# Ecological and physiological characteristics of snow algae from Czech and Slovak mountains

## Ekologické a fyziologické charakteristiky kryosestonních řas z českých a slovenských hor

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## Abstract

Algal communities growing in melting snow represent an interesting example of organisms living in extreme conditions. The aim of this work was to study ecological and physiological demands of algae from Czech and Slovak mountains' snowfields in situ and in culture. During the seasons 2000-2002 algae were sampled, transferred to laboratory, examinated, determined and four strains were isolated – *Chloromonas nivalis*, *Chlorella vulgaris*, *Stichococcus bacillaris* and *Xanthonema hormidioides*. Crossed gradients of temperature and light were used to learn about their ecophysiological limitations. The species *Chloromonas nivalis* expectedly appeared to be psychrophilic while the other species turned out to be psychrotolerant with temperature optima higher than 20°C. Light optima were very similar for all the species. Two cyanobacterial species new for the Czech Republic were found - *Romeria nivicola* in the locality Luční hora and *Aphanocapsa nivalis* in the locality Plešné jezero.

## Introduction

The occurence of snow algae is limited to permanent and semipermanent snowfields or glacier surface and, therefore, to higher altitudes and latitudes. The main season comes when there is no more snowfall and the snowfields melt; in high mountains it is usually a short period (according to altitude late spring till early fall), in polar areas it can last for the whole summer season or a longer part of it (KOL 1968, HOHAM 1980).

In the Czech Republic, populations of snow algae were recorded in the Krkonoše Mts. (FOTT et al. 1978, KOCIÁNOVÁ et al. 1989) and in the Šumava Mts. (LUKAVSKÝ 1993); the research on snow algae has a long tradition in Slovakia (e.g. KOL 1968, 1975, KOMÁREK et al. 1973). The aims of this study were firstly to find localities where snow algae occur, to observe algal communities in their environment and to learn about their life cycles; secondly to find their ecophysiological demands in laboratory, and lately to compare the strains regarding their relation to light and temperature.

**Localities**

Krkonoše Mts., Czech Republic

**Labský důl:**

In the upper part of the valley (900-1100 m a.s.l.) by the meandering River Labe, snowfields persist until May or June. The slope declination varies from 0° to 10°, and exposure is to SE-E. Fields are shaded by an open spruce stand; plant dominants are *Vaccinium myrtillus, V. uliginosum* and *Sphagnum* sp. The snow surface is contaminated with needle fall, soil particles and wind sediment.

**Luční hora:**

On the terraces (1450-1550 m a.s.l.), snowfields persist until July or August. Declination is 10° - 20°, and exposure all round. No shading vegetation is present; the snow surface is contaminated with eolic sediment only.

Šumava Mts., Czech Republic

In these mountains, the occurence of snow algae depends on the amount of snow precipitation and spring temperatures. Snow algae were sampled in one locality:

**Plešné jezero:**

The steep slope above the lake (1090 m a.s.l.) can be covered with snow until late May. Declination is 0° - 40°, exposure to NE. Fields are shaded by a spruce stand; vegetation dominants are *Vaccinium* species. The snow surface is contaminated with litter, soil particles and wind sediment.

Vysoké Tatry Mts., Slovakia

From numerous permanent and semipermanent snowfields in the Vysoké Tatry Mts., one locality was selected:

**Váha saddle:**

From the saddle, snowfields persisting over the whole summer slope to both sides. The SW-exposed field has a lower declination of about 10° and more sunshine, while the E-exposed one is very steep (app. 40°) and shaded a greater part of the day. The snow surface is contaminated with eolic sediment.

**Material & Methods**

Samples were collected five times in seasons 2000 and 2001 in localities Labský důl and Luční hora and once in localities Váha and Plešné jezero. In 2002, several samplings without measuring physical variables were made in April and May in the Krkonoše Mts. and the Šumava Mts. Snow containing algae was inserted into sterile plastic bottles with Z-medium or without medium and the bottles were placed into a pre-cooled vacuum flask.

In localities Labský důl, Luční hora and Plešné jezero, air and snow temperatures, the height of snow layer, the depth of coloration and pH of snow were measured and snow color was registered. Laboratory ethanol thermometer (accuracy 0.5°C) was used for temperature measurement, universal indicator papers for pH measurement and a folding ruler for depth measurement.

The observations were conducted on living material, algal cells were drawn and photographed and their morphological parameters and cell density were measured. An Olympus CX 40 light microscope with an Olympus DP 10 digital camera was used for observation and photodocumentation; photographs were edited in Adobe Photoshop 5.0. Bürker chamber was used for density measurements. Algae were identified according to the following literature: ETTL & GÄRTNER 1995, HOHAM & MULLET 1977, HOHAM et al. 1979, KOL 1968 and KOMÁREK 2001.

The isolation and cultivation of strains were carried out using liquid media as well as agar plates. Media Z, BG11, L-C and L-C with soil extract added were used. Petri dishes and Erlenmeyer flasks with inoculated algae were placed into a cultivation box (IDAF C95, Slovakia) and maintained at temperature 4°C and irradiation 100 $\mu E.m^{-2}.s^{-1}$.

Ecophysiological demands were tested on isolated strains using the method of crossed gradients of temperature and light (KVÍDEROVÁ & LUKAVSKÝ 2001a, b). Temperature range 0-20°C and irradiation range 58-1180 $\mu E.m^{-2}.s^{-1}$ were set up. After having reached the steady phase of growth, absorbance was measured using an iEMS plate reader (Labsystems Finland); maximum values were recalculated after empirically appointed conversion curves.

## Results

### Ecological demands

Table 1 presents physical parameters of the localities. In Labský důl, snow algal communities developed in early May in both seasons when temperature during the day was about 15-16°C; the height of snow cover was 15-20 cm, and the depth of snow coloration 0-12 cm. Cell density was up to $1.10^6$ cells per 1 ml of melted snow. The color of snow was yellow-green to green.

On the terraces of Luční hora, the development of snow algae was a little shifted compared to the previous locality: snow algae occurred from the beginning of June until the snow melted completely (late August in 2000, July in 2001). Temperatures during the day moved up to 20°C; the height of snow cover was 25 cm at the end of June 2000, 10 cm in June 2001 respectively. Coloration reached 2-4 cm deep, or 1-5 cm resp. Cell density was approximately $8.10^5$ cells per 1 ml. The color of snow was pink to reddish.

In the locality of Plešné jezero, a short development of snow algae took place in early May 2001 and 2002. The air temperature was 18-19°C and the height of snow layer up to 70 cm. Coloration depth was 1-12 cm. Cell density was $1.2\ .10^6$ cells per 1 ml. The color of snow was green.

Species diversity

Six species of algae (5 of Chlorophyta and 1 of Xanthophyceae), two cyanobacterial species and two species of fungi were found (Tab.2). The following preview lists all the observed species. Four strains were isolated: *Xanthonema hormidioides* and *Chlorella vulgaris* from the locality Váha, *Stichococcus bacillaris* from the locality Labský důl and *Chloromonas nivalis* from the locality Plešné jezero.

***Aphanocapsa nivalis*** **LAGERHEIM**

Cryophilic Chroococcalean cyanobacterium, a rare species of snow. Small colonies in round mucilaginous envelope, cells round or nearly round, 2-6 μm in diameter, irregularly assembled.

Found in locality Plešné jezero (first record in the Czech Republic).

***Romeria nivicola*** **(KOL) KOM. O. & KOM.**

Cryophilic filamentous cyanobacterium, not very common (KOMÁREK 2001). Trichomes short, solitary, cells cylindrical, 4-10 × 2 μm.

Found in locality Luční hora (first record in the Czech Republic).

***Chlamydomonas nivalis*** **(BAUER) WILLE**

One of the most common snow algae, widespread in high mountains and polar areas all over the world (KOL 1968). Only non-motile zygotes found, spherical to oval with thick sculptured cell wall, deep-red colored. Cell diameter 15-25 μm (resp. 15-20 × 18-25 μm). Motile reproductive stages were not observed.

***Chlorella vulgaris*** **BEIJERINCK**

Common soil- and subaerophytic alga (ETTL & GÄRTNER 1995). Cells spherical or oval, diameter 7-10 μm. Reproduction by autospores.

***Chloromonas brevispina*** **(FRITSCH) HOH., ROEM. & MULL.**

Common snow alga. Non-motile zygotes found (formerly *Cryocystis brevispina*, see HOHAM et al. 1979). Oval-shaped cells, 14-23 × 9-13 μm. Cell surface covered with short spines (up to 1 μm). Reproductive cells not observed.

***Chloromonas nivalis*** **(CHOD.) HOH. & MULL.**

Very common snow alga. Vegetative cells with two flagella, planozygotes with four flagella, non-motile zygotes with sculptured cell surface (spiral flanges) and reproducing cells without flagella (in locality Plešné jezero only) observed. Life cycle of this alga will be described in a separate paper.

***Stichococcus bacillaris*** **NÄGELI**

One of the most abundant soil- and aerophytic algae, cosmopolitan, ubiquitous (ETTL & GÄRTNER 1995). Cylindrical cells 3-6 × 2-3 µm with chloroplast occupying two thirds of the cell, in short trichomes (max. 10 cells). Reproducing by simple division and trichome fragmentation.

***Xanthonema hormidioides*** **(VISCHER) SILVA**

Common alga of mountain forest soils (ETTL & GÄRTNER 1995). Filamentous Xanthophycean; trichomes long and compact, cells 7.5-12.5 × 4-5 µm. Mostly initial spherical cells 5-6 µm in diameter present in snow. Only asexual reproduction by simple cell division observed.

Temperature- and irradiation demands

The strain *Chlorella vulgaris* reached the steady phase of growth after 40 days. Maximum growth (absorbance /A/= 0.8, which corresponds to dry mass /DM/ of 540 mg per liter) was at 20°C and 70 $\mu E.m^{-2}.s^{-1}$. The highest average growth was in temperature 20°C and irradiation 58 $\mu E.m^{-2}.s^{-1}$ (Fig. 1).

The strain *Stichococcus bacillaris* reached the steady phase after 45 days. The highest growth (A= 0.37, corresponds to DM 155 mg per liter) was at 20°C and 70 $\mu E.m^{-2}.s^{-1}$ and at 16°C and 270 $\mu E.m^{-2}.s^{-1}$. The highest average growth was in temperature 20°C and irradiation 58 $\mu E.m^{-2}.s^{-1}$ (Fig. 2).

The strain *Xanthonema hormidioides* reached the steady phase of growth after 45 days. The highest growth (A= 0.43, corresponds to DM 195 mg per liter) was at 20°C and 725 $\mu E.m^{-2}.s^{-1}$ and at 16°C and 58 $\mu E.m^{-2}.s^{-1}$. The highest average growth was in temperature 20°C and irradiation 58 $\mu E.m^{-2}.s^{-1}$ (Fig. 3).

The strain *Chloromonas nivalis* reached the steady phase after 35 days. Maximum growth (A= 0.1, corresponds to DM 27 mg per liter) appeared at 0°C and 58 $\mu E.m^{-2}.s^{-1}$. The highest average growth was in temperature 0°C and irradiation 100 $\mu E.m^{-2}.s^{-1}$. The limiting temperature for the growth of *C. nivalis* was 10°C (Fig. 4).

Strains *Chlorella vulgaris*, *Stichococcus bacillaris* and *Xanthonema hormidioides* have very similar temperature and irradiation demands – they reach the highest growth at 20°C and optimum irradiation is 58-70 $\mu E.m^{-2}.s^{-1}$. The highest absolute growth was reached by the *Chlorella* strain.

Unlike the former strains, the strain *Chloromonas nivalis* has its optimum at 0°C, while its optimum irradiation is basically the same – 58-70 $\mu E.m^{-2}.s^{-1}$. The absolute growth is the lowest compared to the previous strains.

## Discussion

For more detailed studies of life cycles of snow algae, a complex approach comprising both natural observations and laboratory cultivations is needed. However, a very low number of such studies is the evidence of its difficulty. The culturing of snow algae is very uneasy and, therefore, information about these organisms in pure cultures is considerably limited (e.g. HINDÁK & KOMÁREK 1968, LING & SEPPELT 1993).

Although the cells of *Chloromonas nivalis*, *Chloromonas brevispina* and *Chlamydomonas nivalis* were kept in conditions regarded as optimal (temperature up to 4°C and irradiation cca 100 $\mu E.m^{-2}.s^{-1}$) and grown on various media, their division was observed only in the case of non-motile vegetation stages of *Chloromonas nivalis*. The cause could be the sensitivity of snow algae to any changes of their environment, which of course are unavoidable during their sampling and manipulating; this sensitivity is typical particularly for cells with flagella (HOHAM et al. 1979).

The temperature of melting snow represents in fact the lower limit of permanent life. Organisms living or surviving in these conditions belong in principle into two groups: the first are psychrophilic with life optima within these conditions, for which higher temperatures are lethal; the other are psychrotolerant with optima in higher temperatures, while low temperatures are suboptimal or sublethal for them.

Very few experiments concerning the ecophysiological demands of this kind of algae have been carried out so far. HOHAM (1975a) tested several species of snow algae at five different temperatures from 1 to 20°C (*Chloromonas nivalis* was not among them) and found their optima between 1 and 10°C. Therefore, this interval was proposed to be the criterion for the „genuine“ snow algae (HOHAM 1980). Similar limits were found in several cryophilic *Koliella* strains (HINDÁK & KOMÁREK 1968). Studies dealing with irradiation and its influence on snow algae did not concentrate on finding the growth optima or limitations (HOHAM et al. 1998, THOMAS & DUVAL 1995).

This study focused on the co-incidence of two basic factors – temperature and light – continuously changing in space and on the growth response to these conditions. The results show that algae isolated from snow belong to both the above mentioned groups. *Chloromonas nivalis* turned out not to grow in higher temperatures, 10°C was already lethal; its growth optimum was at the temperature of melting snow, i.e. 0°C. Most studies dealing with optimum temperatures for snow algae mention higher values (2-4°C); in these experiments the highest growth was truly at 0°C. However, some temperature fluctuation could happen. On the contrary, the strains of *Chlorella vulgaris*, *Stichococcus bacillaris* and *Xanthonema hormidioides* grew better in higher temperature with optima obviously higher than 20°C. Thus, *C. nivalis* can be regarded as the „genuine“ snow alga while the others belong to psychrotolerants being brought on snow by wind with soil particles, surviving and even reproducing in these suboptimum conditions.

A very interesting and unexpected result is the fact that the absolute growth at 0°C is higher in the case of psychrotolerants. It would imply that in natural conditions *C. nivalis* should be outcompeted by the „aliens“; this situation however does not happen (see also LING & SEPPELT 1990). The most probable explanation is the above mentioned sensitivity of snow algae. Their viability might be reduced but optima remain unchanged.

Irradiation demands seem to be very similar for all tested strains, between 50 and 100 $\mu E.m^{-2}.s^{-1}$, which represent rather shaded sites. These results correspond to the fact that in higher light intensities most snow algae produce carotenoid shields (BIDIGARE et al. 1993) and occur deeper in snow than in shaded places.

Basic culturing demands also arise from these results, i.e. temperature between 0-5°C and irradiation between 50-100 $\mu E.m^{-2}.s^{-1}$. Nevertheless, successful culturing is not guaranteed in these conditions and some other unknown factors, which are yet to be found, influence the growth of snow algae.

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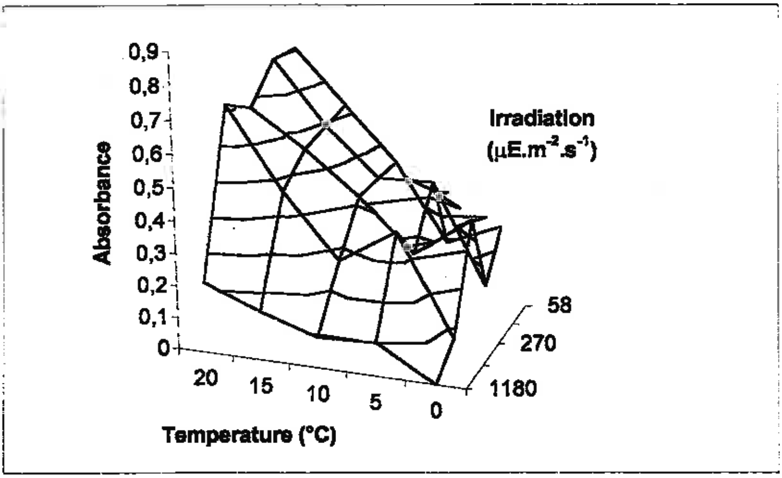
**Tab. 2:** List of species found

| | Vysoké Tatry Váha | Krkonoše Labský důl | Krkonoše Luční hora | Šumava Plešné jezero |
|---|---|---|---|---|
| **cyanobacteria** | | | | |
| *Aphanocapsa nivalis* | | | | + |
| *Romeria nivicola* | | | + | |
| *algae* | | | | |
| **Chlamydomonas nivalis** | + | | + | |
| *Chlorella vulgaris* | + | + | | + |
| *Chloromonas brevispina* | | + | + | + |
| *„Cryocystis brevispina"* | | + | + | + |
| *Chloromonas nivalis* | | + | + | + |
| *"Scotiella nivalis"* | | + | + | + |
| *"Scotiella cryophila"* | | + | + | + |
| "*Chloromonas* palmeloid cells" | | | | + |
| *Stichococcus bacillaris* | + | + | | + |
| *Xanthonema hormidioides* | + | | | |
| **fungi** | | | | |
| *Chionaster nivalis* | + | + | + | + |
| *Selenotila nivalis* | + | + | + | + |

**Tab. 1:** Physical parameters of localities

| | | | *Air temperature [°C]* | *Snow temperature [°C]* | *pH of snow* | *Height of snow cover [cm]* | *Depth of coloration [cm]* |
|---|---|---|---|---|---|---|---|
| **Labský důl** | 2000 | 1.3. | -2 | 0,5 | 5 | 180 | 0 |
| | | 9.4. | 2 | 0,5 | 5 | 155 | 0 |
| | | 6.5. | 16 | 0 | 4,5 | 20 | 0-10 |
| | | 29.6 | 23 | - | - | 0 | 0 |
| | | 1.8 | 20 | - | - | 0 | 0 |
| | 2001 | 28.2 | 0 | 0,5 | 5 | 85 | 0 |
| | | 10.4 | 8 | 0 | 4,5 | 40 | 0 |
| | | 8.5. | 15 | 0 | 4,5 | 15 | 0-12 |
| | | 17.6 | 25 | - | - | 0 | 0 |
| | | 6.8 | 24 | - | - | 0 | 0 |
| **Luční hora** | 2000 | 1.3. | -5 | 0 | 4,5 | 65 | 0 |
| | | 9.4. | -2 | 0 | 4,5 | 55 | 0 |
| | | 6.5. | 11 | 0 | 5 | 45 | 0 |
| | | 29.6 | 19,5 | 0,5 | 4,5 | 25 | 2-4 |
| | | 1.8 | 18 | 0,5 | 4,5 | 10 | 2-4 |
| | 2001 | 28.2 | -2 | 0 | 4,5 | 60 | 0 |
| | | 10.4 | 3 | 0 | 4,5 | 50 | 0 |
| | | 8.5. | 18 | 0 | 4,5 | 20 | 0 |
| | | 17.6 | 20 | 0 | 4,5 | 10 | 2-5 |
| | | 6.8 | 20 | - | - | 0 | 0 |
| **Plešné jezero** | | 4.5. | 18,5 | 0 | 4,5 | 70 | 0-12 |



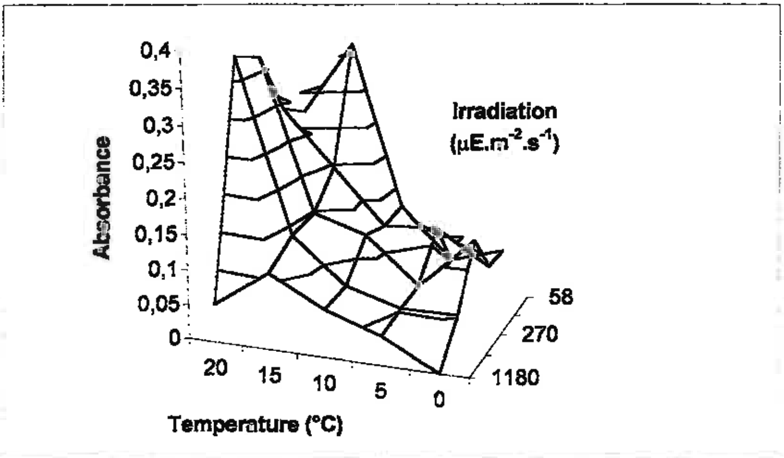
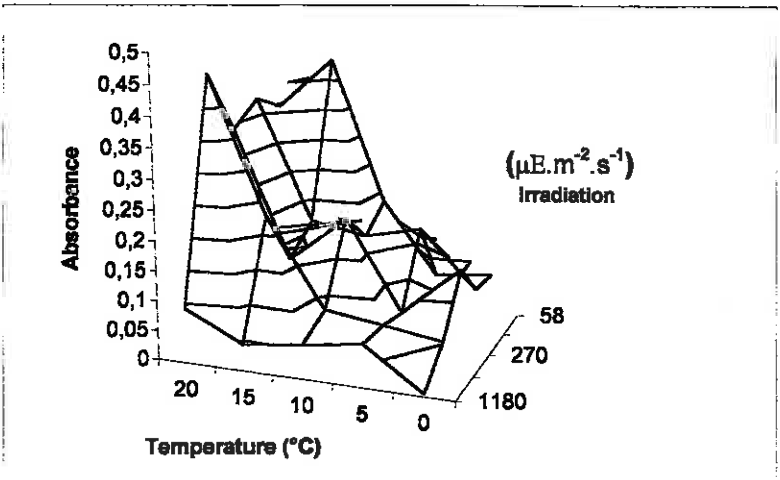




Fig. 1: Temperature and irradiation demands of the strain *Chlorella vulgaris*

Fig. 2: Temperature and irradiation demands of the strain *Stichococus bacillaris*



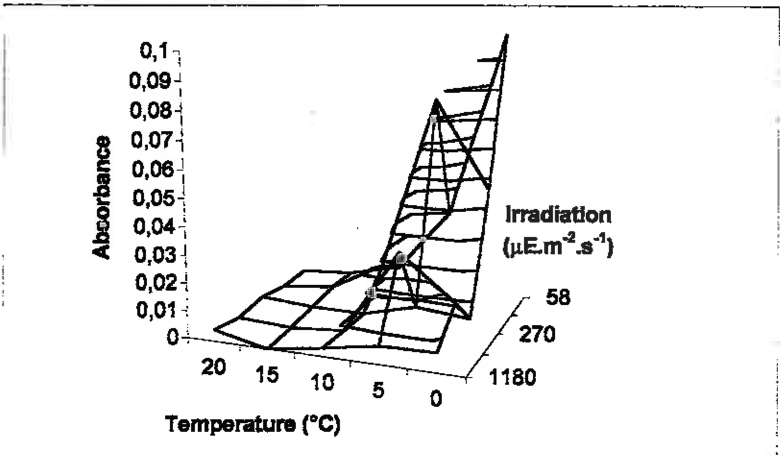




Fig. 3: Temperature and irradiation demands of the strain *Xanthonema hormidioides*

Fig. 4: Temperature and irradiation demands of the strain *Chloromonas nivalis*

Table to Fig. 1

| | 20 | 15 | 10 | 5 | 0 |
|---|---|---|---|---|---|
| 1180 | 0,219625 | 0,15025 | 0,094125 | 0,10225 | 0 |
| 725 | 0,717625 | 0,466375 | 0,270875 | 0,378 | 0,072 |
| 270 | 0,6725 | 0,556125 | 0,420625 | 0,2575 | 0,38375 |
| 125 | 0,796375 | 0,592125 | 0,4365 | 0,439375 | 0,117125 |
| 58 | 0,806125 | 0,62525 | 0,4455 | 0,1885 | 0,260125 |

Table to Fig. 2

| | 20 | 15 | 10 | 5 | 0 |
|---|---|---|---|---|---|
| 1180 | 0,055 | 0,108625 | 0,067875 | 0,0415 | 0,000375 |
| 725 | 0,381625 | 0,137625 | 0,073875 | 0,051375 | 0,05225 |
| 270 | 0,367875 | 0,147375 | 0,1235 | 0,0575 | 0,132625 |
| 125 | 0,278 | 0,195875 | 0,11475 | 0,11925 | 0,07 |
| 58 | 0,258125 | 0,36675 | 0,123125 | 0,045875 | 0,069 |

Table to Fig. 3

| | 20 | 15 | 10 | 5 | 0 |
|---|---|---|---|---|---|
| 1180 | 0,09175 | 0,045125 | 0,05625 | 0,071714 | 0,005 |
| 725 | 0,4525 | 0,227375 | 0,081 | 0,067 | 0,05225 |
| 270 | 0,3425 | 0,12575 | 0,206875 | 0,05475 | 0,141 |
| 125 | 0,375875 | 0,166375 | 0,148375 | 0,170875 | 0,074875 |
| 58 | 0,344 | 0,434 | 0,18475 | 0,059375 | 0,07 |

Table to Fig. 4

| | 20 | 15 | 10 | 5 | 0 |
|---|---|---|---|---|---|
| 1180 | 0,0043 | 0 | 0,0031 | 0,00655 | 0,00705 |
| 725 | 0,0016 | 0,00035 | 0,00195 | 0,03265 | 0,01715 |
| 270 | 0,00255 | 0,0011 | 0,0011 | 0,0061 | 0,0043 |
| 125 | 0 | 0,0023 | 0,01305 | 0,07725 | 0,0461 |
| 58 | 0 | 0,00135 | 0,006 | 0,02975 | 0,09875 |