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Changes in Algal Species Composition of Alpine Lake Nesamovyte (Eastern Carpathians, Ukraine) from 100 Years Ago to Present †

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Abstract: The species diversity of algae in different ecotopes of the alpine lake Nesamovyte was studied. In total, 233 species were identified comprising globally and regionally rare species. We also documented a temporal change in species composition from the first investigations conducted 100 years ago to present. An increase in the species diversity of *Bacillariophyta* and *Chlorophyta*, the constancy of the number of *Euglenophyta* and *Cyanoprocaryota* species, a decrease in the composition of *Charophyta* and the disappearance of *Dinophyta* species were revealed. We further documented some changes in the most abundant species and indicator species for certain limnological conditions, the disappearance of montane species and the colonization of species with a wide ecological-geographical amplitude. Benthic and plankton-benthic conditions became dominant and indicators of low-alkaline mesotrophic waters as well as mass development ("blooming") of some species increased. The studied features of the species diversity of the "algoflora" indicate high species richness and high number of indicator species, as well as lake ecosystem degradation and an increase in organic pollution. Overall, there was a shift of the lake from oligotrophic to mesotrophic state, as indicated by certain algal groups. The noted changes in the species diversity of algae in Lake Nesamovyte could be attributed to the increased anthropogenic influence leading to change of the trophic status of the lake.

Keywords: algae; diversity; Eastern Carpathians; mountains lakes; ecosystems; Nesamovyte

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1. Introduction

Lakes of the Carpathian mountain system have a rich and distinctive biodiversity of algae and algal communities. They constitute an integral part of the highland landscape, serve as a core algae habitat in the region, and as a shelter for rare and characteristic algal taxa [1–5]. At the end of 20th century, the alpine lake algal biodiversity was actively studied in the western part of the Carpathians (Carpathian-Tatra region). However, much less is known about the species composition and taxonomic structure of the algal flora in the analogous Eastern Carpathian lakes [6]. In this study, we summarise the data from the three stages of algal biodiversity research in one the high-elevation lakes in the Eastern Carpathians—Lake Nesamovyte. First, we conduct a comparative analysis of the changes in algal biodiversity that span a period of more than one hundred years [7–10]. We then characterise

these changes by analysing bioindicator species and establish the main ecological features of Lake Nesamovyte algal flora.

2. Materials and Methods

Lake Nesamovyte is a part of glacial cirque valley and has a glacial erosion origin. It is a part of the Carpathian-Danube algofloristic sub-province within the algofloristic zoning of Ukraine classification scheme [11]. It is located on the southern-western slope of Mt Turkul, at the altitude of 1748 m (Figure 1). It has an area of 0.3 ha and a maximum depth of 2 m (Table 1). It is mainly fed by atmospheric water supply and has a β-mesosaprobic water quality status [4]. It is a well-known natural object of the Ukrainian Carpathians and a part of Carpatsky National Park.

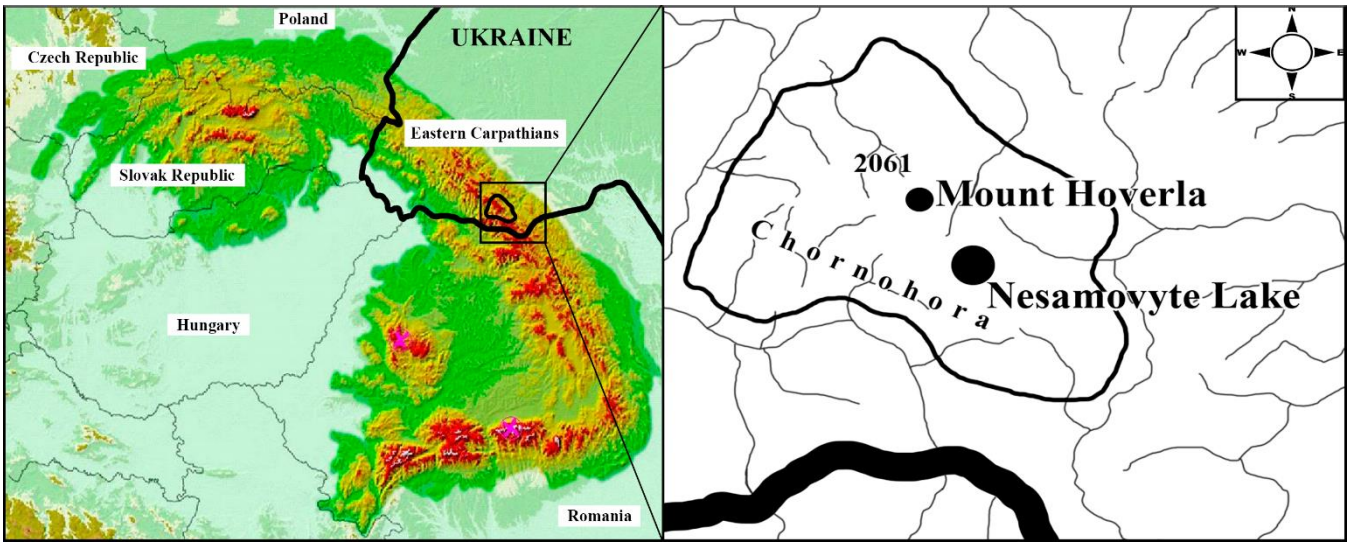

Figure 1. Map of the Carpathian region and the location of the lake Nesamovyte.

Table 1. Morphometry and basic physic-chemical water parameters of Nesamovyte Lake (original measurements).

Location (Coordinates)	Altitude a.s.l., m	Surface, ha	Depth Max., m	Temperature H_2O	pH	Conductivity, µS/cm	dO_2, mg/L
48°07′36.6″ N 24°32′26.4″ E	1748	0.3	~2.0	18.3 (16.2)	6.2–6.4	5.1–6.0	10.7

Our study is based on the original samples collected from different ecotopes of Lake Nesamovyte (plankton, benthos, periphyton, squeezes from the moss) in 2013–2018. We also analysed historical samples (fixed in 4% formaldehyde) of 1967 that are stored in the collection of M.G. Kholodny Institute of Botany NAS of Ukraine (collection acronym: KW-A). Taxonomic identification was done after consulting the following references [12–19]. The samples were studied by means light and scanning electron (SE) microscopy. Diatom identification was done using the 35% H_2O_2 treated frustules material [20]. SE microscopy was done on the JEOL-1230 microscope in the Centre of Electron Microscopy, M.G. Kholodny Institute of Botany NAS of Ukraine. Some of the species were identified after live samples cultivation on liquid and agarised Bold's media. Image processing was done in Axiovision 4.3.7. and GIMP 2.8.10 packages. Identified taxa were validated using the AlgaeBase system [21] and "Algae of Ukraine …" [22] monographic series.

Ecological bioindicator species analysis was based both on the historical data (1910–1920 and 1967–1978 collections) and on the results of original studies (2013–2021). It was based on the following individual algae species characteristics: habitat type, flow velocity, oxygen saturation, pH, salinity, general trophic status and the level of organics pollution [23–28].

3. Results and Discussion

Overall, 233 algae species (including 245 infraspecific taxa, inft) were found in Lake Nesamovyte over a hundred-year period. These species belong to eight taxonomic groups, 15 classes, 33 orders, 55 families, and 99 genera of cyanobacteria and algae (Table 2).

Bacillariophyta and *Charophyta* form the basis of this diversity, accounting for about 57% of the genera richness and 84% of the species and intraspecific richness of algae of all ecotopes. The diversity of *Chlorophyta* and *Euglenozoa* is relatively low (ca. 4.9–5.7% of the taxonomic list), and the diversity of *Cyanobacteria* and *Ochrophyta* is even lower-near 1.7% of the taxonomic list on average. Finally, the diversity of *Cryptophyta* and *Miozoa* accounted for less than 1% of the total taxonomic list (Figure 1). The dominant algal orders were—Desmidiales (15 genera–66 species–75 inft), Naviculales (10 genera–50 species), Cymbellales (5–25), Achnanthales (5–11), Eunotiales (1–11), Sphaeropleales (6–7), Fragillariales (4–9), Zygnematales (6–6) and Euglenidida (2–5). Together, these orders account for 56% of the genera and species composition of Lake Nesamovyte algal biodiversity. The dominant genera of the lake's algal flora were *Staurastrum* (21–22 inft), *Pinnularia* (14–16), *Euastru*m (12–15), *Navicula* (12), *Cosmarium* (12), *Eunotia* (11), *Gomphonema* (10), *Encyonema* (5), *Planothidium* (5) and *Closterium* (4). These 10 genera account for almost a half (44.5%) of all species composition. At the same time, nearly 60% of algal genera of the lake were monotypic, i.e., represented by a single species.

This taxonomic structure represents a generalization of the 100 years research of Lake Nesamovyte algal biodiversity studies [6–8,10,29–31]. The comparative analysis of the taxonomic data from the three phycological research stages–early 20th century, middle of the 20th century, and early 21st century–shows a gradual change the lake algal biodiversity. This is evident on the high, taxonomic groups, level, and especially remarkable on the lower, genera diversity, level (Figure 2).

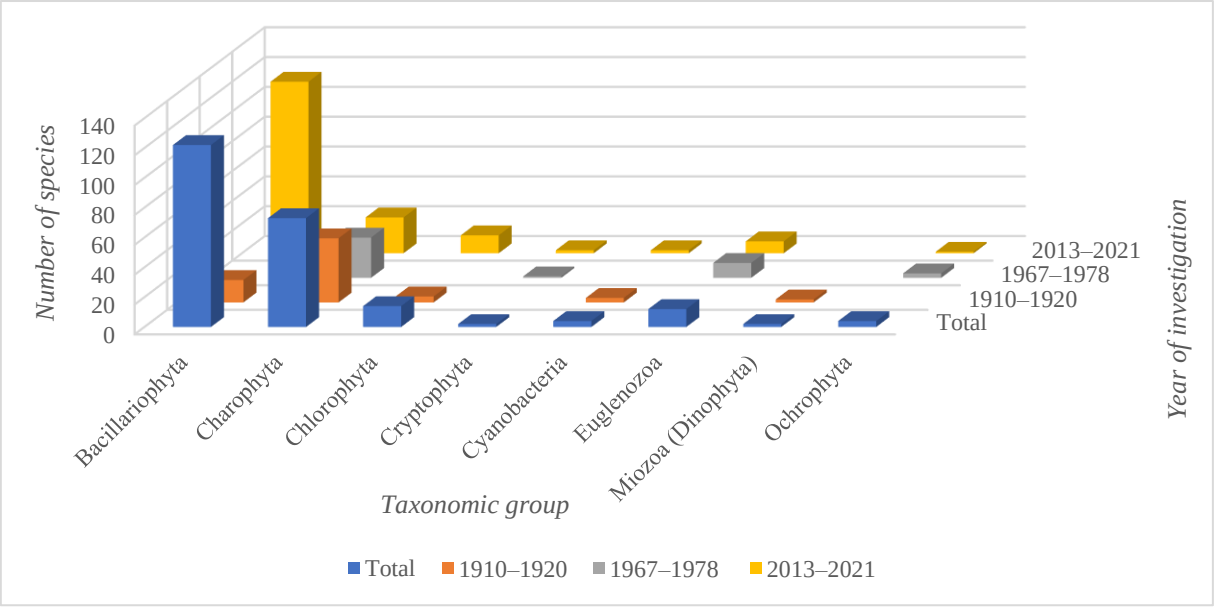

Figure 2. Species composition of algae in Lake Nesamovyte (split by taxonomic groups and three stages of research in 1910–2021).

The change of dominating taxonomic groups of algae in terms of species richness–from *Charophyta* (*Desmidiales*) and diatom algae (together 87% of the total species composition) is established for the period 1910–1920 [7]. The specific high impact *Desmidiales* (~58%) is maintained along with the emergence of representatives of *Euglenozoa* (~15%) and flagellated algae—*Cryptophyta* and *Ochrophyta* (~2–5%) in 1967–1978 [6,9], and now–extremely high rates are noted for *Bacillariophyta* (115 species–117 inft, or ~70%), much lower-*Charophyta* (~15%), *Chlorophyta* (~7.3%) and *Euglenozoa* (~5.0), low for *Cryptophyta*

(>1%) and *Cyanobacteria* (>1%) and absence of *Dinophyta*. A high variety of modern species composition has been established for *Bacillariophyta* [31] sharply contrasting (sevenfold increase) with the literature data [7]. The high role of the diversity and composition of this group for "flora" of Lake Nesamovyte has been noted. The following genera are characterized by high species diversity: *Pinnularia* (12.1%), *Navicula (9.5%), Eunotia* (8.6%), *Gomphonema* (8.6%), *Encyonema* (4.3 %). The typical diatom species in different ecotopes of the lake were *Tabellaria flocculosa* (Roth) Kützing, *Eunotia minor* (Kützing) Grunow and *Frustulia crassinervia* (Brébisson) Lange-Bertalot et Krammer (4–5–according to Starmach scale [32]. At the same time, the regionally rare species for the flora of Ukraine were found (*Cymbella lange-bertalotii* Krammer, *Encyonema neogracile* Krammer, *Eunotia tetraodon* Ehrenberg, *Pinnularia macilenta* Ehrenberg etc.), and also *Pinnularia falaiseana* Krammer, which has a pronounced disjunctive distribution in the world representing a rare species. The current high diversity of *Bacillariophyta* in the lake is probably partly due to the intensive study of diatoms in the lake flora. Apparently, this can also be influenced by a set of ecological and geographical reasons, in particular with the possible settlement of widespread forms and invasive species of plain areas. Likewise, the presence of European and regionally rare species of *Bacillariophyta* is an additional argument for the protection of the ecosystem of Lake Nesamovyte and its diversity as a habitat for their existence.

Original studies of the diversity of the green phylum of the algae flora (*Charophyta* and *Chlorophyta*) of this waterbody confirm the richness and floristic significance in the lake ecosystem (Figure 2) and show a decrease in the species diversity of *Charophyta* (from 43 species (45 inft) in 1920 to 27 (31)–in 1967–1978 and till 24 (25) nowadays), as well as growth-*Chlorophyta* (4 species–12 species, respectively 1910–2021) [6]. The representatives of *Zygnemaphyceae* (14.6%) and *Chlorophyceae* (7.3%) classes comprise about one-fifth of the total algal species of Lake Nesamovyte nowadays. A significant role in the formation of this diversity belongs to the order *Desmidiales* (11.6%), however the species richness of *Zygnematales* and *Sphaeropleales*–insignificant and is at the level of 3.7% and 3.0%, respectively. Among genera by species diversity stand out *Euastrum* and *Staurodesmus* (5 species–3.0%, each of them). 18 genera are presented only by one species each. In turn, compared to the beginning of the 20th century, the presence of six genera has not been confirmed, and from 1967–1978—four genera. Also, for modern period of investigations, the rare species from genera *Hyalotheca, Euastrum* and *Tetmemorus*, that are typical for the flora of waterbodies in mountain regions are revealed. In addition to rare forms, for the first time the presence of filamentous Charophyta algae and mucilage-forming coccoid and flagellar green algae was noted (*Mougeotia, Spirogyra, Zygnema, Oedogonium, Botryococcus, Chlamydomonas, Mucidosphaerium, Chlorella, Mychonastes, Westella*). These findings indicate the probable degradation of the alpine ecosystem from oligotrophic waterbody in the early 20th century into its transformed status to mesotrophic type and settlement of its ecotopes by common species with a wide ecological amplitude [4,6,33].

According to the results of comparative analysis of species composition *Charophyta* of Lake Nesamovyte for 100-year period [7] a change in the complex of leading species by quantitative indicators (=frequency of occurrence) was noted for *Desmidiales*/*Zygnematales*, namely: from communities of *Cylindrocystis brebissonii* (Ralfs) De Bary, *Actinotaenium cucurbita* (Brébisson ex Ralfs) Teiling ex Růžička, *Cosmarium staurastriforme* Gutwinski, *C. venustum* (Brébisson) W. Archer var. *excavatum* (Eichler et Gutwinski) West et G.S. West, *Euastrum insigne* Hassall ex Ralfs and others, to the similar in the 60–70s of the 20th century [9]—*Staurastrum senarium* f. *senarium* and f. *tatricum* Raciborski, *Euastrum pinnatum* Ralfs and others, and modern composition of this species—*Hyalotheca dissiliens* Brébisson ex Ralfs, *Netrium digitus* (Ehrenberg ex Ralfs) Itzigsohn et Rothe emend. Ohtani, *Euastrum humerosum* Ralfs var. *affine* (Ralfs) Raciborski, *E. ansatum* Ehrenberg ex Ralfs and *Staurastrum polytrichum* (Perty) Rabenhorst.

The striking manifestation of the instability of the ecological state of the lake, violation of its ecosystem and increasing of its trophic level is the process of "blooming" of the water of this reservoir, due to the massive development of green colonial coccoid algae

Botryococcus terribilis Komarek et Marvan (Trebouxiales, Trebouxiophyceae), which was highlighted in the summer of 2015 for the first time [6].

The initial investigations revealed the affinity of the mountain-"alpine" part of the species composition of the studied "flora" for the Carpathian lakes with the similar lakes of the Tatra, Sudeten and Alps and the presence of specific 27 alpine holarctic species of desmidiaceen and coccoid green algae [7]. However, the current studies of the algal species diversity of the lake do not confirm the presence of the majority of the species composition (over 80%) observed in the 20-s of 20th century of alpine flora of the Alpine-Carpathian region. However, the presence of another complex of conditionally arctic algae species, including diatoms (*Cavinula pseudoscutiformis* (Hustedt) D.G. Mann & Stickle, *P. obscura* Krasske, *P. rhombarea* Krammer, *P. rupestris* Hantzsch, *P. subanglica* Krammer, *P. subrupestris* Krammer) were revealed.

The ecological analysis is conducted based on types of indicators, which are grouped based on following characteristics: habitat preference, streaming and oxygenation, pH, salinity, trophic state, organic pollution (class of water quality) [26]. With the help of ecological preferences of species that were grouped for time intervals (I—1910–1920; II—1967–1978; III—2013–2021), we present the ecological characteristics (on the example of indicators of trophic and saprobity of the lake) of these periods. The trophic state of the lake is characterized on the basis of characteristics of 55.6–64.7% taxa from the total amount indicated for each time period. There is a tendency of changing the number of indicator species from oligo-mesotraphentic (o-m)—48.8% from the total amount of indicator species characterizing the minimal trophic level of the Lake (20-ies of 20th century) to prevailing of mesotraphentic (m) indicator species (48%). However, the meaningful role oligo-mesotraphentic (o-m) (44%) indicator species in between 60-es of 20th century is maintained. The forming of oligo-mesotraphentic (o-m) (24.1%), meso-eutraphentic (me) 23.1% and oligotraphentic (ot) (22.2%) indicator species along with the emergence of hypereutraphetic (he) and increasing the amount of eutraphetic (e) indicators is embracing for modern period of investigations. The presence of these species nowadays characterizes the general trend of deterioration of the trophic status of the studied reservoir.

Peculiarities of water saprobity indicators (54.3–70.7% of species composition), as well as saprobity indices allowed to characterize an organic pollution and to correlate these data with the classification adopted in Ukraine, highlighting water quality classes [34]. Comparative analysis of the number of indicator species of quality class I varies from 44.7% (1910–1920) to 39.3% and the emerging of indicators of class IV water quality, which indicates a tendency to deterioration of the water status and increasing of organic pollution (1967–1978). And in the modern period - also the predominance of indicators of class II water quality (39%) and the presence of indicators of class IV quality. Thus, we observe a gradual increase in organic pollution of the reservoir from 1920 to the present period.

4. Conclusions

As a result of studying the algal species composition of Lake Nesamovyte during 100-year period of investigations, the 233 species (245 infr) composing 8 taxonomic groups, 15 classes, 33 orders, 55 families, 99 genera were revealed. Modern species diversity of algae is over 70% of the total number of species. During the compared period, significant structural changes in the taxonomic composition of the algoflora of the lake, as well as complexes of dominant species and their diversity were noted. The basis of taxonomic and species diversity of the waterbody is formed by *Bacillariophyta* and *Charophyta*, which combine more than half of the genera level and more than 2/3 of the species and intraspecific composition of algae. The diversity of *Chlorophyta* and *Euglenozoa* were insignificant (about 5%), for *Cyanobacteria* and *Ochrophyta*–quite low (less than 2%). The representatives of *Miozoa* according to the materials of modern research was not revealed. The modern species composition of algae in Lake Nesamovyte is characterized by the presence of a

significant number of regionally rare species and is an additional argument for the protection of the ecosystem of the reservoir and the preservation of the specific species composition.

On the basis of the ecological characteristics of algae, an ecological analysis of the species composition of the lake was carried out. Its results showed a number of changes that took place with the ecosystem of the lake in different historical periods 1910–2021. In particular, the indication of the habitat preferences reaffirmed the hydrological changes in the Lake. The indication of the pH confirmed this conclusion, and moreover, decreasing the leading group of acidophil algae, indicating the presence among the species composition of the "bog component" of flora, and the increase in the role of indicator indifferents in the modern period, testified not only hydrological changes in the lake, but also ecological changes that have occurred with the ecosystem in Lake Nesamovyte. The analysis of the trophic state indicators, namely the presence of hypereutraphentic (he) taxa in modern period of investigations, in comparison with the previous period (1967–1978), as well as increasing amount of eutraphentic (e) indicators in comparison with the earliest period (1910–1920), characterizes the deterioration of the current trophic state of the investigated Lake. The analysed indicators of saprobity in the Lake indicate a change in organic pollution at the present stage, with a change in the class of water quality from I to II class with the occurrence of IV class indicators, which were not noted in 1910–1920. Thus, it becomes obvious that the ecosystem of Lake Nesamovyte is undergoing changes, which were recorded by researchers in 1967–1978, and are aggravated today. In general, there is a tendency for the degradation of the ecosystem in Lake Nesamovyte and the deterioration of the ecological state in comparison with earlier periods.

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Conflicts of Interest: The authors declare no conflict of interest.

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