists of counting the number of plants, noting the life stage of the plants (flowering, seedling), color intensity, size, and the location (including proximity to the trail). The data will be obtained during the flowering season and counts will take place two or three times a week over a 2-month period.

#### 3.3 Assessment of trail widening, root exposure and soil composition

Two hiking trails will be assessed in each of the three closed sections and six hiking trails in open sections of the parks in total. Their relative changes over time will be measured with respect to trail width and the frequency of root exposure. Measured changes will be compared between the open and closed trails to assess the impact of the humans. Data will be collected once a year at the end of summer season for all trails. In both open and closed trails, trail width is going to be recorded where there is trail widening occurs for at least 1m long and the width of minimum 10 cm. Observations are going to be made in the same recorded spots during the project. Number of problems (unnaturally wide parts of the trails), length of the problem, width of the problem and number of exposed roots in the trails are going to be recorded. Measurements are going to be normalized with respect to length and mean width of the measured trail. Additionally, soil samples will be collected from every kilometre of both open and closed trails. These samples will be stored in the refrigerator at the project headquarters in Zakopane, and once a week will be transport to Kraków, where they will undergo pH, metal content and soil composition tests in the designated university laboratories.

#### 4. Expected Outputs

#### 4.1 Assessment of occurrence and behaviour of large mammals

As there are many tourists in Tatra Mountains especially on the Polish side in the summer, it is expected that the absence of people will make the bears feel more at ease and increase the area of their feeding and migration. Potentially, the difference in their behaviour could be more visible in Polish part of the mountains as trails there have higher tourist density and more common bear sightings. Similarly, we predict changes in locations and behaviour of chamois within the open and closed regions of the park. We may see for example, that chamois are avoiding largely crowded areas, and migrating into the closed sections, where they do not have any disturbances. A significant increase in the populations in the short time of three years is not expected, as bears have an average life span of 20-30 years, and chamois 15-20 years, but such a possibility is not excluded. However, the changes in behaviour and regions which the animals occupy may be significant and can show the severity of human impact and potentially the need for reducing the number of hikers.

#### 4.2 Assessment of rare plant species

The assessment of three rare plant species (*Saxifranga retusa, Chamorchis alpina and Ranunculus glacials*) will provide data on how the populations alongside the trails change with and without human activity. For the plant population in the closed off section of the Tatra Mountains, it is expected that the number of plants will grow overtime and that there will be larger plant populations, compared to the section that remained accessible for hikers. Additionally, we expect that there will be more flowering plants. It is to be expected that the sections with open trails will have fewer number and density of plants, as intentional and unintentional trampling and picking of flowers is a serious problem.

#### 4.3 Assessment of trail widening, root exposure and soil composition

It is known that intense human activity will create trampling which causes further problems such as erosion and loss of plant species. Evaluation of trail widening and root exposure frequencies, magnitudes of the widening, differences in the soil composition and changes in these values by the time will be used to create inference about the human influence on soil quality, erosion, and vegetation loss. Trail widening, root exposure and soil content are expected to be highly related with the number of hikers using the trail and it is expected to find a directly proportional relationship between these observations. Heavy metal concentration, nitrogen and phosphorous levels, pH, number of root exposures and trail widening are all expected to be lower in closed trails than the open trails. Natures' regeneration ability without the human threat and the species' behaviours affected by landscape changes are also other side outcomes that expected to be interpreted from the data.

#### **5. Expected Timescales**

The project will be begun in May of 2022, which is the beginnings of spring in the mountains. First, a brief (2-3 days) paid training will be provided to the workers on how to take and record observations, as well as safety training about working in the field. Then, observations will begin, for flowers, chamois, bears, and trails, for various time periods, as outlined in the methods section. All observations for the first year will conclude by the end of September 2022. The winter months will allow for data input and primary analysis.

For the next two years, the same process will be used, with field observations taken from May to September. Every year measurements will be made in designated times: plants, soil, and trails over 2 months, and bears and chamois over 4 months. The final months of the project (October-December 2024) will allow for final data analysis and results preparation, as well as writing the final report and finalizing the website for results publication. The website will be created at the start of the project, to inform tourists and post regular updates about the project, and also create a forum allowing for comments and feedback from the public.

#### 6. Practical Implementation

We predict that the findings of our study will show that the unlimited number of tourists in both TPN and TANAP parks negatively impacts the environment including plants, animals, and soil ecosystems. Although this is not a comprehensive overview of all the impacts humans may have, we believe it is a good starting point to prompt future research and draw attention to the problems associated with tourism in the Tatra region. This project also opens the opportunity for other scientists or universities to conduct research in the closed regions of the park at the same time. For example, testing water quality is also an important factor, but is beyond the scope and budget of our research plan, however such studies could be coordinated with other interested parties. Although 3 years may be insufficient to observe major changes in nature , we expect to observe regeneration of the nature in selected species and areas. Outcomes of this project can be used for research purposes and can create a pathway for future projects, and the methods can be applicable to other mountain ecosystems and protected areas to observe human impact. It is possible that our research will result in a recommendation to limit the number of tourists, through policy changes or limiting the number of park entry tickets sold.

#### **Additional questions:**

### What are the risks to the health and safety of those involved in the project and how are these risks to be minimised?

For this project, we will employ several dozens of part-time workers to aid with the transect studies and observations, along with research staff and students from Jagiellonian University. As they will be walking through the park, they will face risks associated with typical alpine tourism including adverse weather conditions, personal injuries, or problems such as dehydration/lack of food, and threats from wildlife. Many of these are avoidable and possible to prepare for. While hiring employees, prior wildlife experience, and physical fitness will be an important criterion. All of the field workers will have an insurance with a coverage of potential health risks. We will also provide all employees with training on basic first aid and bear/animal safety prior to starting work. Throughout their time working, water and supplies will be provided at the headquarters, for them to take to prepare for a day in the field. All employees will be regularly updated on any current issues in the park which may affect their health or safety, such as high avalanche risks or severe weather forecasts (in which case work would be postponed), or any recent bear attacks or other dangers to humans. In the case of any emergencies, any workers will also be able to shelter or seek help in any of the TPN or TANAP's mountain shelters.

### Please provide details of agreed collaborations and project partners that will facilitate the proposed project:

This project will rely on collaboration with TPN and TANAP, to provide information on the number of visitors daily, as well as current and historic data regarding bear and chamois populations and any ongoing counts. Potentially we can also collaborate with a university in Slovakia, for example Comenius University in Bratislava, which has an excellent environmental science department, who may be interested in participating in such research. Jagiellonian University may also assist with this project by connecting us with student workers, possibly allowing them to earn ECTS points while participating in this project. Lastly, we will coordinate with the mountain shelters in Poland and Slovakia, asking them to take in any of our workers who need food, water, shelter, or first aid, especially in emergency situations.

### Please provide details of the suitability of the institution where the work will be carried out and the availability of equipment and facilities required for the work:

The main institution where the data analyses will be carried out, planning will be done, and the location of researchers' offices is the Jagiellonian University in Krakow, Poland. The distance between the University and the TPN is 108km which is approximately 2 hours by car. The office space in the Zakopane town will be rented out and used as headquarters which will create more easily accessible space near the park. This space will be used to store all the essential equipment that is needed in the park for field observations, and to store the refrigerated soil samples. Field assistants, coordinator and the researchers will also use this point as a main meeting spot near the park. All the collected data will be stored in the computer in the office and then distributed later for further analysis. To overcome the possible emergency situations in the research field; designated sleep shelters will be provided to field workers as a result of the collaboration with the Board of The Polish National Parks Federation and the administrations of the TPN and TANAP.

#### Please provide details of necessary permits/licences obtained, if applicable:

First, it is important to complete all formalities at the Jagiellonian University as an institution whose research team is applying for the program. Due to the fact we plan to hire employees to help with the project, it is necessary to formulate contracts in cooperation with the university or a legal advisor. Perhaps it will be needed to start an official activity as a non-profit organization. It is meaningful to contact and obtain consent from the Tatra National Park in Poland and Slovakia to conduct research and close the sections. It is obligatory to inform people that their presence is going to be monitored during research and only for the purposes of the project. An agreement is also planned with the authorities of the city of Zakopane, the townships of Kościelisko, Poronin and Bukowina Tatrzańska, the Małopolskie voivodship. It assumes the appearance of joint benefits from running the project and the development of legal rules that will be introduced exceptionally during the project duration. In the same way, it will be necessary to contact the local government in Slovakia.

### Please indicate how you will assess the scientific impact of the project and the benefits for non-academic stakeholders:

We predict that our study will impact large and diverse groups of people such as the local community, tourists, but also business owners, the national park itself and the volunteers who have worked on this project. In order to assess the impact, an accessible free website will give an opportunity for each stakeholder to share their thoughts and notes about the changes implemented after our study. Besides that, we also want to provide a way for stakeholders to talk in person about the impact by conducting interviews.

Furthermore, we predict that decreased overcrowding as a result of limited ticket sales will positively impact the local community and the tourists themselves. The decrease of visitors to the parks will provide benefits for the hiking tourists, as they will have a more enjoyable and peaceful experience in the park, without having to deal with mobs of people. We also predict that as a result of limited ticket sales every day the number of people visiting will be more evenly spread throughout the year, instead of a high concentration of visitors during summer days. This development could positively impact the business stakeholders by providing clients year-round. However, there is also the possibility that the business will suffer financial losses related to the changes. Local community may experience a better quality of life related to the reduction of traffic jams and crowds. All of it would be implemented under the influence of stricter conservation laws introduced by local government. If the number of people entering the parks will be limited, the national parks could increase their ticket prices to outbalance the loss of numbers of people (this can also be used as a method to deter as many visitors as possible). However, these changes will not affect the residents of Zakopane and other local towns, as they will still be allowed free entrance into the park as per the current system.

#### How do you intend to make your research data publicly available?

In order to make our data available for a large audience, both scientists and non-scientists, we would create the beforementioned website with our results explained both on scientific and popular science level. Also, the raw data will be free and available here. The online platform will allow us to create surveys among users and get know what opinions they have. This website will be online for the 10 years after the starting date of the project. The national park websites and social media accounts can advertise our website and project details, so visitors easily find our research. Besides that, our goal is to publish at least one high impact paper in a scientific journal focused on nature preservation. In addition, flyers will be given to the visitors to inform them about the ongoing project and will show closed sections on the mini map.

#### Please provide details of any published papers relevant to this project:

- Kołodziejczyk, Krzysztof. (2020). "The negative impact of hiking on the mountain environment: the position of Polish scientists in comparison to global literature." *Folia Turistica* 55, 85-115.
- Matysek, Marcin, Gwiazda, Robert, Zięba, Filip, Klimecki, Maciej, Mateja, Radosław, Krzan, Piotr. (2020).
  "High Tourism activity alters the spatial distribution of Hazel Grouse (*Tetrastes bonasia*) and predation on artificial nests in a high-mountain habitat." *Ornis Fennica* 97, 53-63.
- Pęksa, Łukasz and Ciach, Michał. (2015). "Negative Effects of Mass Tourism on High Mountain Fauna: The Case of the Tatra Chamois Rupicapra Rupicapra Tatrica." *Oryx*, v. 49, 500-505.
- García-Rodríguez, Alberto, Nuria Selva, Tomasz Zwijacz-Kozica, Jörg Albrecht, Clement Lionnet, Delphine Rioux, Pierre Taberlet, Marta De Barba (2021). "The bear-berry connection: Ecological and management

implications of brown bears' food habits in a highly touristic protected area." *Biological Conservation*, 264, 109376.

#### Budget

#### Equipment

Item	Quantity	Description	Total Cost (PLN)
Office Computer 1 For data input and analysis,		For data input and analysis,	3,000
		organization in Zakopane	
Printer	1	For internal office use, printing data	2,000
		sheets for field work	
Refrigerator	1	For storing soil samples in the office	500
Website creation	1	Domain name and website design	5,000
Website hosting and	10	Server hosting for 10 years	12,000
domain (annual)			
Reusable water bottles	20	Provided to all workers	500
First aid kits	15	Provided to all workers for safety	750
Bear spray	15	Provided to all workers for safety	1,500
Binoculars	8	For bear and chamois observations	800
Tape measure	2	For trail measurements	100
Soil sampling probe	4	For obtaining soil samples	200
and sieve			
Signs for park closure	6	To notify hikers at park entrance and	2,400
(large)		start of trail	
Signs for park closure	50	To notify hikers, placed throughout	5,000
(small)		the park, at trail intersections	
Overall Equipment Cost	•	I	33,750

#### Consumables

Item	Quantity	Description	Total Cost (PLN)
Notebooks	50	For workers to take field notes	500
Pens and Pencils	200	For workers to take field notes	100
Plastic containers with	50	For soil sampling	300
lids (reusable)			
Glass beakers (100 mL)	200	For testing metals and pH of	2,000
		soil	
Information pamphlets	100,000	To inform tourists about the	30,000
for park closures		ongoing project, closures, etc.	

Overall Consumables Cost:		32,900

Personal and Field Travel/Accommodation/Subsistence

Description	Total Cost
Office space in Zakopane (including furniture, 15 months, 5 for each year	15,000
of the project)	
Transportation to Zakopane (1 trip per week for 15 months, from Kraków	8,000
and return - 200 km)	
Transportation Poland-Slovakia (6 trips per week for 15 months – 50 km)	10,000
Accommodation for 1 person (project manager) for 15 months in	30,000
Zakopane	
Overall Travel Cost:	63,000

#### Employment (note only casual, short term assistance will be considered)

Position	Description of role	Rate & Duration of	Total Cost
		employment	(PLN)
Field coordinator	Point of contact in Zakopane	25 pln/hour, 6 days per week	57,600
	headquarters, plans	(8-hour days) for 4 months	
	observations for all assistants,	each year	
	fills in taking measurements		
	when needed. Assists with		
	data input and organization.		
4 Field assistants	Workers will be taking plant	20 pln/hour	61,440
(plants)	observations in the field.	4 days per week (8 hours/day)	
		for 2 months each year	
4 Field assistants	Workers will be walking	20 pln/hour	122,880
(chamois and	transects, observing chamois	4 days per week (8 hours/day)	
bears)	and bear appearance and	for 4 months each year	
	behaviour.		
4 Field assistants	Workers will measure trail	20 pln/hour	61,440
(trails and soil)	width and other factors for	4 days per week (8 hours/day)	
	each trail once per year.	for 2 months each year	
Paid training for all	Safety training and teaching	20 pln/hour	15,600
employees	them how to properly take	13 workers, 20 hours training	
	and record measurements,	every year	
	and familiarization with park		
	terrain		

Insurance policy	Covers up to 250,000 PLN	200 PLN per person per year	8,000
	per person for on-site injury		
Overall Employment	Cost:		326,960

Total project cost	456,610 PLN