Carbon, Nitrogen and Sulphur concentration and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ values in *Hypogymnia physodes* within the montane area – preliminary data

Monika Cieżka*, Maria Kossowska, Piotr Paneth, Maciej Górka

Monika Cieżka: Institute of Geological Sciences, University of Wrocław, Cybulskiego Street 32, 50-205 Wrocław, Poland

Maria Kossowska: Department of Biodiversity and Plant Cover, Institute of Plant Biology, University of Wrocław, Kanonia 6/8, 50-328 Wrocław

Maciej Górka: Institute of Geological Sciences, University of Wrocław, Cybulskiego Street 32, 50-205 Wrocław, Poland

Piotr Paneth: Institute of Applied Radiation Chemistry, Lodz University of Technology, 90-924 Lodz, Poland

Record

The chemical (S, C, N) and isotopic composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) of the lichens from the Karkonoski National Park record information about the temporal changes in the air quality (pollution).

Abstract

The contribution of C, N and S, as well as the isotopic composition of C and N of atmospheric pollutants, are assumed to be reflected in the organic compounds inbuilt into the lichen thallus. The chemical and isotopic analyses were carried out on lichen *Hypogymnia physodes* samples gathered from *Picea abies* and *Larix decidua*, collected in 13 sampling points located in Karkonoski National Park and its closest vicinity in 2011. The results for %C, %N and %S varied from 43.44 to 46.79%, from 0.86 to 1.85% and from 0.07 to 0.27 %, respectively. The $\delta^{13}\text{C}$ values ranged from -26.6 to -24.6‰, whereas $\delta^{15}\text{N}$ values varied from -13.0 to -6.8‰. The ranges in isotope composition suggest different sources of C and N for Karpacz compared to the remaining sampling sites. For Karpacz, the $\delta^{13}\text{C}$ values suggest (in case the fractionation product-substrate does not exist and $\Delta=0$) that the dominant sources are coal combustion processes, whereas for remaining sampling points, the $\delta^{13}\text{C}$ values are ambiguous and are masked by many mixed natural and anthropogenic processes. With the same assumption that $\Delta=0$, the $\delta^{15}\text{N}$ values suggest that transport is not a dominant source of nitrogen within Karpacz city. Moreover, in this study we tested the possible fractionation (Δ) for carbon and nitrogen, assuming that within the investigated area, the source of carbon is probably CO_2 and/or DIC (HCO_3^-) dissolved in precipitation, while the source of nitrogen is NO_x and/or NO_3^- ion. The calculated fractionation factors were: (i) for gaseous carbon compounds $\Delta_{\text{CO}_2\text{-Corg}}$ value from -13.4 to -11.4‰, whereas for the ions form $\Delta_{\text{HCO}_3^-\text{-Corg}}$ value from -16.6 to -14.6‰, (ii) for nitrogen gaseous compounds $\Delta_{\text{NOx-Norg}}$ value between apx. -17 and -5‰, whereas for the ions form $\Delta_{\text{NO}_3^-\text{-Norg}}$ value between -9.9 and -3.7‰.

Keywords

Hypogymnia physodes • Karkonosze Mountains • CNS concentration • $\delta^{13}\text{C}$ • $\delta^{15}\text{N}$

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1. Introduction

Pollutants in the atmosphere constitute one of the major problems in the urban environments owing to anthropogenic activities like industry, vehicular traffic, etc. (Sawidis et al., 2011; Cekstere et al., 2015). The influence of air pollution on living organisms has been investigated for several decades (Conti and Cecchetti, 2001; Kłos, 2007). The increase of anthropopressure factors and a new quality of pollutants affect the organisms in their physiology and morphology. Species, both plants and animals, which accumulate pollutants in their body and show physiological, morphological or anatomical changes under the pollutants' influence (Kłos, 2007) are called bioindicators or biomonitors. According to Conti and Cecchetti (2001), bioindicators are organisms which are used

for qualitative identification of environmental factors generated by humans (Tonneijck and Posthumus, 1987), whereas the organisms used for the quantitative information of contaminants are called biomonitors. The biomonitors should be sensitive (the morphological changes are optically visible) and with an ability to build the contaminants in their tissue for further investigation of the concentration of contaminants in the environment (Conti and Cecchetti, 2001).

In environmental studies worldwide, researchers use different types the bioindicators for assessing the quality of air. Needles of yew (*Taxus baccata*) (Samecka-Cymerman et al., 2011), Scotch pine (*Pinus sylvestris*) (Manninen et al., 1991, Rautio and Huttunen, 2003, Gorshgkov et al., 2008) and spruce (*Picea abies*) (Jędrysek and Kałużny, 2002) have been used as tracers to de-

*E-mail: monika.ciezka@uwr.edu.pl

termine the environmental pollution such as SO_2 , S deposition, metal concentration PAHs, as well as S and O isotopic ratio. Bark can also provide information about air pollution, and can be used as a bioindicator for receiving data on long term contamination caused due to, for example, heavy metals (Sawidis et al., 2011). The bark structure stores the pollutants longer because of its porosity (Berlizov et al., 2007). According to Sawidis et al. (2011), many tree species were used for its bark sample analysis such as oak, willow, elm, pine, cedar, poplar, olive, or eucalyptus (Poikolainen, 1997; Mandiwana et al., 2006; Berlizov et al., 2007 in Savidis et al., 2011). Another set of organisms which are found to be very good bioindicators are mosses. The mosses are used as a tool for controlling the atmospheric pollution because: (i) cation exchange sites are present in their cell walls, (ii) they have simple root system, and (iii) there is lack of cuticula in their leaves, hence the pollutants are absorbed into their bodies very easily (Galsomies et al., 1999; Fernández et al., 2000; Gerdol et al., 2000; Carballeira and Fernández, 2002; Zechmeister et al., 2003 in Kosior et al., 2015). Mostly, moss samples are used for monitoring the atmosphere and the environment contaminated by heavy metals (Lippo et al., 1995; Migaszewski et al., 2011; Misra and Tandon, 2014; Cowden et al., 2015) and for assessing the anthropopressure by sulphur (Kosior et al., 2015) or nitrogen inputs (Kosior et al., 2008; Bonanno, 2013). One of the most popular and often used bioindicators, playing a very important role in monitoring the air quality, are lichens. Numerous lichen species may be classified as effective organisms able to absorb and accumulate atmospheric trace element pollutants from ambient air and rain due to their structure and slow growth (van Dobben et al., 2001; Gombert et al., 2003). Similar to other bioindicators, lichens are used for determining the degree of environment contamination by heavy metals (Conti and Cecchetti, 2001; Jóźwiak, 2007; Bosch-Roig et al., 2013), sulphur (Manninen et al., 1991; Wadleigh et al., 1996; Wadleigh, 2003), nitrogen (Fuentes and Rowe, 1998; Gombert et al., 2003;) or gaseous pollutants (LeBlanc and DeSloover, 1970; Conti and Cecchetti, 2001; van Dobben et al., 2001). Numerous studies published the data of isotopic composition of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) of various lichen species, namely *Hypogymnia physodes*, *Pseudevernia furfuracea*, *Xanthoria parietina*, *Usnea* spp. (van Dobben, 2001; Munzi et al., 2007; Beck and Mayr, 2012) from different countries such as Germany, Romania, Norway and Portugal (Bruteig, 1992; Maguas et al., 1995; Cuna et al., 2007; Beck and Mayr, 2012; Boltersdorf and Wrener, 2014). In Poland, similar investigations were carried out by Gałuszka (2005), in Holy Cross (Świętokrzyskie) Mountains.

Several publications present the concentration of main elements, such as C, N, S as well as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Hypogymnia physodes*, (e.g. Migaszewski et al., 2000; Gałuszka, 2005; Cuna et al., 2007; Jeran et al., 2007; Biazrov, 2012a). For this study, the area of Karpacz city (15°44'E, 50°47'N), which is located in the closest vicinity of Karkonoski National Park, was chosen. At the same time, the

significant anthropopression in the city (e.g., weak ventilation, traffic, different types of home furnaces where diverse types of fuels are burnt, low emission, etc.) was noted. The general aim of the study was to check whether the bioindicators, such as *Hypogymnia physodes*, reflect the atmospheric pollutants in their concentration of main elements (C, N, S) and their isotopic composition, by a comparison of the results obtained in this study and published by other researchers. Furthermore based on this comparison, our study aimed at assessing the pollutant levels and trying to point at the main source of pollution in the investigated area, which is dominated by tourism. Moreover, because the database of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from lichen samples for Lower Silesia region was still incomplete, this study yielded the information that would fill the gap in data for chemical and isotopic analyses for the montane area.

2. Study area

The samples were gathered in the Karpacz city and their vicinity in the Karkonoski National Park area. The Karpacz city is a typical mountain resort, located in the eastern part of the Karkonosze Mountains (the Sudetes, SW Poland), at the base of Śnieżka Mt. (1603 m a.s.l.). Its southern areas adjoin the Karkonoski National Park, and come under the National Park's protected zone.

The town, along with its settlements, extends in the range of 480–885 m a.s.l. and lies within the lower montane zone, which in the Karkonosze Mountains covering an altitude range of 500 to 1000 m. Within the city and in its suburbs, there are fragments of forests, consisting mainly of spruce (*Picea abies*) and larch (*Larix decidua*). Originally, the lower montane zone was dominated by natural deciduous forests with a high proportion of beech (*Fagus sylvatica*) and sycamore (*Acer pseudoplatanus*), but in the nineteenth century, these trees were replaced by fast-growing conifers. Above this zone, spruce is natural fauna and forms the upper montane zone, extending up to approximately 1250 m a.s.l.

The study area is characterized by one of the harshest climates in Poland, because of heavy rainfalls, significant temperature fluctuation, fog and turbulent wind. The Karkonosze Mountains are influenced by the montane climate, modified by polar-marine air masses from the North-West direction (Sobik et al., 2014). One of the most typical weather complexes influencing the climate within the investigated area are the foehn processes (Kwiatkowski and Holdys, 1985). The mean annual air pressure reaches ca. 932 hPa, whereas slightly higher values are noted from May till October (mean monthly values vary between 934 and 936 hPa). Due to the prevalence NW wind direction, 58.8% of windless periods are noted. In Karpacz, about 28% of windy days witness foehn processes, whereas about 11 days per year (mostly during autumn and winter), high winds are noted (more than 15 m s⁻¹). In Karpacz, the mean annual temperature is 6.7°C (Sobik et al., 2014). The coldest month is January with the mean day tempera-

Figure 1. Sampling point localization.

ture being -5°C , while during July and August, the average daily temperature is 15°C (Kwiatkowski and Hołdys, 1985). The average yearly rainfall is 997 mm (Sobik et al., 2014).

3. Materials and methods

The investigations were carried out in 2011 on 13 sampling points, located in the vicinity of Karpacz (Fig. 1). Material from the Karkonoski National Park (stands 5–13) was collected during the lichen monitoring of environmental state in KNP (Kossowska et al., 2014).

The sampling points were differentiated in terms of altitude, air humidity, exposition on local air pollution (generated by road

traffic, smoking chimneys etc.) and anthropopressure degree (Table 1). The thalli of lichen species *Hypogymnia physodes* were collected from trunks or branches of coniferous trees, namely pine (*Picea abies* - most cases) and larch (*Larix decidua* - stands 2, 3 and 4) from ca. 2 m height, depending on material availability. All the locations were situated between 702 m a.s.l. and 1253 m a.s.l. (Table 1).

For the study, a common corticolous lichen *Hypogymnia physodes* was chosen. This species forms discrete foliose thalli (Fig. 2), attached to the tree trunks or branches by adhesive discs present on the lower cortex. Lobes are grey, smooth and shining, often forming rosettes. On the ends of its lobes, lip-shaped soralia are usually present. Because of its frequent occurrence, the relative resistance to air pollution and ease of identification, *Hypogymnia*

Table 1. Description of sampling points.

Coordinates					
Sampling point No.	N	E	Height [m a.s.l.]	Tree species	Comment
1	50°45'35.6"	15°45'37.2"	756	<i>Picea abies</i>	KNP, forest, no pollution emitters
2	50°45'40.8"	15°45'14.5"	782	<i>Larix decidua</i>	KNP, near Urszula mountain route, influence of pollution from Karpacz
3	50°46'00.3"	15°45'04.9"	702	<i>Larix decidua</i>	KNP, near the road to Information Centre of KNP, minor traffic influence
4	50°45'35.9"	15°46'00.8"	746	<i>Larix decidua</i>	Vicinity of KNP, near Wilczka brook, no traffic,
5	50°44'54.2"	15°44'38.6"	1014	<i>Picea abies</i>	KNP, in the vicinity of Nad Łomniczką hostel, tourism influence
6	50°45'15.8"	15°44'59.8"	955	<i>Picea abies</i>	KNP, mountain route from Nad Łomniczką hostel to Karpacz, influence of traffic and low emission
7	50°44'59.8"	15°42'24.5"	1253	<i>Picea abies</i>	KNP, mountain route from Strzecha Akademicka hostel to Samotnia hostel, tourism influence
8	50°45'30.5"	15°42'12.5"	1105	<i>Picea abies</i>	KNP, road to Strzecha Akademicka hostel, low transport influence
9	50°44'45.8"	15°44'15.0"	1093	<i>Picea abies</i>	KNP, near Łomniczka Valley, no anthropopressure
10	50°45'33.1"	15°44'32.9"	920	<i>Picea abies</i>	KNP, mountain route from Łomniczka Valley to Karpacz, low traffic influence
11	50°45'59.6"	15°44'12.0"	985	<i>Picea abies</i>	KNP border, near Orlinek ski jump and big parking, influence of road
12	50°45'21.0"	15°42'06.6"	1143	<i>Picea abies</i>	KNP, mountain route from Samotnia hostel to glade (near Łomnica brook), low tourism influence
13	50°45'57.7"	15°42'38.3"	1035	<i>Picea abies</i>	KNP, Pławsa Valley (near Bronek Czech mountain route) in the vicinity of glade, no pollution emitters

Figure 2. Lichen thalli of *Hypogymnia physodes*.

physodes is one of the so-called "monitoring species", commonly used in biomonitoring investigations and experimental studies (Kłos, 2007; Sawicka-Kapusta et al., 2010).

The samples were separated from needles and bark as soon as they were brought to the laboratory. Each sample was washed two times using deionized water (Gałuszka, 2005; Kosior et al., 2015) in order to remove the dust particles, pollens and dead insects deposited on the lichen surface. Then the samples were dried at an ambient temperature (22°C). Finally, the dry samples were homogenized in a mill and sealed in HDPE containers for further analyses. For determination of the weight concentration of H, C, N and S, the samples were put into tin capsules, and the analyses were carried out with the use of Vario Micro CUBE elemental analyzer (Elementar). The isotopic concentration of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) were determined using EA-IRMS (Sercon). The results of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were presented relative to the international standards - PDB (Pee Dee Belemnite) and atmospheric air (N₂-Air) respectively, where the $\delta^{13}\text{CPDB}$ and $\delta^{15}\text{NAir}$ isotopic signature was defined as the relative difference between the isotope ratio of the sample and that of the standard. The results were reported in parts per thousand (‰). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data was normalized with the use of IAEA600 standard.

Table 2. C, N and S concentration, as well as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic composition of *Hypogymnia physodes* samples.

Sampling point No.	C [%]	N [%]	S [%]	$\delta^{13}\text{C}$ [‰]	$\delta^{15}\text{N}$ [‰]
1	46.43	1.59	0.27	-26.6	-9.7
2	44.61	1.10	0.12	-24.6	-10.1
3	44.40	0.86	0.07	-25.7	-10.5
4	44.39	1.64	0.14	-24.9	-13.0
5	45.48	1.74	0.13	-26.4	-10.1
6	43.44	1.16	0.10	-25.8	-9.5
7	44.25	1.70	0.10	-26.3	-10.3
8	44.46	1.30	0.10	-25.5	-6.8
9	46.79	1.57	0.11	-26.2	-11.1
10	45.08	1.85	0.11	-26.4	-10.1
11	44.39	1.77	0.12	-26.5	-10.0
12	45.30	1.77	0.10	-25.6	-7.5
13	44.47	1.81	0.11	-26.2	-9.7
Minimum	43.44	0.86	0.07	-26.6	-13.0
Maximum	46.79	1.85	0.27	-24.6	-6.8
Average value	44.88	1.53	0.12	-25.9	-9.9
Median	44.47	1.64	0.11	-26.2	-10.1
Mode	44.39	1.77	0.11	-26.4	-10.1
Standard deviation	0.92	0.32	0.05	0.6	1.5

4. Results

The results of concentration of C, N and S are given in table 2 (Table 2).

The values ranged from 43.44 to 46.79% (average $44.88 \pm 0.92\%$, median 44.47%, mode 44.39%), from 0.86 to 1.85% (average $1.53 \pm 0.32\%$, median 1.64%, mode 1.77%) and from 0.07 to 0.27% (average $0.12 \pm 0.05\%$, median 0.11%, mode 0.11%), respectively for C, N and S. The $\delta^{13}\text{C}$ values ranged from -26.6 to -24.6‰ (average $-25.9 \pm 0.6\%$, median -26.2‰, mode -26.4‰), whereas $\delta^{15}\text{N}$ values varied from -13.0 to -6.8‰ (average -9.9 $\pm 1.5\%$, median -10.1‰, mode -10.1‰).

5. Discussion

The sulphur concentration values obtained in this study reached from 0.07 to 0.27%. The results are higher than found in the Świętokrzyski National Park, where the S concentration varied from 0.006 to 0.101% (in the heating season) and from 0.069 to 0.206% (in the vegetative season) (Ciężka et al., 2016). The samples from the same sampling area (ŚNP), but collected in 1994 by Migaszewski et al. (1995), were characterized by the range of 0.364–0.512% S, whereas within the whole Świętokrzyskie Moun-

tains, the range was 0.230–0.372% S. Also, Migaszewski et al. (2001) have done the investigations on *Hypogymnia physodes* as well in ŚNP, obtaining the results: 0.049–0.058, 0.058 and 0.103% S gathered from pine, birch and oak bark respectively. Sawicka-Kapusta and Zakrzewska (2009) have worked on the same material in the same sampling area. In 2009, the S was 0.141% (Sawicka-Kapusta and Zakrzewska, 2009). According to the results presented in these papers, the decrease in % S in time is noticed indicating that the atmospheric air is getting cleaner. According to the results obtained by Voivodeship Inspectorate for Environmental Protection (VIEP) in Wrocław, the decrease in sulphur concentration in lichens could be caused due to a decrease of sulphur input into the atmosphere in gaseous form (SO_2) and as sulphur ions dissolved in precipitation (SO_4^{2-}) form. Between 2006 and 2011, the yearly SO_2 concentration in atmosphere measured in the urban monitoring points in Wrocław decreased from ca. 15 $\text{mg}\cdot\text{m}^{-3}$ to 6 $\text{mg}\cdot\text{m}^{-3}$, whereas SO_4^{2-} ion dissolved in precipitation for the complete Lower Silesia Voivodeship decreased from 24.42 kg/ha-year to 14.71 kg/ha-year (VIEP, Report, 2011).

The nitrogen concentration in lichen *Hypogymnia physodes* noted in literature for other localization ranged from 0.49 to 0.71% (Laxton et al., 2010), from 0.6 to 1.1% (Biazarov, 2012a) and from 0.75 to 2.60% (Poikolainen et al., 1998), whereas the results obtained in this study varied from 0.86 to 1.85%. The results obtained in 2013 for ŚNP area were characterized by a wider range, and varied from 0.9 to 3.1% during the winter season and from 1.2 to 2.6% during the vegetative season (Ciężka et al., 2016). The varied sampling locations (elevation) were affected by different pollution levels, causing the difference in concentration of nitrogen. The relation between elevation and content was tested for this study, as described in Biazarov (2012a), but no relationship was found (rank Spearman, $p < 0.05$). The nitrogen concentration was not found to be related to the altitude, but it could be dependent on the distance from main roads or low emission areas, as well as on wind direction. Nevertheless, there was no clear relationship found between the sampling points and the influence of roads or being in close vicinity of low emission and higher nitrogen concentration areas. Hence, it can be concluded that there is more than one factor responsible for such data. The $\delta^{15}\text{N}$ values of N_{org} in lichens obtained in this study varied from -13.0 to -6.8‰ and were close to those obtained in Świętokrzyski National Park, which ranged from -10.9 to -7.2‰ (heating season) and from -12.2 to -7.3‰ (vegetative season) (Ciężka et al., 2016).

The concentration of carbon in lichen samples varied from 43.44 to 46.79%, whereas in the ŚNP it varied from 35.1 to 48.2% during the winter season and from 43.3 to 49.4% during the summer season (Ciężka et al., 2016), which is wider range than obtained in this research. Although Biazarov worked on different lichen species (*Xanthoparmelia camtschadalis*), the results were quite similar (33.1–42.7%) (Biazarov, 2012b). Nevertheless, it should be mentioned that Biazarov collected lichens from sampling points

Figure 3. Tri-plot combination between altitude and C concentration and $\delta^{13}\text{C}$ of lichens in analyzed area.

Table 3. Results of rank Spearman correlation matrix of lichen samples, where $p < 0.05$ was applied. None of the correlation is significant.

Variable	%N	%C	%S	$\delta^{13}\text{C}$ [‰]	$\delta^{15}\text{N}$ [‰]
%C	0.17				
%S	0.27	0.29			
$\delta^{13}\text{C}$ [‰]	-0.50	-0.24	-0.34		
$\delta^{15}\text{N}$ [‰]	0.14	0.01	-0.26	-0.04	
Height [m a.s.l.]	0.35	0.06	-0.39	-0.07	0.36

located at higher altitudes (1550 to 3250 m a.s.l.), whereas in Karkonoski National Park (KNP), the samples were gathered from sampling points at heights between 701.5 and 1253.5 m a.s.l. The $\delta^{13}\text{C}$ values obtained in this study varied from -26.6 to -24.6‰, whereas ŚNP has a wider range and reached from -27.4 to -25.6‰ and from -28.8 to -25.6‰ during summer and vegetative seasons, respectively (Cieźka et al., 2016). Cuna et al. (2007) obtained slight variation in results of $\delta^{13}\text{C}$ with the altitude gradient. The values ranged from -24.47 to -21.21‰, making an increase of ca. 3‰ (~0.5‰ per 100 m). Such effect is probably caused by the photosynthetic lichen activity, which vary depending on environmental factors present at high altitudes. It is claimed that the highest ozone levels occur at high elevations, and the ozone effect decreases in photosynthesis. Hence, the

lesser negative $\delta^{13}\text{C}$ value at higher elevations is caused due to ozone (Cuna et al., 2007).

Because the data was not normally distributed (test Shapiro-Wilk was applied), it was not possible to use linear Pearson correlation. Hence for this study, the non-parametric rank Spearman correlation was used (Table 3). The p-value (significance level) was at 0.05, as is the norm in environmental studies.

According to other studies carried out by Cuna et al. (2007) and Biazrov (2012a, b), it was expected that there would be significant correlation between height and other factors such as $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$. Nevertheless, the correlation is insignificant (Table 3). Also, Biazrov (2012a, b) found a relation between height and %C, as well as %N. The correlation matrix point for such a relation was not found in these studies.

Despite the fact that the investigated samples did not show any significant correlations, the results can be interpreted quantitatively.

The lichens are supplied by macro elements such as carbon and nitrogen, depending on the availability and conditions of their growth, in both gaseous and ion dissolved in precipitation forms. According to Dahlman et al. (2004), Hauck (2010) and Boltersdorf and Werner (2014), it is claimed that epiphytic lichens (e.g., *Hypogymnia physodes*) take up nitrogen directly from the atmosphere. In case of nitrogen, it can be NH_3 , NO_x , NO_2 , HNO_3 , NH_4^+ or NO_3^- . As reported by Gombert et al. (2003), the main nitrogen source for lichens in rural environments is ammonia (NH_3), whereas in urban environments, it is nitrogen oxide (NO_x). As the sampling was done in the environment where NH_3 input is negligible, we can take into consideration that lichens are supplied with nitrogen mainly from NO_x and NO_3^- ions from precipitation. For carbon, it is believed that the main source for lichens is CO_2 and HCO_3^- (Dissolved Inorganic Carbon - DIC) from precipitation.

The $\delta^{15}\text{N}$ values of N_{org} in lichens obtained in this study varied from -13.0 to -6.8‰ whereas the $\delta^{13}\text{C}$ values of C_{org} varied from -26.6 to -24.6‰. To explain the possible ways of carbon and nitrogen budget in lichens, two hypotheses were proposed. The first hypothesis implied that in lichens, the isotopic fractionation for both carbon and nitrogen is not noted ($\Delta = 0$), and $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{org}}$ (products) is the same for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the source (substrate), and as a dominant source, we take NO_x and NO_3^- as well as CO_2 and HCO_3^- . The results presented in Fig. 3 yields the information that based on $\delta^{13}\text{C}$ and %C values, we can point that dominant source of carbon for the analyzed samples located within Karpacz city (sampling points No. 2, 3 and 4 - Fig. 3) is coal combustion. In spite of the fact that point No. 1 lies in the city area, the urban character was not observed in the isotopic composition of lichens from this location. For the remaining sampling points, the $\delta^{13}\text{C}$ values are ambiguous and are masked by many mixing processes, for example, natural (assimilation/respiration of CO_2) and anthropogenic (fossil fuel combustion input) (Fig. 3).

Surprisingly, the $\delta^{15}\text{N}$ results (Fig. 4) noted in the same sampling locations (2, 3 and 4) showed that transport is not a dominant

source. This is contrary to the results from the locations No. 8 and 12, in which $\delta^{15}\text{N}$ values suggested that nitrogen was derived from fossil fuel combustion (Fig. 4).

As we assumed previously, the main sources for carbon in the investigated area are probably CO_2 and DIC (HCO_3^-), and for nitrogen, NO_x and NO_3^- . If we take into consideration the second hypothesis that the fractionation exists, then based on the results from literature and the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of lichens from this study, we can try to calculate the fractionation (Δ) for both carbon and nitrogen isotopic composition.

The average values for $\delta^{13}\text{C}_{\text{CO}_2}$ of atmospheric carbon dioxide and $\delta^{13}\text{C}_{\text{DIC}}$ from HCO_3^- from precipitation, obtained in 2008 in Wrocław by Górka et al. (2011), were -13.2‰ and -10.0‰ respectively. Hence, if we assume that the dominant source for lichen samples is CO_2 , so based on $\delta^{13}\text{C}_{\text{org}}$ values from this study (range from -26.6 to -24.4‰), we can calculate $\Delta_{\text{CO}_2\text{-Corg}}$ value, which varies from -13.4 to -11.4‰ . Whereas, if we take the DIC in precipitation as a main carbon source for lichens, then the $\Delta_{\text{HCO}_3^-\text{-Corg}}$ value varies from -16.6 to -14.6‰ . However, a very low concentration of ionic carbonaceous form in the precipitation (Górka et al., 2011) rather excluded DIC as an important component in C budget of lichens. It is not clear if the lichens have a C3 or a C4 photosynthesis pathway (Ahmadjian, 1993), hence our data suggests that in our region, C3 is more probable.

Similar to carbon, we are able to calculate Δ value for nitrogen. Assuming the main source nitrogen being the nitrogen oxides (NO_x), the $\Delta_{\text{NO}_x\text{-Norg}}$ will be dependent on the NO_x origin.

If NO_x is due to the automobile exhaust, where the $\delta^{15}\text{N}_{\text{NO}_x}$ varies between 3.7 and -1.8 (after Moore (1977) and Fryer (1978) in Fryer (1991)) respectively, then the $\Delta_{\text{NO}_x\text{-Norg}}$ values will vary from -16.7 to -10.5‰ and from -11.2 to -5.0 respectively. According to Heaton (1987) in Freyer (1991), if NO_x is derived from coal-fired power station, then $\delta^{15}\text{N}_{\text{NO}_x}$ is 5.2‰ , which gives $\Delta_{\text{NO}_x\text{-Norg}}$ for our samples ranging from -18.2 to -12.0‰ . When we take diesel engine exhaust as a NO_x source, then $\Delta_{\text{NO}_x\text{-Norg}}$ value will vary from -11.4 to -5.2‰ . However, we can exclude the coal-fired power station input, and in our area, transport origin will dominate with average $\Delta_{\text{NO}_x\text{-Norg}}$ varying between c.a. -17.0 and -5.0‰ .

The other potential source for nitrogen in lichens is precipitation with NO_3^- ion dissolved, in which $\delta^{15}\text{N}_{\text{NO}_3^-}$ obtained by Freyer (1978) is -3.1‰ . It allows us to calculate $\Delta_{\text{NO}_3^-\text{-Norg}}$ value between -9.9 and -3.7‰ . The obtained nitrate ranges are similar, and lie in range for nitrous oxide as described above.

To sum up, the complex studies concerning lichens presented by Michener and Lajtha (2007) suggest that the lower $\delta^{15}\text{N}$ values reported in mosses are found within areas with agricultural activity, mostly dominated by NH_3 emissions. Whereas the more heavily influenced by NO_x emissions in industrialized areas show $\delta^{15}\text{N}$ that is more enriched in ^{15}N . Unfortunately, our results do not confirm this assumption. Because we excluded NH_4^+ and NH_3 as sources for nitrogen on the investigated area, the $\Delta_{\text{NH}_3\text{-Norg}}$ and $\Delta_{\text{NH}_4^+\text{-Norg}}$ values were not calculated here.

Figure 4. Tri-plot combination between altitude and N concentration and $\delta^{15}\text{N}$ of lichens in analyzed area.

6. Conclusions

The study on lichen *Hypogymnia physodes* collected in Karkonoski National Park and its closest vicinity provided critical information about the concentration of carbon, nitrogen and sulphur, as well as the isotopic composition of carbon and nitrogen, which is valuable due to the fact that there is lack of such data from this region. We showed that bioindicators record information about atmospheric pollutants range in their thallus. However, we conclude that for more complex analysis more sampling points should be taken into account. Moreover, any correlation between the analyzed factors and height was not found. Nevertheless, this study yielded the information that in due course of time, the SO_2 and SO_4^{2-} ion decreased, which is reflected in decreased %S in lichens. For sampling points located within Karpacz city (sampling points No. 2, 3, 4), based on the $\delta^{13}\text{C}$ results, we suggest that the dominant source of C are coal combustion processes. Whereas for remaining sampling points, the $\delta^{13}\text{C}$ values are ambiguous and the sources are masked by many mixed processes, both natural (assimilation/respiration of CO_2) and anthropogenic (solid/liquid fossil fuel combustion input). The $\delta^{15}\text{N}$ values suggest that the transport is not a dominant source within Karpacz city, as against $\delta^{15}\text{N}$ values measured in the sampling points No. 8 and 12. Moreover, in this study, we attempted to calculate the fractionation (Δ) for carbon and nitrogen. To explain the possible ways of car-

bon and nitrogen budget in lichens, two hypotheses were proposed: (i) the isotopic fractionation for both carbon and nitrogen for substrates (gaseous/ions form) and products (organic C and N) does not exist ($\Delta = 0$), hence the composition of lichens reflects the composition of the sources. For the second hypothesis for carbon compounds, the calculated $\Delta_{\text{CO}_2\text{-Corg}}$ value varies from -13.4 to -11.4‰, whereas the $\Delta_{\text{HCO}_3^-\text{-Corg}}$ value varies from -16.6 to

-14.6‰; and both paths suggest that in our region the C3 cycle is dominant in lichens compared to the C4 cycle. Moreover, for the second hypothesis, $\Delta_{\text{NO}_x\text{-Norg}}$ value varies between c.a. -17.0 and -5.0‰, whereas $\Delta_{\text{NO}_3^-\text{-Norg}}$ value varies between -9.9 and -3.7‰, and both paths suggest transport origin of N in our investigation area.

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