# ASSESSMENT OF MOSSES AND LICHENS IN ANTARCTICA AND BULGARIA BASED ON REMOTE SENSING AND CHLOROPHYLL FLUORESCENCE

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***Keywords:*** *Chlorophyll fluorescence, Destination Earth, lichens, LST, moss, NDGI, photogrammetry*

***Abstract:*** *Chlorophyll fluorescence is the emission of light by chlorophyll molecules when they are excited by absorbed light. Chlorophyll is the pigment responsible for photosynthesis, the process by which plants and other photosynthetic organisms convert light energy into chemical energy, which varies in different values for different latitudes.*

*The aim of the research is an attempt to compare the spectral characteristics of mosses and lichens, and note any variations in their fluorescence intensity from the region of Livingston Island, Antarctica and Vitosha Mountain in Bulgaria during the summer season in the southern and northern hemispheres.*

*Field research was carried out in Antarctica and Bulgaria, in order to verify the data from Sentinel 2MSI, drone photography and photogrammetry, as well as photography by a thermal camera with a measurement accuracy of +/- 2°C and a wavelength of 8 - 14 μm .*

*A spectrometer was used to analyze the visible range from 380 to 780 nm and the spectral range in which Sentinel 2MSI and Sentinel 3 SLSTR images are generated.*

*The main research methods are through chlorophyll fluorescence response and the use of several optical indices for remote sensing monitoring such as NDVI (Normalized Difference Vegetation Index), NDGI (Normalized Difference Greenness Index), composite images and LST (Land Surface Temperature) analysis.*

*The spectral curves from different days of the field research, which were carried out in the summer season in Antarctica and Bulgaria in 2022 and 2023, were analyzed.*

*The assessment was made by mapping with GIS technologies, laboratory and field studies, Open and spatial data, which are published for free access.*

*The research is part of project No. 70-25-59/10.08.2022, on the topic: "Destination Earth Antarctica - Digital data space, pilot project", funded under the Polar Scientific Research for Young Scientists program, part of the National Polar Scientific Program of the Bulgarian Antarctic Institute.[1].*

# ОЦЕНКА НА МЪХОВЕТЕ И ЛИШЕИТЕ В АНТАРКТИКА И БЪЛГАРИЯ НА БАЗА ДИСТАНЦИОННИ ИЗСЛЕДВАНИЯ И ХЛОРОФИЛНА ФЛУОРЕСЦЕНЦИЯ

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***Ключови думи:*** *Хлорофилна флуоресценция, Дестинация земя, лишеи, LST, мъхове, NDGI, фотограметрия*

***Резюме:*** *Флуоресценцията на хлорофил е излъчването на светлина от хлорофилните молекули, когато те се възбуждат от абсорбираната светлина. Хлорофилът е пигментът, отговорен за фотосинтезата, процесът, при който растенията и другите фотосинтезиращи*

*организми преобразуват светлинната енергия в химическа енергия., която варира в различни стойности за различните географски ширини.*

*Основната цел на изследването е опит за сравнение на спектралната характеристика на мъхове и лишеи, и отбелязване на всякакви вариации в интензитета на флуоресценцията им от района на о-в Ливингстън, Антарктика и планина Витоша в България през летен сезон в южното и северното полукълбо.*

*Направени са теренни изследвания в Антарктика и България, с цел да се верифицират данните от Sentinel -2MSI чрез дрон заснемане и направена фотограметрия, а също така и заснемане чрез термокамера с точност на измерване +/- 2°C и дължина на вълната 8 - 14 µm.*

*Използван е спектрометър за анализ във видим диапазон от 380 до 780 nm и спектралният диапазон, в който генерират изображения Sentinel 2MSI и Sentinel 3 SLSTR.*

*Основните методи за изследване са чрез реакция на Флуоресценцията на хлорофила и използването на няколко оптични индекси за дистанционен мониторинг като NDVI (Нормализиран разликов вегетационен индекс), композитни изображения и анализ на температурата на земната повърхност (LST – Повърхностна земна температура).*

*Анализирани са спектралните криви от различни дни на теренните изследвания, които са извършени в сезон лято в Антарктика и в България през 2022 и 2023 година.*

*Оценката е направена чрез картографиране с GIS технологии, лабораторни и теренни изследвания, Отворени и пространствени данни, които са публикувани за свободен достъп.*

*Изследването е част от проект № 70-25-59/10.08.2022 г., на тема: "Дестинация Земя Антарктика – Дигитално пространство за данни, пилотен проект", финансиран по програма Полярни научни изследвания за млади учени част от Националната полярна научна програма на Български Антарктически Институт. [1].*

**Introduction**

Antarctica's icy landscape has become decidedly greener in recent years [2].

About 100 species of mosses have been recorded from Antarctica. They are typically small leafy plants, either upright or creeping [3]. In the climatically milder northern part of the Antarctic Peninsula, there are 93 species of lichen, along the coast of East Antarctica - 70 species. In total, about 300 lichen species grow in Antarctica [4].

326 species of mosses are known in Vitosha Nature Park, which represents about 47% of the moss flora of Bulgaria. They are distributed in all mountain belts and inhabit different types of natural habitats. There are 13 rare species of mosses, 8 vulnerable and 7 endangered species [5].

About 500 species of freshwater algae have been established on the territory of the "Vitosha" PP. About 360 lichen species have been described, and 22 of them are found only within the Vitosha Nature Park [5].

By drilling down into layers of moss that have accumulated on the southern continent over the last 150 years, researchers discovered that those diminutive plants have done more growing than usual in the last five decades [6].

Temperature increases over roughly the past half century on the Antarctic Peninsula have had a dramatic effect on moss banks growing in the region.

If this continues, and with increasing amounts of ice-free land from continued glacier retreat, the Antarctic Peninsula will be a much greener place in the future [6]

Mosses in general grow very slowly, and in cold Polar regions, they accumulate rather than decompose at the end of the growing season. This provides researchers with growth records going back thousands of years, similar to peat accumulations in other regions of the world [6]

The Antarctic Peninsula harbors the highest diversity of terrestrial vegetation on the Antarctic continent [7, 8], which must cope with extreme temperatures, high radiation, and strong wind conditions [7, 8, 9, 10, 11], as well as freeze–thaw daily cycles. Among the organisms that stand out in these environments are lichens; these are considered stress-tolerant due to their longevity, low nutritional requirements, and specific adaptations [12, 13].

The aim of the research is an attempt to compare the spectral characteristics of mosses and lichens, and note any variations in their fluorescence intensity from the region of Livingston Island, Antarctica and Vitosha Mountain in Bulgaria during the summer season in the southern and northern hemispheres.

The main research methods are through chlorophyll fluorescence response and the use of several optical indices for remote sensing monitoring such as NDVI (Normalized Difference Vegetation Index), composite images and LST (Land Surface Temperature) analysis.

Spectral curves from different days of in situ research, which were carried out in the summer season in Antarctica and in Bulgaria in 2022 and 2023, were analyzed.

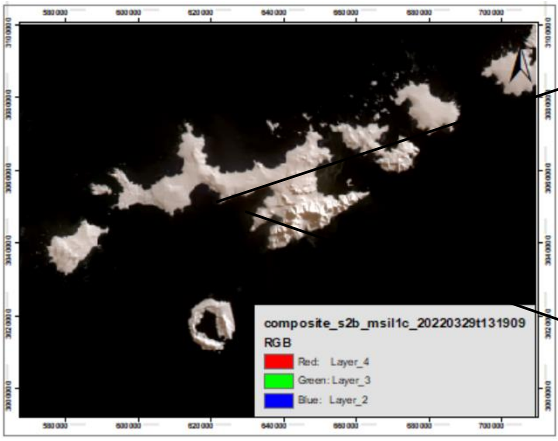
The assessment was made by mapping with GIS technologies, laboratory and field studies, Open and spatial data, which are published for free access.

In the research, a complex approach was used, which was used in an identical way for both territories.

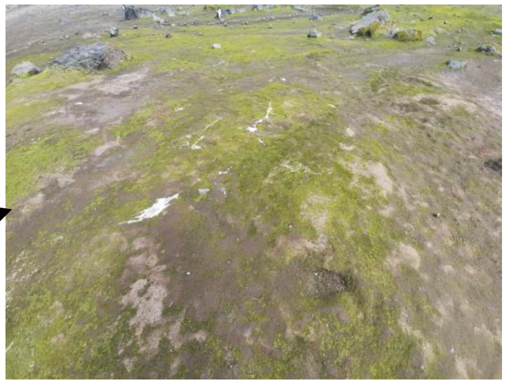
In the study, experiments were carried out to check the ability of mosses and lichens from places with significant differences in climate to absorb and use sunlight thanks to the presence of chlorophyll in them and through chlorophyll fluorescence.

**Study Area**

In situ studies of mosses and lichens from two different locations were made. One is from Cape Hana Point and the Bulgarian Antarctic Base St. Kliment Ohridski, Livingston Island, Antarctica, and the other is Aleko Hut, Vitosha Mountain, Bulgaria (Fig. 1, 2).
The territories were selected due to available field research of the author during the summer of 2022 and 2023 in both hemispheres of the Earth.




a) Sentinel-2 composite images (Bands 4, 3, 2)

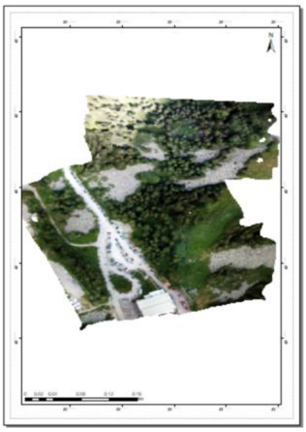


b)

c)

Fig. 1. Area of interest - Livingston Island (a), b) Cape Hannah, (c) territory around the Bulgarian Antarctic base, object 1, author Temenuzhka Spasova

a



b

Fig. 2. Area of interest (a) mountain Vitosha b) Photogrammetry of a drone image from mountain Vitosha, object 2, author: Temenuzhka Spasova

## Materials and Methods

### Satellite data

The satellite data used in the study are from the Sentinel-2-B, Sentinel 3A and Sentinel 3B satellites of the European Space Agency (ESA) [14, 15] Sentinel-2B is a Multi-Spectral Instrument (MSI) [16] recording data in the optical range with different spectral resolutions. Data by dates and satellites used are represented in Table 1.

Table 1. Image acquisition dates, Copernicus (ESA)[15]

| Satellite | Date | Satellite | Date |
|---|---|---|---|
| Sentinel-2-A<br>MSI | 22/07/2022 | Sentinel 3A | December and July<br>22/07/2023<br>29/07/2023<br>12/12/2022<br>07/12/2022<br><br>LST<br><br>Land Surface Temperature |
| | 07/12/2022<br>16/01/2023 | Sentinel 3B | |

The data from Sentinel 3 are from the month of December and July for 2022 and 2023. The selected months are from the summer seasons in the different hemispheres of the Earth. The data are from the two satellites S3A and S3B.

Sentinel 3 SLSTR Level-2 LST [17] provides land surface parameters at 1 km resolution. In addition, the file is measured with the Land Surface Temperature (LST) value calculated and specified for each pixel transformed to degrees Celsius.

### Spectrometer data

A Sekonic C-800-U mobile spectrometer was used for field research. The device measures natural light and flash, from 380 to 780 nm, a wide measurement range of color temperature (1600 to 40,000 K). Also measures: color rendering properties (CRI, SSI, TLCI, TLMF, TM-30-18), color control parameters (Hue/Saturation, CIE 1931 x, y chromaticity coordinate) and other. The C-800 Series Utility software can be open downloaded from Sekonic's official website [18].

### Ground Control points (GCP)

The following ground control point (GCP) locations were generated and applied for the purpose of the survey: generated on 12/12/2022, 15.12.2022 for Antarctica and 30/07/2023 for Vitosha mountain. The points were generated using OruxMap in offline mode and published for open access.

### Thermal camera data

Thermal Camera HT-19 has an IR image resolution of 320x240 and a temperature range of -20°C to 300°C with Measurement accuracy of +/-2°C, 5 types of color palettes and wavelength 8 - 14 μm.

### Drone data

The last data source is the DJI Mavic 2 drone. The drone photography was used to correctly verify the environment, in which the experiment was made and for the complex remote sensing monitoring approach. The Drone images are from the days of field measurements 12/12/2022 for Antarctica and 30/07/2023 for Vitosha mountain (figure 2). The shooting height is below 100 meters and the accuracy is high enough for the research needs.

### Laboratory experiment

A laboratory experiment for chlorophyll fluorescence was carried out in the following sequence [19, 20, 21]:

1) mosses from both areas of interest were carefully washed to remove any sand particles accompanying their rhizoids;

2) the green parts of the mosses were carefully separated from the rhizoids and placed in test tubes. 30ml of 70% ethyl alcohol was added to the test tubes (the lower percentages are in order not to damage the structure of the mosses and the chlorophyll that is extracted from them). This step was performed at different times for the two sites of interest since the summer season is selected for the northern and southern hemispheres in the methodology;

For Antarctica, the experiment takes place in May 2023 due to the transportation of the materials and for the mosses from Vitosha in the area around the Aleko hut on July 31, 2023 from Vitosha Mountain.

Several types of mosses were taken for in situ research. For the purposes of the experiment, the mosses of the class Bryopsida (Fig. 3) were used for Bulgaria, and for Antarctica: Polytrichastrum alpinum, Schistidium antarctici are species found only in Antarctica: Bryum pseudotriquetrum and Ceratodon purpureus.

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Fig. 3 A species used for a laboratory experiment from Vitosha Mountain [19]

3) the tubes with the pieces of mosses and the ethyl alcohol were placed in a steam bath at approx 100ºC until the chlorophyll leaves the moss cells and separates into the solution. The solution is fine strained;

4) The tube with the extracted chlorophyll was placed in a dark place and illuminated lengthwise of the tube with a flashlight by UV light [22, 23, 24];

### Model of the Methodology

The description of the model includes the elements that are detailed in Fig. 4.

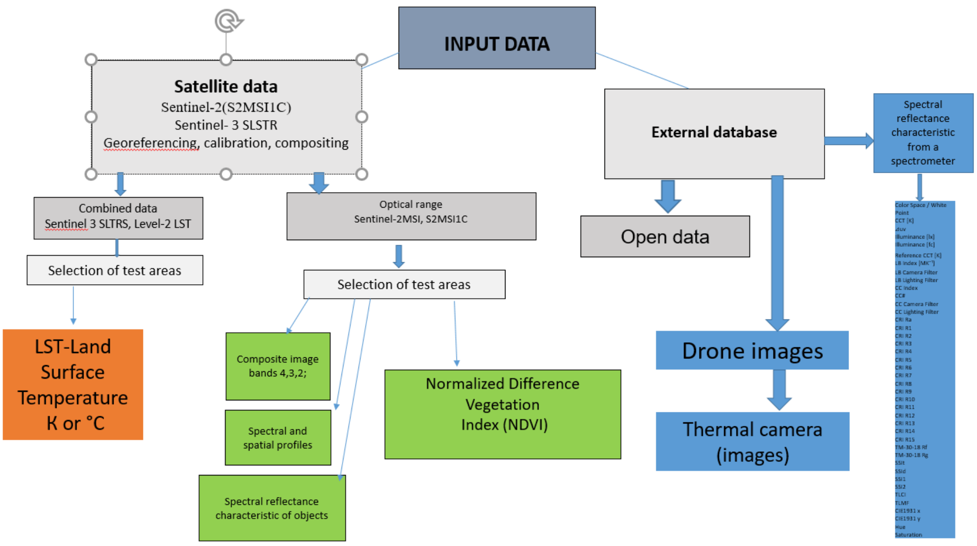



Fig. 4. Model of the methodology for mosses and lichens Author: Temenuzhka Spasova [25]

**Results**

The use of optical indices is not an innovation in this study, but rather a way to quickly indicate the presence of vegetation and mosses allow analysis based on satellite data (Fig. 5).

Site 2 is definitely in an area with very high vegetation and this is clear from the field surveys and from the NDVI index values (Fig. 5).

As a result of the transformation of LST (Fig. 6) images from Kelvin in degrees, it is possible to clearly evaluate the climatic changes in a specific territory and to distinguish the places where points such as heat islands are formed.

In any case, from an already done study based on spectral curves [25, 26] and indices proved that the indices can be used as indicators for the presence of mosses and other vegetation especially in Antarctica , as we have seasonality of in situ studies.

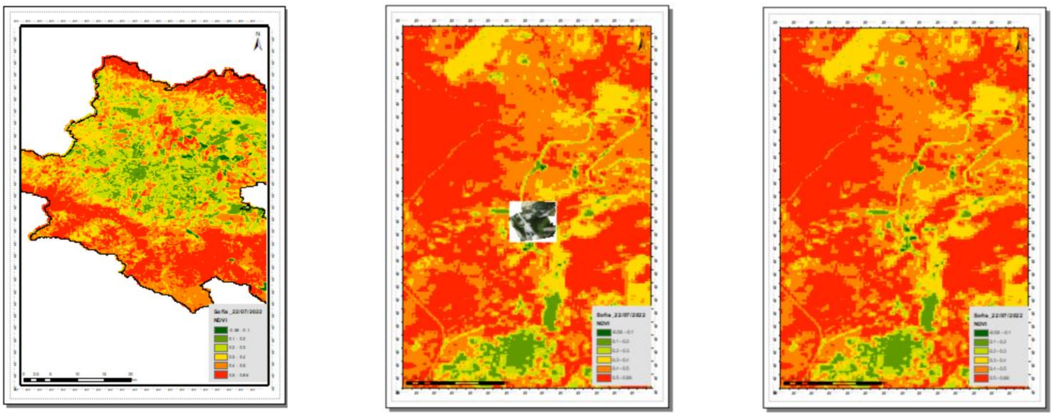


Fig. 5. NDVI- Sofia city and area of interest (AOI), Sentinel 2MSI -22/07/2022

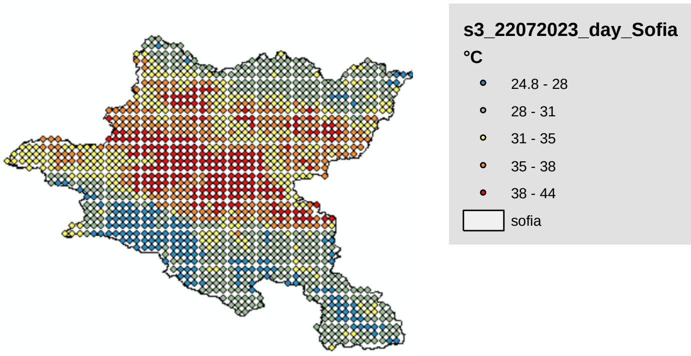



Fig. 6. LST- Sofia city, Transformed image in °C, Sentinel 3, 22/07/2023 day

Regarding the NDVI values in Antarctica (Fig. 7), it can be said that of the sites that were visited in the field research, Hana Point is definitely one of the greenest places, and this is also due to the higher temperature and the presence not only of flora but also of fauna. And since the weather there is very dynamic, the differences in LST in the 5-day period are also visible, which is very noticeable in the colors of Fig. 8.

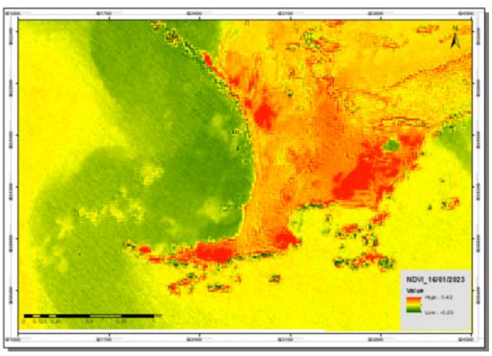


Fig. 7. NDVI- Cape Hanna and area of interest (AOI), Sentinel 2MSI -16/01/2023

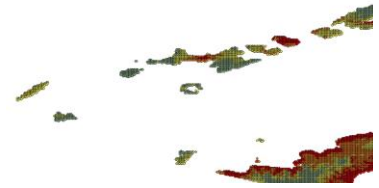
| | | |
|---|---|---|
|  S3_12/12/2022_day |  S3_07/12/2022_day |  S3_12/12/2022_day |

Fig. 8. LST- Livingston Island, Transformed image in °C, Sentinel 3, 07/12/2022 day and 12/12/2022 day

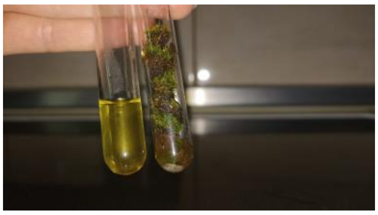
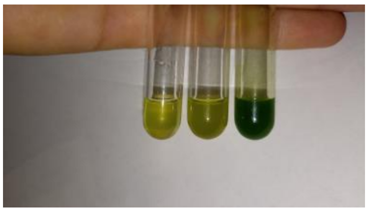
| | | |
|---|---|---|
|  |  |  |
| a | b | c |

Fig. 9. Species used for chlorophyll fluorescence measurement from object 1 and object 2

Figure 9 shows the experiment step by step. The third figure represents the test tube with material from site 1, number two is from site 2, and number three is different fresh vegetation for experiment verification. It is clearly seen that the amount of chlorophyll varies in different values.

The light causes the green chlorophyll molecules to fluoresce red, which is actually chlorophyll fluorescence itself. It was observed that the effect of chlorophyll fluorescence was not strongly manifested even after changing the amount of ethyl alcohol in a test tube from site 1, the amount of mosses and even the temperature they are subjected to in the extraction of chlorophyll.

The spectral curves are similar at first glance, but differ in color temperature values because much higher values are visible for Antarctica.

| | | |
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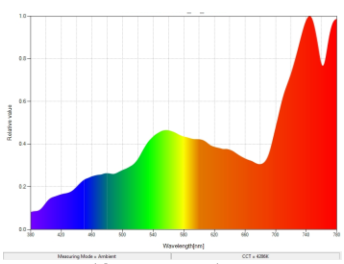
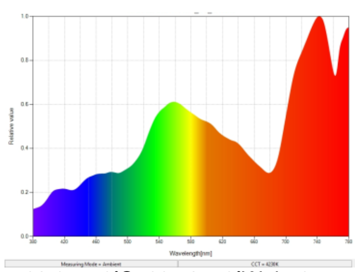
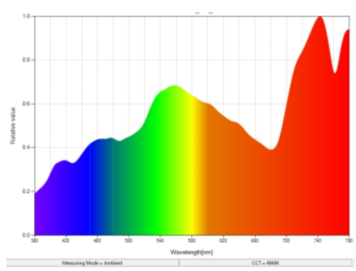
|  62.64802˚S, 60.58698˚W, h=3m |  62.64776˚S, 60.58440˚W, h=0m |  62.64778˚S, 60.58468˚W, h= -17.4m |
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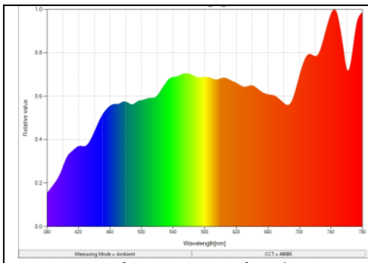
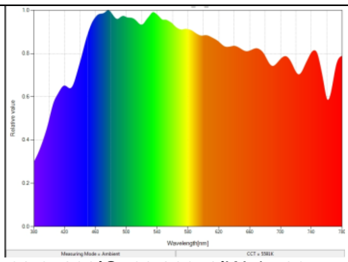
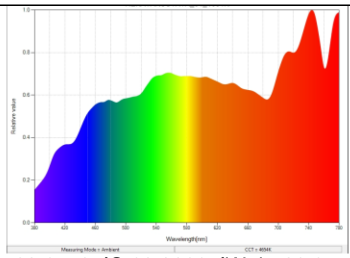


62.64197˚S, 60.36661˚W, h=31m

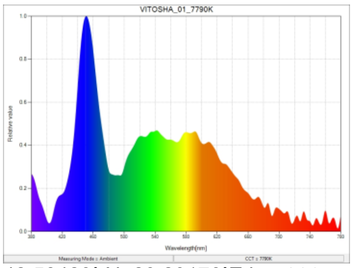


62.64202˚S, 60.36678˚W, h=36.7m

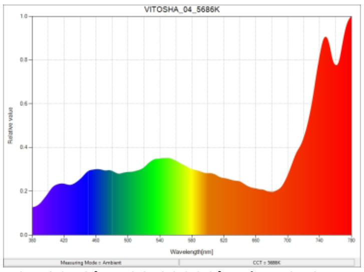


62.64194˚S 60.36665˚W, h=30.6m

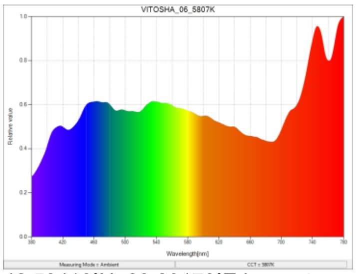
Fig. 10. Spectral reflectance characteristics of mosses and lichens by a spectrometer;
BAB, Antarctica and Cape Hannah, object 1



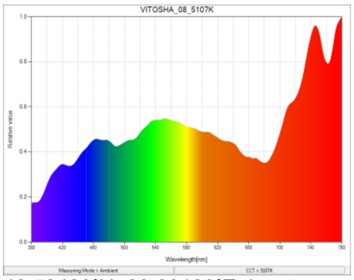

42.58423˚ N, 23.29176˚E,h=1806 m



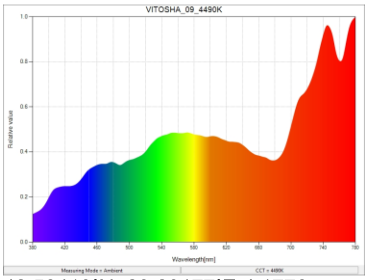

42.58273˚N, 23.29236˚E, h=1850 m



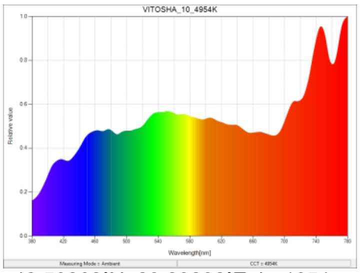

42.58416˚N, 23.29178˚E h=17791 m




42.58420˚N, 23.29180˚E, h=1811.m




42.58419˚N, 23.29177˚E, h1776 m




42.58262˚N, 23.29232˚E, h=1851m

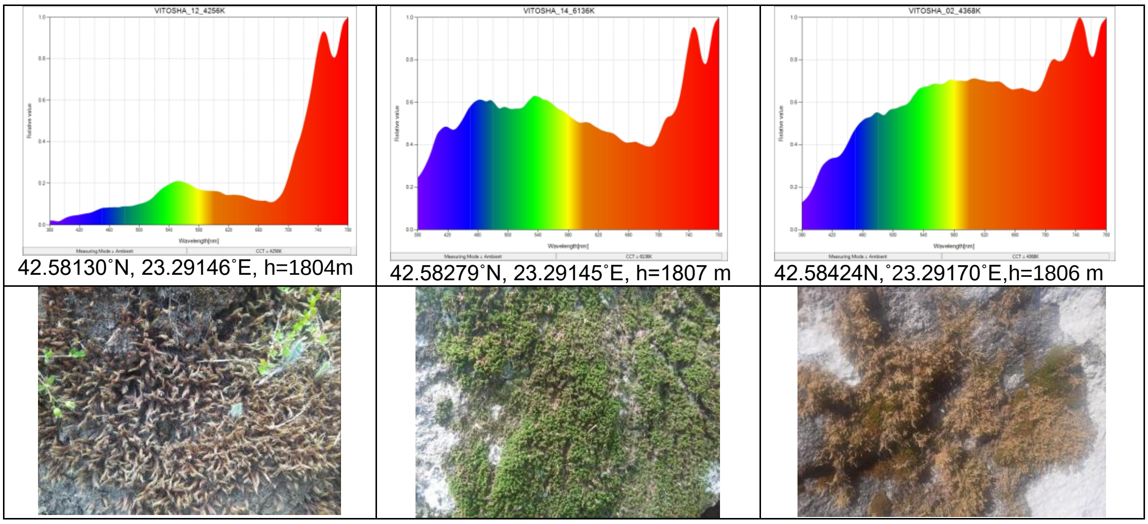



Fig. 11. Spectral reflectance characteristics of mosses and lichens by a spectrometer, Vitosha Mountain, object 2

The thermal images (Fig. 12) are only from site 2, but they can successfully verify the average temperature data, since the average temperatures are the same values of about 24 degrees as in the LST images. The minimum temperatures are lower, but we are talking about the Vitosha Mountain, shadier and moist places with the presence of moss and above all on rock surfaces, which is a sure sign of maintaining higher humidity.

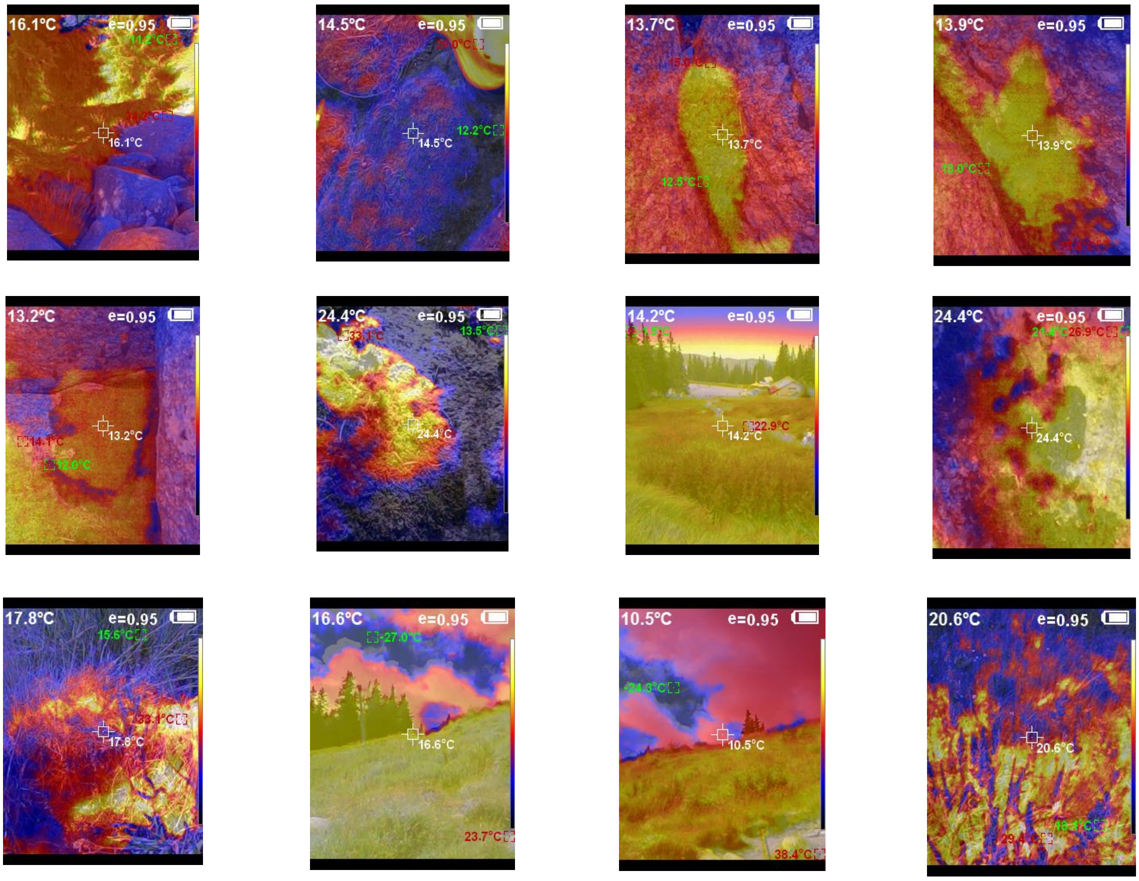



Fig. 12. Thermal images from Bulgaria (object 2) Vitosha Mountain, object 2

**Conclusion**

The use of data from the Copernicus program gives a sufficiently clear idea of the terriotrial dynamics in the development of mossy vegetation, but it would be more difficult to use them in the analysis of lichens due to their specific structure regardless of the geographical location. Through in situ research, reliable information was obtained to be used in validating the results of a comparative analysis of the two territories, and the availability of drone image further verifies the satellite data.

The data from the spectral profiles made by a spectrometer are extremely close in values when examining dry or wetter mosses, and therefore the geographical coordinates also testify. High values of the color temperature, which is measured in Kelvin, are higher in shadier places, but this is a sure sign of a lower ambient temperature.

LST (Land Surface Temperature ) from Sentinel 3 SLSTR is a reliable indicator of the studied terrain and of climate change.

Conclusions for chlorophyll fluorescence:

1: Mosses from Vitosha are greener, wetter, with significantly more chlorophyll than those in Antarctica; (this is due to the fact that the mosses from Antarctica were drier than those from Vitosha, due to their transport time to Bulgaria. For more accurate results in the following studies, it is good to conduct them on site in Antarctica to have with more precise results and fresher samples with sufficient moisture and chlorophyll);

2. The chlorophyll solution from the Antarctic mosses is lighter, less dense, with less chlorophyll compared to that of the Bulgarian mosses. However, the results show that the presence of chlorophyll in the mosses is not sufficient for chlorophyll fluorescence to occur. For such a process, the cells must be significantly richer in chlorophyll for there to be enough of it in the sample for its cells to fluoresce red. For comparison, the experiment was carried out with leaves from other vegetation (eg. celery) where the chlorophyll content is significantly higher, and the effect was achieved.

3. Lichens were not used in this experiment due to the impossibility of extracting their chlorophyll. As a result, instead of excreting their chlorophyll, they absorb the ethyl alcohol, making it impossible to extract chlorophyll from their cells.

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