The diversity of diatom assemblages developed on fallow soil in Pogórka Wola near Tarnów (southern Poland)

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Abstract

The aim of this work was to investigate the species diversity of diatoms growing on fallow fields on loose sand. The study site was located in the western part of Pogórka Wola near Tarnów (southern Poland). Samples were collected from fallow land once a month from April to December 2011. 57 diatom taxa from 19 different genera were found. The most numerous genera were *Fragilaria* (8) and *Luticola* (7). The highest species richness was observed in December, November and July, while the highest values of species diversity (Shannon–Wiener index) were recorded between August and October. Four taxa – *Pinnularia borealis* Ehrenb. var. *borealis*, *Stauroneis borrichii* (Petersen) Lund, *Hantzschia amphioxys* (Ehrenb.) Grunow, and *Luticola nivalis* (Ehrenb.) D.G. Mann, were found to be the dominant species. Neutral species (a pH of about 7) dominated in all months (from April to December), reaching an over 90% share in the assemblage. In terms of nitrogen content, nitrogen-autotrophic taxa, tolerating an elevated concentration of organically bound nitrogen, occurred most abundantly, as was indicated by the low content of nitrogen in the soil.

Keywords: soil algae; loose sand; Bacillariophyceae; species diversity

Introduction

Over recent years, soil algae have been the object of many ecological, physiological and biochemical studies. They play a significant role in the colonization of low productive soils, which results from volcanic activity, retreating of glaciers, fires, or mining activity. The biomass of photoautotrophic soil algae is a source of humus and nitrogen. They produce organic matter and other organic compounds, e.g. amino acids, organic acids, polysaccharides and vitamins, which influence the life cycles of other soil organisms and vascular plants [1–3].

Edaphic ecosystems are characterized by instability of environmental conditions. The key factors that determine algal growth in soils are insolation, soil moisture, temperature, and water availability. Sunlight penetrates only a few millimeters into the soil and therefore soil algae are exposed to high sunlight as well as some deficits. They live in varying temperatures (from extremely high to extremely low), so they can colonize deserts in both tropical and Antarctic climates. Soil algae can fix some mineral components from the soil, thereby taking part in the process of soil stabilization. Thanks to durable valves, composed of silica, diatoms are an important part of fossil flora, where they occur in the form of diatomite (diatomaceous earth) [4–9].

An important factor affecting the species composition of algal communities is soil pH. Most algae (diatoms as well) prefer neutral or slightly alkaline soils [4–8].

Green and blue-green algae are among the most studied soil algae. Green algae occur in a wide range of pH, while soil cyanobacteria dominate on neutral and slightly alkaline soils. Some species of edaphic algae, which are able to assimilate nitrogen, can colonize specific ecosystems, such as deserts. Diatoms are rarely identified in soil algae studied [10–20]. Although some research has been carried out on the ecology and species composition of soil diatoms [10,13,21–28], no studies have been found which concern soils developed on fallow land. Also, no research studies have been conducted on the diversity of soil diatom communities in the area of the Małopolska region (southern Poland). Only two studies have attempted to investigate diatom communities growing in cultivated fields in Dąbrowa and Boguchwała in the Podkarpackie region [27,28].
The aim of this study was to investigate the species diversity of diatoms growing on fallow fields situated on loose sands and to attempt to identify the factors that have a direct effect on the variation in diatom assemblages.

Material and methods

Study area

The study area is situated in Pogórka Wola, in the municipality of Skrzyszów located within two major morphological units: Pogórze Karpackie and Kotlina Sandomierska [29] (Fig. 1).

Fig. 1 Location of the sampling site in Pogórka Wola (grey color indicates forested areas).

The study sites were located in the western part of Pogórka Wola, on a small flat hill where the bedrock consists of loose sand. There is no watercourse near the study site, so the research area was never flooded. The field had been fallow for about eight years. Rye and triticale had been grown there previously. The land surrounding the study site is still farmed.

The climate in the study area is transitional (between oceanic and continental). The area near Tarnów is known as a “heat island”, with the longest summer in Poland (about 80 days). The area near Tarnów is known as a “heat island”, with the longest summer in Poland (about 80 days). The area around Tarnów receives 700 mm of rainfall per year (maximum rainfall is in July and minimum rainfall in February) [30]. The average total precipitation for the meteorological station in Tarnów was 600.7 mm in 2011 (data provided by the Organization Department for the meteorological station in Tarnów). The average total precipitation for the meteorological station in Tarnów was 600.7 mm in 2011 (data provided by the Organization Department for the meteorological station in Tarnów). The area near Tarnów was about 90 g). At the same time, samples were taken from the top soil layer (0–5 cm) and basic physico-chemical analysis was performed (moisture, pH, organic matter and nitrogen content, granulometric composition). Additional soil samples were collected in Kopecki cylinders (in three replications) to examine soil moisture.

The analysis of soil moisture was carried out in a laboratory using the drying-weighing method. A small amount of soil material (about 10 g) from the Petri dishes was placed in beakers with a capacity of 100 ml and they were filled with a chromic acid cleaning mixture in order to obtain pure valves of diatoms. Then, the samples were cleaned by centrifugation. The diatoms were mounted on permanent diatom slides with synthetic resin – Pleurax. The material was prepared according to the methods applied by Kawecka [31], Stanek-Tarkowska and Noga [27,28].

Diatoms were identified using a Nikon ECLIPSE 80i light microscope, according to the following keys: Krammer and Lange-Bertalot [32–35], Ettl and Gärtner [18], Krammer [36], Hofmann et al. [37].

Pictures of diatoms were taken using an optical microscope (Nikon ECLIPSE 80i) and a scanning electron microscope (HITACHI S-4700) at the laboratory of field emission microscopy, scanning electron microscopy and microanalysis (Institute of Geological Sciences of the Jagiellonian University in Cracow).

The number of diatoms was obtained by counting all valves in randomly selected microscopic fields of view, up to a total of 400 valves. Species whose percentage in a given community was 5% or more were classified as dominant [27,28,31].

According to the red list of algae [38], diatom taxa were assigned to threat categories.

The assessment of ecological preferences of diatoms was performed using the list proposed by van Dam et al. [39]. The following indicators were taken into consideration: pH, moisture and nitrogen uptake metabolism diatom type.

The following physico-chemical analyses of soil were performed: pH in KCl, organic matter content was determined using Tiurin’s method [40], granulometric composition was determined by the application of Bouyoucos-Casagrande’s method, with Pruszyński’s modifications (according to the Polish Standard PN-78/9180-11), nitrogen content using Kjeldahl method [41], and soil moisture – using the drying-weighing method.

The species diversity of diatom assemblages was determined by the Shannon–Wiener (H′) species diversity index:

\[ H' = \sum_{i=1}^{s} \frac{n_i}{n} \times \log_2 \left( \frac{n_i}{n} \right) \]

where:

- \( n_i \) – number of individual diatoms of the species \( i \)
- \( n \) – total number of individual counts (\( n_i/n \) – relative abundance of the species \( i \))
- \( s \) – total number of diatom taxa occurring in the sampling site.

A graph of moisture distribution in each month was made using STATISTICA software (version 10.0).
Results

The soil in the study site in Pogórska Wola was characterized by an acidic reaction (4.1–5.3) and the lowest pH values were recorded in the summer months. The granulometric composition analysis showed that the studied soil can be classified as loose sand (65% of sand, 24% of silt, 1% of clay). The content of organic matter ranged from 1.37% in November to 2.04% in May 2011. The highest soil moisture occurred in the autumn and winter months, the lowest one in summer (Fig. 2). Physico-chemical examination showed a low level of soil nitrogen concentrations, similar in all months (Tab. 1).

The largest populations were formed by *Pinnularia borealis var. borealis*; it always reached an over 20% share in the assemblage, while in April and July even more than 60%. *Hantzschia amphioxys* (15–27.5%) and *Stauroneis borrichii* also occurred numerously, reaching the highest percentage in August – 32%, while *Luticola nivalis* were numerous only in autumn (Tab. 3).

Alkaliphilous taxa dominated (more than 90%) in all samples. In respect of nitrogen uptake metabolism, nitrogen-autotrophic diatom taxa, tolerating an elevated concentration of organically bound nitrogen, were most frequent. Diatoms growing mainly on wet or temporarily dry places were the most numerous in all months (Fig. 5–Fig. 7).

Among 57 diatom taxa identified, seven were on the Polish red list of algae [38]. Three taxa from the genus *Pinnularia* were in the endangered category (E): *P. schoenfelderi* Krammer, *P. subrastreas* Krammer and *P. viridiformis* Krammer, whereas two in the vulnerable category (V): *Aulacoseira crenulata* (Ehrenberg) Thwaites and *Fragilaria tenera* (W. Smith) Lange-Bertalot. Two taxa from the rare category (R) were also found: *Luticola acidoclinata* Lange-Bertalot and *Stauroneis termicola* (Petersen) Lund (Tab. 2).

Discussion

The organic matter content of the soil is one of the most important parameters, affected not only by the system of cultivation and fertilization, but also by high agricultural culture and the character of the bedrock. In the past, arable land was often left fallow in order to improve the physical and chemical conditions of the soil, whereas currently arable land is left fallow due to the unprofitability of agricultural production. The field on which the present study was conducted had been left fallow for about 8 years for economic reasons. During the study period, the organic matter content was variable – the highest value was recorded in May (2.04%), while the lowest one was noted on November (1.37%). Czyż et al. [42] indicated a correlation between organic matter content and the amount of water in the soil – the more organic matter, the greater amount of water in the soil. The present study did not confirm the above relationship, since the highest moisture occurred in the autumn and winter months (Tab. 1).

The highest rainfall was recorded in the second half of July (181 mm) and in June (112.9 mm), while in the remaining months of the growing season the rainfall was low and ranged from 21.4 mm in September to 61 mm in April (data provided by the Organization Department for the Hydrological and Meteorological Services Center for Poland’s Climate Monitoring Institute of Meteorology and Water Management National Research Institute IMGW-PiB Warsaw). As regards the weather conditions that occurred in 2011, the dates of sampling coincided with the lack of rainfall and the present study showed a relatively high species richness of diatoms – 57 taxa were noted from 19 different genera. Two similar studies carried out in the neighboring region, but on cultivated soils, showed a similar species richness of diatoms – 47 diatom taxa were found at the study site in Boguchwała, whereas in Dąbrowa – 62 taxa [27,28].
### Tab. 2
The list of diatom taxa identified on fallow soil in Pogórska Wola near Tarnów and the categories of threats (according to Siemińska et al. [38]): E – endangered, V – vulnerable, R – rare. “+” means the taxon was found in a given study month. RL means the category of threat.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>2011</th>
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<tbody>
<tr>
<td></td>
<td>IV</td>
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<tr>
<td>Achnanthidium pyrenaicum (Hustedt) Kobayasi</td>
<td>+</td>
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<tr>
<td>Amphora pediculus (Kützing) Grunow</td>
<td></td>
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<tr>
<td>Amphora sp.</td>
<td>+</td>
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<tr>
<td>Aulacoseira crenulata (Ehrenberg) Thwaites</td>
<td>+</td>
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<tr>
<td>Aulacoseira distans (Ehrenberg) Simonsen</td>
<td>+</td>
</tr>
<tr>
<td>Aulacoseira granulata (Ehrenberg) Simonsen</td>
<td>+</td>
</tr>
<tr>
<td>Aulacoseira cf. crenulata (Ehrenberg)/Thwaites</td>
<td></td>
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<tr>
<td>Cyclotella maneghiniana Kützing</td>
<td>+</td>
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<tr>
<td>Diadesmis sp.</td>
<td>+</td>
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<tr>
<td>Diatomas vulgaris Bory</td>
<td>+</td>
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<tr>
<td>Encyonema reichardtii (Krammer) D.G. Mann</td>
<td>+</td>
</tr>
<tr>
<td>Encyonema silesiacum (Bleisch) D.G. Mann</td>
<td>+</td>
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<tr>
<td>Encyonema ventricosum (Agardh) Grunow</td>
<td>+</td>
</tr>
<tr>
<td>Encyonema vulgare Krammer var. vulgare</td>
<td>+</td>
</tr>
<tr>
<td>Emolonta minima (Grunow) Lang-Bertalot</td>
<td>+</td>
</tr>
<tr>
<td>Eunotia curtagrunowii Nörpel-Schmepp &amp; Lange-Bertalot</td>
<td>+</td>
</tr>
<tr>
<td>Eunotia minor (Kützing) Grunow</td>
<td>+</td>
</tr>
<tr>
<td>Eunotia cf. minor (Kützing) Grunow</td>
<td>+</td>
</tr>
<tr>
<td>Fragilaria bicapitata Mayer</td>
<td>+</td>
</tr>
<tr>
<td>Fragilaria biceps (Kützing) Lang-Bertalot</td>
<td>+</td>
</tr>
<tr>
<td>Fragilaria construens (Ehrenberg) Grunow f. construens</td>
<td>+</td>
</tr>
<tr>
<td>Fragilaria construens f. venter (Ehrenberg) Hustedt</td>
<td>+</td>
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<tr>
<td>Fragilaria exiguiformis Lang-Bertalot</td>
<td>+</td>
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<tr>
<td>Fragilaria pinnata Ehrenberg</td>
<td>+</td>
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<tr>
<td>Fragilaria tenera (W. Smith) Lange-Bertalot</td>
<td>+</td>
</tr>
<tr>
<td>Fragilaria sp.</td>
<td></td>
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<tr>
<td>Hantzschia abundans Lange-Bertalot</td>
<td>+</td>
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<tr>
<td>Hantzschia amphioxys (Ehrenberg) Grunow</td>
<td>+</td>
</tr>
<tr>
<td>Hantzschia calcifuga Reichardt &amp; Lange-Bertalot</td>
<td>+</td>
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<tr>
<td>Luticola acidolinata Lange-Bertalot</td>
<td>+</td>
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<tr>
<td>Luticola goeppertiana (Bleisch) D.G. Mann</td>
<td>+</td>
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<tr>
<td>Luticola mutica (Kützing) D.G. Mann</td>
<td>+</td>
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<tr>
<td>Luticola nivalis (Ehrenberg) D.G. Mann</td>
<td>+</td>
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<tr>
<td>Luticola paramatica (Bock) D.G. Mann</td>
<td>+</td>
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<tr>
<td>Luticola ventricosa (Kützing) D.G. Mann</td>
<td>+</td>
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<tr>
<td>Luticola sp.</td>
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<tr>
<td>Navicula gregaria Donkin</td>
<td>+</td>
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<tr>
<td>Navicula lanceolata (Agardh) Ehrenberg</td>
<td>+</td>
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<tr>
<td>Navicula cf. reichardtiana Lange-Bertalot</td>
<td>+</td>
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<tr>
<td>Navicula sp.</td>
<td></td>
</tr>
<tr>
<td>Nitzschia abbreviata Lange-Bertalot &amp; Rumrich</td>
<td>+</td>
</tr>
<tr>
<td>Nitzschia palea (Kützing) W. Smith</td>
<td>+</td>
</tr>
<tr>
<td>Nitzschia sp.</td>
<td></td>
</tr>
<tr>
<td>Pinnularia borealis Ehrenberg var. borealis</td>
<td>+</td>
</tr>
<tr>
<td>Pinnularia schoenfelderi Krammer</td>
<td>+</td>
</tr>
<tr>
<td>Pinnularia subpraepastris Krammer</td>
<td>+</td>
</tr>
<tr>
<td>Pinnularia viridisformis Krammer</td>
<td>+</td>
</tr>
<tr>
<td>Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot</td>
<td>+</td>
</tr>
<tr>
<td>Planothidium lanceolatum (Brebisson) Lange-Bertalot</td>
<td>+</td>
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<tr>
<td>Stauroeis borrichi (Petersen) Lund</td>
<td>+</td>
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<tr>
<td>Stauroeis obtusa Lagerstedt</td>
<td>+</td>
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<tr>
<td>Stauroeis reichardtii Lange-Bertalot</td>
<td>+</td>
</tr>
<tr>
<td>Stauroeis termicola Lange-Bertalot &amp; Alles</td>
<td>+</td>
</tr>
<tr>
<td>Thalassiosira cf. pseudonana Hasle &amp; Heimdal</td>
<td>+</td>
</tr>
</tbody>
</table>

**Total taxa in a given month**: 14 14 16 21 19 20 17 21 23

**Shannon–Wiener (H') species diversity index**: 1.60 1.78 2.09 1.94 2.49 2.38 2.37 1.87 1.97
cultivated soil in northeastern Italy, Zancan et al. identified only 10 diatom species at four sites studied [13].

The study conducted on fallow soil in Pogórska Wola showed that in all the months of the study the most numerous taxa were *Pinnularia borealis* var. *borealis* and *Hantzchia amphioxys*, which are typical soil diatoms [18,32,36,37].

*Pinnularia borealis* var. *borealis* is probably a cosmopolitan species, frequent in aerial habitats, such as rocks, walls, soil and mosses [18,36]. It was also often found on cultivated soils in Boguchwala and Dąbrowa in the Podkarpacie region, but it was not the dominant taxa [27,28].

*Hantzchia amphioxys* is a cosmopolitan species, one of the most frequent diatoms growing in dry places and on the soil [18,32,37]. Studies on soil algae concern mainly green algae and cyanobacteria, while diatoms are rarely found in the soil and among them *H. amphioxys* is the most frequently mentioned [12,14,15,43,44]. *Hantzchia amphioxys* is defined as a ubiquitous species, with a very wide geographical

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**Fig. 3** Selected diatom taxa: *Stauroneis borrhii* (J.B. Petersen) J.W.G. Lund (a–i), *Luticola nivea* (Ehrenberg) D.G. Mann (j–r), *Pinnularia borealis* var. *borealis* (Ehrenberg) Rabenhorst (s–z).
range, which creates dwarf forms in dry habitats [45,46]. This species has also been noted on agricultural soils in the Podkarpackie Province, but did not exceed 5% in the assemblage [27,28].

The other two dominant species – Stauroneis borrichii and Luticola nivalis – occurred less frequently, constituting 5–20% of the assemblage. Both species are cosmopolitan and aerophitic [18,32].

According to van Dam et al. [39], Stauroneis borrichii is a neutrophilous, oligosaprobous, fresh water species. Its first record in Poland comes from the Kobylanka stream, where it occurred rarely, mainly in samples with Vaucheria sp. [47,48]. However, Stanek-Tarkowska et al. [49] considered these algae to be a typical soil species, similar to H. amphioxys and P. borealis var. borealis.

The ecological preferences of the found diatom taxa for pH, nitrogen uptake metabolism and moisture were determined. It was noted that neutral species (a pH of about 7) dominated in all months (from April to December), reaching an over 90% share of the assemblage. In terms of nitrogen

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Fig. 4 Selected diatom taxa: Hantzschia amphioxys (Ehrenberg) Grunow (a,c–i), Stauroneis thermicola (J.B. Petersen) J.W.G. Lund (b,j–o), Achnanthidium pyrenaicum (Hustedt) Kobayasi (p–s).
**Tab. 3** The dominance structure of diatom communities on fallow soil in Pogórka Wola.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>2011</th>
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<tr>
<td></td>
<td>IV</td>
</tr>
<tr>
<td><em>Pinnularia borealis var. borealis</em></td>
<td>&lt;5%</td>
</tr>
<tr>
<td><em>Hantzchia amphioxys</em></td>
<td></td>
</tr>
<tr>
<td><em>Stauroneis borrichii</em></td>
<td></td>
</tr>
<tr>
<td><em>Luticola nivalis</em></td>
<td></td>
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</tbody>
</table>

![Diagram](image)

**Fig. 5** Classification of ecological indicator values (according to van Dam et al. [39]). pH range: 1 – acidobiontic, 2 – acidophilous, 3 – neutral, 4 – alkaliphilous, 5 – alkalibiontic, 6 – indifferent, no apparent optimum, 7 – unknown. IV–XII – months from April to December.

![Diagram](image)

**Fig. 6** Classification of ecological indicator values (according to van Dam et al. [39]). Nitrogen uptake metabolism type of diatom: 1 – nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen, 2 – nitrogen autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen, 3 – facultatively nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen, 4 – obligately nitrogen-heterotrophic taxa, needing continuously elevated concentrations of organically bound nitrogen, 5 – unknown. IV–XII – months from April to December.
content, nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen, occurred most abundantly, as additionally indicated by the low content of nitrogen in the soil (Tab. 1). Diatoms growing mainly in wet, moist or temporarily dry places, primarily species of the genus *Eunotia* and *Stauroneis* [39], occurred in all the studied months. A slight increase in taxa from the groups that never or only very rarely occur outside water bodies was recorded in summer (VIII) and late autumn (XI–XII).

In addition to the cosmopolitan diatoms, which are common in different types of habitats, taxa from the Polish red list of algae [38] were also noted. Some of them, such as *Pinnularia schoenfelderi* and *Stauroneis thermicola*, occur very rarely in water habitats, as it seems that they find favorable growth conditions on soils.

Taxa from the genus *Pinnularia*, with the status of endangered species (category E) – *P. schoenfelderi*, *P. subrupesrtris* and *P. viridiformis* – are species occurring in European freshwaters from oligo- to dystrophic, with low to moderate electrolyte content [36,37]. In the area of Podkarpacie region, they were found in many rivers and streams, always as individual specimens, while they did not occur on cultivated soils [50]. *Pinnularia schoenfelderi* is probably a cosmopolitan species, often found in the sub-mountainous and mountainous regions of Europe [36] and occurring in waters with a neutral pH, i.e. in springs of central Poland [51]. In the Podkarpacie Province, it is very rarely found in aquatic ecosystems [50], whereas the study conducted on soils near Tarnów showed that it grows better in a soil habitat.

According to Ettl and Gärtner [18], *Stauroneis thermicola* grows among wet mosses isolated on soils (England, Germany, the Czech Republic, Slovakia, Iceland, Denmark). According to van Dam et al. [39], it is an aerophilous and fresh brackish water species. In Poland it is a very rare species [52], regarded as rare (R) on the Polish red list of algae [38]. It was reported from a peat bog in Kraków [53], in the Raba River [54], central Poland [55], the Szczecin Lagoon [56], and the Kobylanka stream [47,48]. The study conducted on cultivated soils in the Podkarpacie region revealed that it best grows in soil habitats where it often reaches more than 20% in the total numbers and is a dominant species [27,28,49].

The studied soil was characterized by high diatom species richness, mainly cosmopolitan species and typical for soil habitats. As a result of our study, we could not identify the factors that have a direct effect on the variation in diatom assemblages. The occurrence of soil diatoms may be associated with many habitat factors, so further studies are needed to better understand these relationships.

**Acknowledgments**

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**Authors’ contributions**

The following declarations about authors’ contributions to the research have been made: concept of the study: JST, TN; field work: JST, EK; writing and statistical analysis: JST, TN, NK, ŁP, AP.

**Competing interests**

No competing interests have been declared.

**References**

45. Wojtal A. Diatom flora of the Kobylnka stream near Kraków.
Różnorodność zbiorowisk okrzemek rozwijających się na odłogowanej glebie w Pogórskiej Woli koło Tarnowa

Streszczenie