Taxonomic structure of cyanoprokaryotes and microalgae in the steppe soil of Askania Nova

ANATOLIY SOLONENKO
Melitopol State Pedagogical University named after Bogdan Khmelnitsky
OLEKSANDR BREN (oscillat8@gmail.com)
Institute of Botany of the Czech Academy of Sciences
OKSANA BREN
Melitopol State Pedagogical University named after Bogdan Khmelnitsky

---

Research Article

Keywords: Cyanoprokaryota, microalgae, steppe, Askania Nova, taxonomic structure

Posted Date: May 18th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2914738/v1

Abstract

This study investigated the species composition and diversity of cyanoprokaryota and microalgae in the steppe soil of Askania Nova. There were identified a total of 135 species belonging to 6 phyla, 8 classes, 33 orders, 48 families, and 76 genera, with Chlorophyta, Cyanoprokaryota, and Xanthophyta being the most predominant in terms of species richness. The partial soil algoflora is diverse and stable over time due to the strict conservation regime in the reserve. The leading orders were Chlamydomonadales, Mischococcales, and Oscillatoriales, and the leading families were *Chlamydomonadaceae*, *Pleurochloridaceae*, and *Leptolyngbyaceae*. The most diverse genera were *Chlamydomonas*, *Leptolyngbya*, *Phormidium*, and *Nostoc*. There were also registered 39 species with the highest frequency of occurrence, including *Hantzschia amphioxys*, *Klebsormidium flaccidum*, and *Pinnularia borealis*. The prevalence of Chlamydomonadales representatives at all taxonomic levels suggests that anthropogenic factors may be impacting the area. Cluster and correspondence analyses showed conformity for certain associations in terms of similarity of species composition and species richness of cyanoprokaryota and microalgae.

INTRODUCTION

The tendency of the steppe area to decrease is an extremely negative phenomenon. Destruction of the sod layer in these regions leads to a lack of the possibility of retaining snow and water and protecting the soil from frost and wind. The granulometric composition of the soil changes, and humus depletion is observed. All these changes have changed the climate and relief of the area, which also has an impact on the biota of the region and, of course, on people. The protection of virgin steppe areas is important from the point of view of both conservation of the biome and biological diversity within it. Such unique steppe areas can be observed in Askania Nova.

Askania Nova is a biosphere reserve located in the south part of Ukraine in the steppe climate zone. The total area of Askania Nova is 825 km². It was established in 1898 by Friedrich-Jacob Falz-Fein. In 1983, it was reorganized into a biosphere reserve. The territory includes a zoological park, an arboretum (2.1 km²), and a virgin steppe area (110 km²). The Askanian steppe has great value from the point of scientific research and nature conservation; nowadays, the area of untouched steppes of Askania Nova is the largest in Europe.

Today, Askania Nova is one of the largest scientific, education and tourism centres in southern Ukraine. Despite the considerable study of animals and higher plants of the reserve, microalgae and cyanoprokaryotes of these territories remain the least studied. Understanding the importance of these organisms stems from the fact that they are a crucial element of ecosystems and the primary link in trophic chains. As constituents of the soil microflora, they participate in various processes, react to changes in soil conditions, and are therefore convenient bioindicators [1]. The latter is particularly important for monitoring the conditions of the steppe territories and their preservation.

The study of the steppe zone of Ukraine in terms of phycology is quite uneven. Thus, information on cyanoprokaryotes and algae in saline soils and hyperhaline continental water bodies is covered in a number of publications [2–4]. Partial results of soil algal flora studies conducted in 1989–1991 are presented in monographs [5, 6]. There are still no data in the literature regarding species composition, taxonomic structure of cyanoprokaryotes, and microalgae of the virgin Ukrainian steppe in modern conditions. Understanding the microorganisms that are most important in soil formation processes is crucial. It is also relevant to use cyanoprokaryota and algae as indicators of the state of soils in protected areas. Thus, the aim of this study was to investigate the species composition and systematic structure of the soils of the steppe areas of the Askania Nova biosphere reserve.

MATERIALS AND METHODS

Field research and sampling were carried out on the eight polygons (in accordance with the map of the reserve – districts 33, 43, 44, 68 in 1988–1991 and 15, 46, 55, 79 in 2019–2021) within Askania Nova territory (Fig. 1, Table 1). The polygons are located in the Kherson region within the Dnieper-Azov geobotanical district [7]. According to algofloristic zoning, the territory belongs to the European algofloristic region, the Eastern European algofloristic province, and the Black Sea-Azov district [8].

The material was sampled according to the generally accepted methods in soil phycology [9]. The combined soil samples included 20–50 individual ones. They were sampled at an area of 4 cm^2 and 0–2 cm depth. In total, 71 samples were collected and processed. The dominant species of higher plant associations in the studied polygons were determined in the field, refined according to [10], and presented in Table 1.

Table 1
Coordinates of polygons of sampling and plant associations and plant associations within them

Polygon of sampling/ district	**Coordinates of the central point of polygon**	**Plant association**
1988–1991		
33	46°28'27.91"N 33°59'45.69"E	Ruderal vegetation
43	46°27'36.04"N 33°54'19.35"E	*Crinitaria villosa + Festuca valesiaca*
44	46°27'40.49"N 33°55'5.56"E	Forb-bunchgrass *+ Elytrigia pseudocaesiae*
68	46°27'27.12"N 34° 0'35.03"E	*Festuca valesiaca + Stipa ucrainica*
2019–2021		
15	46°29'36.81"N 33°59'35.13"E	*Carex praecox + Festuca valesiaca*
46	46°27'45.99"N 33°56'40.65"E	*Carex praecox + Stipa ucrainica*
55	46°28'11.04"N 34° 3'34.76"E	*Elytrigia pseudocaesiae + Stipa ucrainica*
79	46°26'51.44"N 33°59'53.50"E	Forb-bunchgrass *+ Stipa spp.+Festuca valesiaca*

The cultural processing and determination of cyanoprokaryotes and microalgae were carried out in the laboratory of algoecological research on terrestrial and aquatic ecosystems at the Department of Botany and Horticulture of Bogdan Khmelnitsky Melitopol State Pedagogical University.

Samples were studied by direct microscopy and cultural methods (soil, soil-water and agar cultures). Bold's medium with single and triple nitrogen content (1N BBM and 3N BBM) was used during cultivation, with and without the addition of the aqueous extract from the studied soil [9].

The cultures were grown in a lighting stand with fluorescent lamps at 12:12 h dark and light phases. The cultures were examined under a microscope for 30 days. The organisms were determined to be in the live state. Bacillariophyta representatives were additionally identified in the permanent microslides [11]. During determination, we used staining for starch was stained with Lugol's iodine in 1% glycerin, mucus was stained with methylthioninium chloride, and oil was stained with Sudan-III dye. Some organisms have been studied in pure phycological cultures, such as representatives of the Oscillatoriaceae, Chlamydomonadaceae, Chlorellaceae, and Chlorosarcinaceae.

Determination was performed using floristic compilations [12–29]. The names of orders, classes, orders, families, genera, and species are given in accordance with the current nomenclature changes for individual species and intraspecific taxa (www.algaebase.org).

The relative abundances of cyanoprokaryotes and algae were determined using the Starmach scale [30]. Species with scores of «5–7» are classified as dominants.

Frequency of occurrence was calculated for all registered species using the following formula:

$x=(a/b)\ 100\%$,

where x is the frequency of occurrence, a is the number of samples in which the species was registered, and b is the total number of samples.

Cluster analysis was carried out by pairwise comparison of species lists in the study area using the Sørensen-Dice index. A dendrogram was constructed using Orange 3.34.0 (distance metric: non-standardized Euclidian distance; joining rule: single-linkage). A correspondence analysis was conducted using Past 4.12b.

RESULTS AND DISCUSSION

As a result of our research, 135 species of cyanoprokaryotes and microalgae were found in the steppe soil of Askania Nova. These species represented seven phyla, seven classes, 33 orders, 48 families, 76 genera (Table 2, Appendix).

Table 2
Systematic structure of cyanoprokaryotes and microalgae in the steppe soil of Askania Nova

Phylum	Quantity of taxons*				
	Classes	Orders	Families	Genera	Species
Chlorophyta	3	10	22	35	60 (44.4%)
Cyanoprokaryota	1	6	10	16	35 (25.9%)
Xanthophyta	1	2	5	12	18 (13.3%)
Eиstigmatophyta	1	2	4	5	10 (7.4%)
Bacillariophyta	1	12	6	7	9 (6.7%)
Charophyta	1	1	1	1	3 (2.3%)
Total	8	33	48	76	135 (100%)

* Note. The percentage in parentheses is for species within the order in relation to the total number of found species.

An analysis of the leading orders indicated the predominance of Chlorophyta representatives. Therefore, among the nine leading orders, which united 106 species (78,5% of the total number of found species), the first place was occupied by Chlamydomonadales (38 species). The second and third places in this list are Mischococcales (Xanthophyta) and Oscillatoriales (Cyanoprokaryota) – 15 and 14 species, respectively (Table 3).

Table 3
Leading orders of cyanoprokaryotes and microalgae in the steppe soil of Askania Nova

Position (place)	Order	Number of species
1	Chlamydomonadales	38
2	Mischococcales	15
3	Oscillatoriales	14
4	Leptolyngbyales	11
5	Eustigmatales	9
6	Sphaeropleales, Naviculales	8
7	Nostocales	6
8	Chlorellales	5
Totally in the leading orders		106

Fourteen leading families were found in the study area. These included 90 species (66.6% of the total number of species found). The features at this level are similar to the ranking of the leading orders. Thus, the first three places were occupied by chlorophytes, xanthophytes, and cyanoprokaryotes. We should again pay attention to the largest number of representatives of Chlorophyta in this list – five families, which unite 36 species (40% of the number of species in the leading families). Cyanoprokaryota is also represented in this list of numerous and diverse – 3 families, with 25 species (27.8%). The families of other phyla were significantly inferior in these indicators. (Table 4).

Table 4
Leading families of cyanoprokaryotes and microalgae in the steppe soil of Askania Nova

Position (place)	**Family**	**Number of species**	**Share in the total number of species, %**
1	*Chlamydomonadaceae*	13	9.6
2	*Pleurochloridaceae*	11	8.2
3	*Leptolyngbyaceae*	10	7.4
4	*Oscillatoriaceae*	9	6.7
5	*Chlorococcaceae*	8	5.9
6	*Nostocaceae*	6	4.4
7	*Chlorosarcinaceae*, *Protosiphonaceae, Chlorellaceae, Chlorobotryaceae*	5 each	3.7 each
8	*Microcoleaceae*	4	3.0
9	*Naviculaceae, Klebsormidiaceae, Pseudocharaciopsidaceae*	3 each	2.2 each
Totally species in the leading families		90	66.6
Totally species		135	100

At the genus level, there is low saturation of species. Thus, 53 genera were represented by only one specie. The remaining 82 registered species were combined into 25 genera that were identified in the study area. The threshold value to define the leading genera was 1.78 species, i.e., genera that already included at least two species were considered leading. So, the lower part of the list of leading genera (fourth and fifth places) included 50 species, and the first three places were taken by the genus *Chlamydomonas* – 12 species (8.8%), *Leptolyngbya*, *Phormidium* – 8 each (5.9% each), *Nostoc* – 4 (3.0%) (Table 5). The predominance of representatives of Chlorophyta and Cyanoprokaryota at the genus level was again noted at higher taxonomic levels, as mentioned above.

Table 5
Leading genera of cyanoprokaryotes and microalgae in the steppe soil of Askania Nova

Position (place)	**Genera**	**Number of species**	**Share of species for each leading genus relatively to the total number of the found species, %**
1	*Chlamydomonas*	12	8.8
2	*Leptolyngbya, Phormidium*	8 each	5.9 each
3	*Nostoc*	4	3.0
4	*Tetracystis, Chlorococcum, Spongiochloris, Navicula, Klebsormidium, Monodus, Vischeria, Ellipsoidion*	3 each	2.2 each
5	*Microcoleus, Chlorosarcinopsis, Neochlorosarcina, Protosiphon, Bracteacoccus, Microspora, Pseudopleurococcus, Gloeotila, Xanthonema, Botrydiopsis, Nephrodiella, Pleurochloris, Characiopsis*	2 each	1.5 each
Totally species		82	60.7%

Analysis of the leading genera indicated that representatives of different habitats represent the algoflora of the studied area. Species in the genus *Chlamydomonas* are the most diverse. The predominance of monadic microalgae species is also present at the level of leading orders and families. Filamentous cyanoprokaryotes occupy the second (*Leptolyngbya* and *Phormidium*) and third (*Nostoc*) places in the list of leading genera. A rather low threshold for entering the list of leading genera created a situation in which a large number of various genera occupied the fourth and fifth places. The diversity of cyanoprokaryotes is significantly reduced here; the only genus *Microcoleus* is in fifth place, and other genera are representatives of various phyla registered by us.

Soil algae in the studied area were not noticeable to the naked eye and were characterized by dispersed growth. The dominant complex of species throughout all studies was fairly uniform and represented by *Microcoleus autumnalis*, *M. vaginatus*, *Pinnularia borealis*, and *Hantzshia amphioxys*.

The list of species with the highest frequency of occurrence included 39 species (Table 6). The diversity of representatives of various phyla in this list is also noticed: Chlorophyta, 12 species; Cyanoprokaryota, 10; Xanthophyta, 6; Eustigmatophyta, 5; Bacillariophyta, 4. The top five places in the list were species with the highest frequency of occurrence, which was above the threshold of 7.9%: *Hantzschia amphioxys* (share of occurrence: 81.7%), *Klebsormidium flaccidum* (74.6%), *Pinnularia borealis* (71,8%), *Vischeria magna*, *Monodopsis subterranea* (49.3%), and *Sellaphora pupula* (45.1%).

Table 6
Species of cyanoprokaryotes and microalgae with the highest frequency of occurrence values in the studied samples.

Places	Species	Quantity of the samples in which specie was found	Frequency of occurrence, %
1.	*Hantzschia amphioxys*	58	81.7
2.	*Klebsormidium flaccidum*	53	74.6
3.	*Pinnularia borealis*	51	71.8
4.	*Vischeria magna, Monodopsis subterranea*	35 each	49.3 each
5.	*Sellaphora pupula*	32	45.1
6.	*Luticola mutica*	28	39.4
7.	*Monodus dactylococcoides*	26	36.6
8.	*Eubrownia aggregata*	24	33.8
9.	*Microcoleus vaginatus*	23	32.4
10.	*Microcoleus autumnalis, Leptolyngbya foveolarum*	21 each	29.6 each
11.	*Chlorella vulgaris*	20	28.2
12.	*Tetracystis pulchra*	19	26.8
13.	*Ulothrix tenerrima*	17	23.9
14.	*Stichococcus minor*	16	22.5
15.	*Coelastrella rubescens, Mychonastes homosphaera*	15 each	21.1 each
16.	*Bracteacoccus minor, Klebsormidium pseudostichococcus*	14 each	19.7 each
17.	*Tetracystis excentrica*	12	16.9
18.	*Pleurastrum sp.*	11	15.5
19.	*Chlamydomonas aggregata, Botrydiopsis eriensis,*	10 each	14.1 each
20.	*Nephrodiella semilunaris, Pleurochloris commutata, Vischeria helvetica*	9 each	12.7 each
21.	*Xanthonema monochloron, Characiopsis anabaenae, Ellipsoidion oocystoides*	8 each	11.3 each
22.	*Microcystis pulverea, Leptolyngbya fragilis, Leptolyngbya tenuis, Stenomitos frigidus, Pseudopleurococcus sp.*	7 each	9.9 each
23.	*Phormidium bohneri, Phormidium papyraceum, Leptolyngbya valderiana, Xanthonema stichococcoides*	6 each	8.5 each

Comparison of species diversity in different years of the study indicates that during the studies of 2019–2021, 28 species that were observed during 1988–1991 were not found. The relative abundance and frequency of occurrence of such species in the studied samples in a study to 1988–1991 were low; therefore, the probability of finding such species in modern studies was negligible. The study of 2019–2021 expanded the information on the species diversity of the Askania Nova soils; 13 species were found that were not previously recorded within the reserve (Table 8).

An analysis of the floristic spectrum dynamics indicated that under modern conditions, a larger number of cyanoprokaryotes and a slightly reduced number of representatives of other phyla, except for Charophyta, have been identified (Fig. 2). In general, these changes can be characterized as insignificant, and the proportions of different representatives of the phyla in the composition of the studied partial algoflora remained almost unchanged for almost 30 years.

Cluster analysis of the cyanoprokaryotic and microalgal species on the different research polygons of Askania Nova showed four distinct clusters (Fig. 3). Two of them, clusters C1 and C2 (polygons 15 and 33), are separated from the others by the Euclidean distance value of 3.3 and 2.9, respectively. The high-level clustering of C1 and C2 indicated significant differences in the species composition of cyanoprokaryota and microalgae within these sampling sites. The C3 cluster includes polygons 43, 44, 46, and 68 (forked at a value of Euclidean distance 1.9), and the C4 cluster includes polygons 55 and 79 (forked at a value of 2.2). Cluster C4 also demonstrates common features of the species composition of sampling sites 55 and 79. The remaining sampling sites were grouped into a single cluster C3.

A similar situation was observed based on the results of the correspondence analysis of the species richness of cyanoprokaryotes and microalgae to the associations of higher vegetation (Fig. 4, Table 7). The most informative are axes 1 and because of their highest eigenvalues (0.025 and 0.015, respectively) and the highest shares of the total variation explained by the corresponding axis from the overall variation of the original data (51.6% and 31.2%).

There was a large negative value on the scatter plot for ruderal vegetation on axis 1, indicating that this type of vegetation has significantly different ecological requirements for species distribution among the phyla compared to other sampling sites. The differences primarily lie in the relatively small number of identified species, low species richness of cyanoprokaryota and green algae among other phyla, the complete absence of Eustigmatophyta representatives, and the prevalence of cyanoprokaryotic species over chlorophytes.

Table 7
Species richness of cyanoprokaryotes and microalgae in Askania Nova at the phyla level within different associations of higher vegetation

Sample site	**15**	**33**	**43**	**44**	**46**	**55**	**68**	**79**
Association	*Carex praecox+* *Festuca valesiaca*	Ruderal vegetation	*Crinitaria villosa+* *Festuca valesiaca*	*Forb-bunchgrass+* *Elytrigia pseudocaesiae*	*Carex praecox+* *Stipa ucrainica*	*Elytrigia pseudocaesiae+* *Stipa ucrainica*	*Festuca valesiaca+* *Stipa ucrainica*	*Forb-bunchgrass + Stipa spp.+* *Festuca valesiaca*
Cyanoprokaryota	9	6	17	8	16	7	15	11
Chlorophyta	12	3	42	18	27	16	29	17
Bacillariophyta	4	3	8	5	5	4	5	5
Charophyta	2	2	3	3	3	2	3	2
Xanthophyta	8	2	15	5	9	4	8	6
Eustigmatophyta	7	0	8	6	5	4	5	3
Total	42	16	93	45	65	37	65	44

The main common feature of ruderal vegetation and *Carex praecox + Festuca valesiaca* plant associations which move them apart along the axes is that Cyanoprokaryota and Chlorophyta are relatively less abundant compared to other vegetation types. The shares of the sum of the species number in these phyla were 56.3% and 50.0%, respectively, in relation to the total number of species recorded at these sampling sites. Within other associations, their share ranges from 57.78–67.69%. In all other associations, there were more representatives of Chlorophyta than of Cyanoprokaryota. In the case of ruderal vegetation, remote correspondence with the species richness of Charophyta, Bacillariophyta, and Cyanoprokaryota was most pronounced. Carex praecox + Festuca valesiaca is opposed to the species richness of Chlorophyta.

In the third quadrant, the correspondence between the species richness of Cyanoprokaryota and forb-bunchgrass + *Stipa sp.+Festuca valesiaca* and *Carex praecox + Stipa ucrainica*. The same higher vegetation associations were opposed to those of Eustigmatophyta.

Crinitaria villosa + Festuca valesiaca, *Festuca valesiaca + Stipa ucrainica* and *Carex praecox + Stipa ucrainica* were located together in the negative part of axis 2. They are united by the common feature – they have the highest number of cyanoprokaryotic an microalgae species within these sample sites (93, 65 and 65 species respectively), as well as the highest proportion of representatives of Cyanoprokaryota and Chlorophyta among all registered species (63.44%, 67.69%, 66.15%). In this part of the plot, the attraction of Chlorophyta to these associations was most pronounced.

In all of these cases, the correspondence between the species richness of a certain of cyanoprokaryotes and microalgae phyla in relation to the plant association of higher plants is quite distinct. The exception is *Elytrigia pseudocaesiae + Stipa ucrainica* which shows a close relationship with all cyanoprokaryotes and microalgae, of which three stand out the most: Chlorophyta, Xanthophyta and Eustigmatophyta.

Forb-bunchgrass + *Stipa sp. + Festuca valesiaca* is also located within the 95% ellipse of correspondence, but at some comparative distance from the cyanoprokaryota and algae points.

DISCUSSION

It can be argued that sufficient diversity of representatives of different phyla may indicate the absence of strong limiting environmental factors. For example, in different extreme ecosystems, in most cases, the opposite situation is observed; only a certain taxonomic group with a highly specific ecophysiological possibility can remain viable and prevail at all taxonomic levels.

Comparing the proportions of divisions of the partial algal flora of Askania Nova in different years of the study with a thirty-year interval showed consistency and almost complete stability. This can be explained by the strict conservation regime in the reserve, which indicates the effectiveness of protecting rare biotopes in small areas.

Another notable feature is the presence of filamentous cyanoprokaryotes in the list of three different orders. Coccoid phototrophic prokaryotes are not included in this list, as are the leading taxa at a lower level. The species diversity and relative abundance scores of filamentous cyanoprokaryota indicated that these organisms are the primary determinants of the structure and aggregation of soil in the algal flora of virgin steppes.

It is noteworthy that the greatest species diversity at the order level was observed in Chlamydomonadales, which is typical of freshwater habitats. Despite the fact that representatives of this order can often be found in soils, we consider such species as allochthonous in the studied dry steppe soil. These species in our study did not show a high frequency of occurrence (with the exception of *Chlamydomonas aggregata*). This group of organisms probably originated from the Dnieper River through an irrigation canal that runs in the northern and northwestern parts of Nova Askania. The reserve is surrounded by agricultural fields and circular irrigation systems, which spray water from the canal and allow microalgae to spread to adjacent territories. Thus, even despite the presence of a buffer zone 1-1.5 km wide (2.2 km in some parts) along the perimeter of the reserve, there is a direct influence of the anthropogenic factor on the species diversity of microalgae in this area.

The cluster analysis and correspondence analysis showed the weakest relationships between associations Carex *praecox + Festuca valesiaca* and ruderal vegetation in terms of the similarity of the species composition and species richness of cyanoprokaryota and microalgae, while the highest conformity was found for *Elytrigia pseudocaesiae + Stipa ucrainica* and forb-bunchgrass + *Stipa sp.+Festuca valesiaca*. Other associations were equally distant in the ordination scatter plot and had approximately equal correspondence values to the species richness of the studied microscopic autotrophs. As it is known, the absence of significant floristic differences in the species composition of cyanoprokaryota and microalgae of different associations of higher plants within one zonal vegetation type has been established [31]. In our study, we found the same situation with the addition of *Elytrigia pseudocaesiae + Stipa ucrainica* and forb-bunchgrass + *Stipa sp.+Festuca valesiaca* clearly had the greatest influence on the formation of maximum species richness of the studied microscopic autotrophs. Other environmental factors may also play a decisive role in determining species richness in this case. In this study, we relied on data on plant associations and established a stable correspondence. However, this finding requires further confirmation in future studies.

CONCLUSIONS

1. The species composition of cyanoprokaryotes and microalgae in the steppe soil of Askania Nova was represented by 135 species belonging to six phyla, eight classes, 33 orders, 48 families, and 76 genera.
2. Chlorophyta (60 species, 44.4% of the total number of detected species), Cyanoprokaryota (35–25.9%), and Xanthophyta (18–13.3%) were predominant in terms of species richness. The partial soil algoflora of the study area can be characterized as diversely represented by the leading taxa at all taxonomic levels, and it has remained consistent and stable over a thirty-year period, indicating the effectiveness of the reserve's conservation regime.
3. On the top three places in the list of leading orders were Chlamydomonadales, Mischococcales and Oscillatoriales (38, 15 and 14 species respectively). On the first places among the leading families – *Chlamydomonadaceae* (13 species), *Pleurochloridaceae* (11), *Leptolyngbyaceae* (10). The most diversely represented genera are *Chlamydomonas* (12 species), *Leptolyngbya*, *Phormidium* (8 each), Nostoc (4). The prevalence of Chlamydomonadales at all taxonomic levels suggests the influence of anthropogenic factors in the study area.
4. The list of species with the highest frequency of occurrence included 39. The top three are *Hantzschia amphioxys* (81.7%), *Klebsormidium flaccidum* (74.6%), and *Pinnularia borealis* (71.8%).
5. Cluster and correspondence analyses revealed conformity for *Elytrigia pseudocaesiae + Stipa ucrainica* and forb-bunchgrass + *Stipa sp.+Festuca valesiaca*, in terms of similarity in species composition and species richness of cyanoprokaryota and microalgae.

Declarations

Funding

No funding was obtained for this study

Competing interests

The authors declare no conflict of interest.

Availability of data and material

All data generated or analyzed during this study are included in this published article

Code availability

Not applicable

Authors’ contributions

Anatoliy Solonenko: conceptualization, methodology, investigation; Oleksandr Bren: writing- original draft preparation; Oksana Bren: visualization, reviewing and editing

ACKNOWLEDGEMENTS

We would like to thank Dr. Shapoval V. V. for providing us with a map of districts of Askania Nova and for providing us with information on the associations of higher plants within the research area.

ROLE OF THE FUNDING SOURCE

This research was conducted without any special financial support.

References

1. Barinova S., Fahima T. The Development of the World Database of Freshwater Algae-Indicators Journal of Environment and Ecology. 8 (1). – 2017. – 1-7. DOI: 10.5296/jee.v8i1.11228
2. Arabadzhy-Tipenko L.I., Solonenko A.M., Bren A.G. (2019). Cyanoprokaryota of the Salt Marshes at the Pryazov National Natural Park, Ukraine. International Journal on Algae, 21(4), 299-310. DOI: 10.15407/alg29.04.463
3. Solonenko A.M., Bren O.G. . (2020). Floristic Composition and Taxonomic Structure of Algae in the Hyperhaline Reservoirs of the Northwestern Azov Sea Coast (Ukraine). International Journal on Algae, 22(4), 373-382. DOI: 10.15407/alg30.04.393
4. Bren O.G., Solonenko A.M., Podorozhny S.M. Species Structure of Algae of the Saline Coastal Reservoirs of the Pryazov National Natural Park, Ukraine. International Journal on Algae. 24 (2). -2022. – 105-120. DOI: 10.15407/alg32.01.068
5. Kostikov I.Y., Romanenko P. O., Demchenko E.M., Dariyenko T.M., Mikhailiuk T.I., Ribchinskii O. V., Solonenko A. M. (2001) Vodorosti gruntiv Ukraini - istoria ta metodi doslidzhennia, sistema, konspekt flori. Kyiv. Fitosotsiotsentr.
6. Tsarenko P.M., Wasser S.P., Nevo E. (2006. Vol. 1. 713 p.; 2009. Vol. 2. 413 p.; 2011. Vol. 3. 512 p.; 2014. Vol. 4. 703 p.). Algae of Ukraine: diversity, nomenclature, taxonomy, ecology and geography. Ruggell: A.R.G. Gantner Verlag K.-G.
7. Didukh Y.P., Sheliag-Sosonko Y.R. (2003) Geobotanichne raionuvannia Ukraini ta sumizhnikh teritorii. Ukr. Botan. Zhurn. 60 (1) 6-17.
8. Palamar-Mordvintseva G.M., Tsarenko P.M. (2015) Algofloristicheskoe raionirovanie Ukrainy. Algologiia. 25 (4) 355-395.
9. Gollerbakh M.M., Shtina E.A. (1969). Pochvennye vodorosli. L. Nauka. 143 p.
10. Shapoval V.V. Generalized results of large-scale geobotanical surveys of Askanian steppe vegetation.News Biosphere Reserve «Askania Nova», 22, 2020. 29-34. DOI: 10.53904/1682-2374/2020-22/5
11. Hasle G. R., Fryxell G. A. Diatoms: Cleaning and Mounting for Light and Electron Microscopy. Transactions of the American Microscopical Society. Vol. 89, No. 4, 1970, pp. 469-474
12. Andreeva V.M. (1998). Pochvennye i aerofil'nye zelenye vodorosli (Chlorophyta: Tetrasporales, Chlorococcales, Chlorosarcinales). Spb.: Nauka.
13. Dedusenko-Shchegoleva N.T., Gollerbakh M. M. (1962) Opredelitel presnovodnykh vodoroslei SSSR Vypusk 5. Zheltozelenye vodorosli. M. L. AN SSSR.

14. Kondratyeva N. V. (1984) Viznachnik prisnovodnikh vodorostei Ukrainsko RSR Kyiv. Nauk dumka.
15. Matviyenko O.M., Dogadina T.V. (1978) Viznachnik prisnovodnikh vodorostei Ukrainskoi RSR Zhovtozeleni vodorosti. T 1. Kyiv. Naukova dumka.
16. Moshkova N.A., Gollerbakh M.M. (1986) Opredelitel presnovodnykh vodoroslei SSSR. Vyp 10. Zelenye vodorosli. Klass Ulotriksovye. Poriadok Ulotriksovye. L. Nauka.
17. Tsarenko P.M. (1990) Kratkii opredelitel khlorokokkovykh vodoroslei Ukrainskoi SSR. Kiev. Nauk dumka.
18. Vinogradova K.L., Gollerbakh M.M., Zauer L.M., Sdobnikova N.V. (1980). Opredelitel presnovodnykh vodoroslei SSSR. Vypusk 13. Zelenye vodorosli. Klassy Sifonokladovye Sifonovye. Krasnye vodorosli. Burye vodorosli. L. Nauka.
19. Zabelina M.M., Kiselev I.A., Proshkina-Lavrenko A.I., Sheshukova V.S. (1954) Opredelitel presnovodnykh vodoroslei SSSR. Vypusk 4. Diatomovye vodorosli. M. Sovetskaia nauka.
20. Ettl H., Gärtner G. (1988). Süβwasserflora von Mitteleuropa. Bd 10. Jena: G. Fischer Verlag.
21. Ettl H. (1978). Süβwasserflora von Mitteleuropa (T. Bd 3). Stuttgart, New York: G. Fischer Verlag.
22. Huber-Pestalozzi G. (1962). Das phytoplankton des Susswassers: Systematik und biologie. Die Binnengewasser (T. 3). Stuttgart: E. Schweizerbart'sche.
23. Komárek J., Anagnostidis K. (2005). Cyanoprokaryota. 2. Oscillatoriales / Susswasserflora von Mitteleuropa. Bd. 19/2. Munchen: Elsevier Spectr.
24. Komárek J. (2013). Cyanoprokaryota. 3. Heterocytous genera / Süswasserflora von Mitteleuropa/Freshwater Flora of Central Europe. (G. Büdel B., Ред.) Berlin: Springer Spektrum.
25. Komárek J., Anagnostidis K. (1998). Cyanoprokaryota. 1. Chroococcales / Süβwasserflora von Mitteleuropa. Bd 19/1. (G. Ettl H., Ред.) Jena-Stuttgart-Lübeck-Ulm: G. Fischer Verlag.
26. Krammer K., Lange-Bertalot H. (1986). Bacillariophyceae. Teil 3. Centrales, Fragillariales, Eunotiaceae. Gustav Fischer.
27. Krammer K., Lange-Bertalot H. (2004). Bacillariophyceae. Teil 4. Achnanthaceae. Gustav Fischer.
28. Krammer K., Lange-Bertalot H. (1986). Bacillariophyceae. Teil 1. Naviculaceae. Gustav Fischer.
29. Krammer K., Lange-Bertalot H. (1988). Bacillariophyceae. Teil 2. Bacillariaceae, Epithemiaceae, Surirellaceae. Gustav Fischer.
30. Starmach, K. (1955). Metody badań planktonu, PWRiL, Warszawa, 133.
31. Kostikov I.Y. K voprosu o zonalnyh osobennostyah sostava pochvennyh vodorosley. Algologia. – 1991. 1 (4). 15-23.

Figures

Figure 1

Scheme of research polygons. 1988-1991 years of sampling – red squares; 2019-2021 – yellow squares. 1 – protected area, 2 – buffer zone, 3 – anthropogenic landscapes

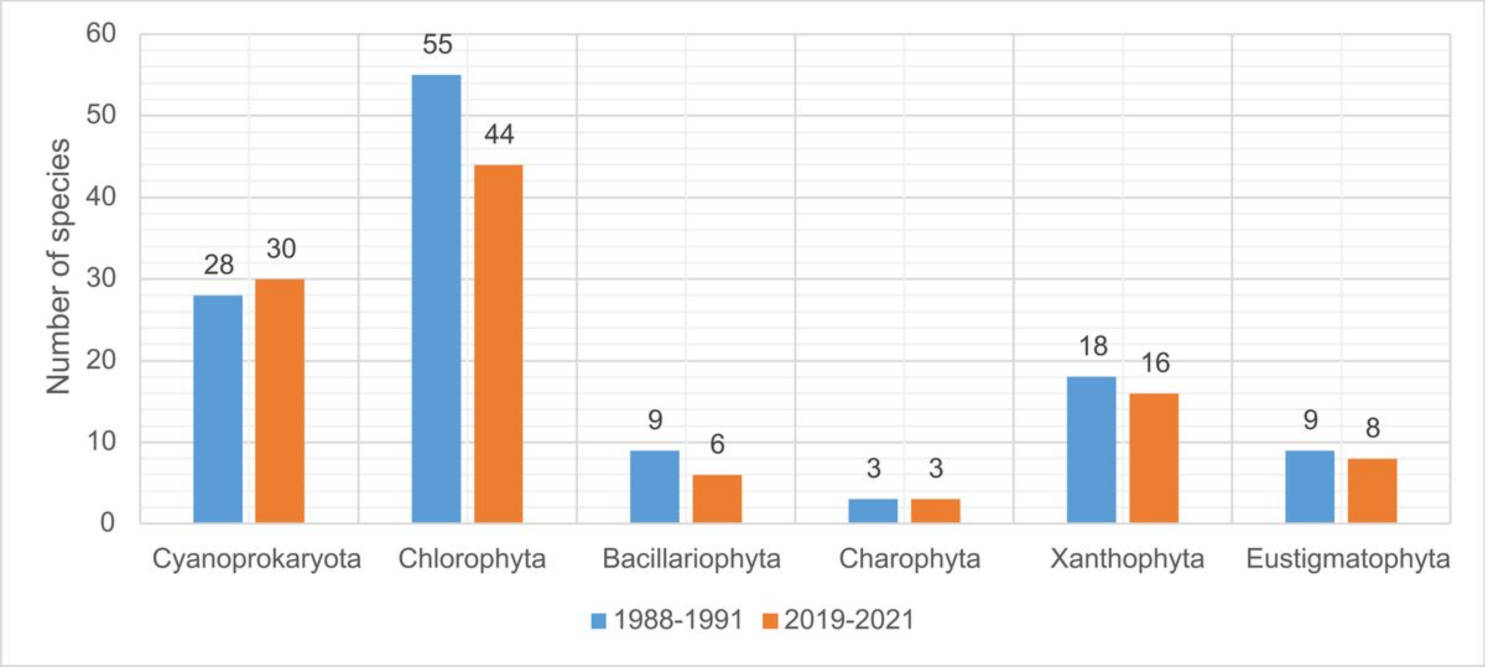

Figure 2

Dynamics of floristic spectrum of cyanoprokaryotes and microalgae in different years of the study in Askania Nova

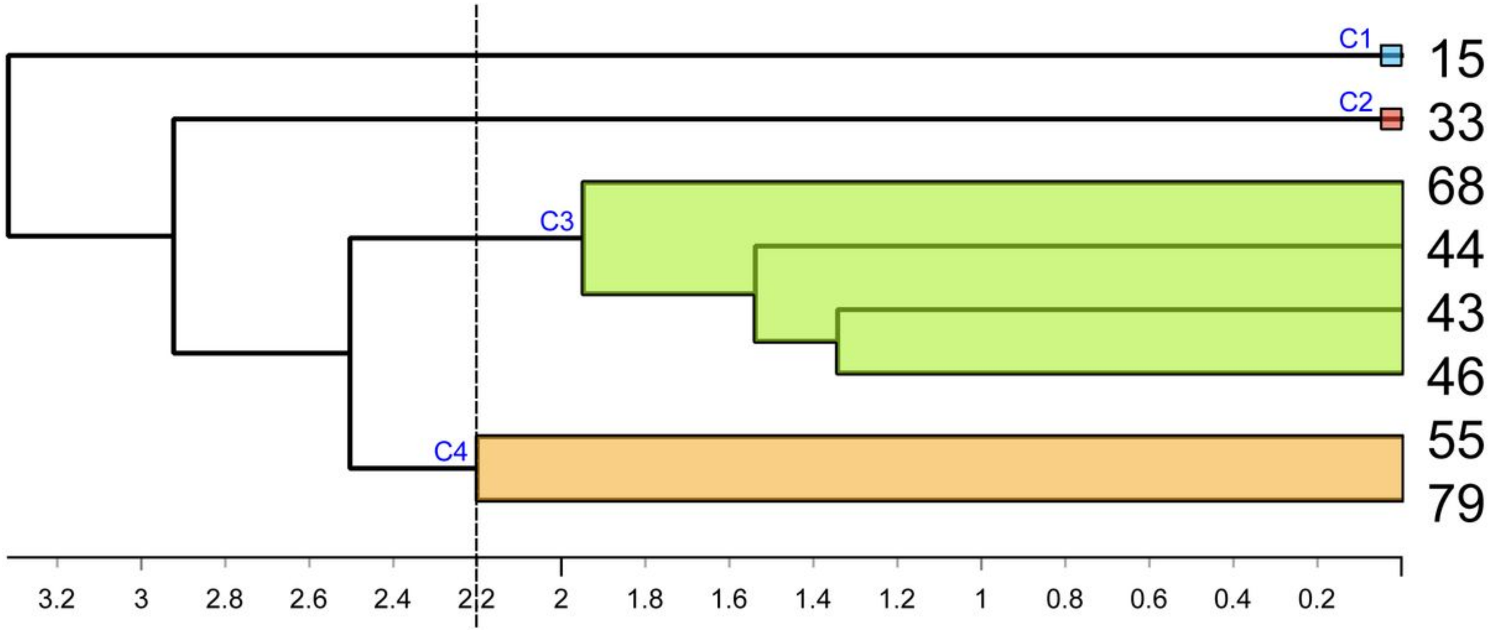

Figure 3

Dendrogram of the floristic similarity of the species of cyanoprokaryotes and microalgae on the different research polygons of Askania Nova

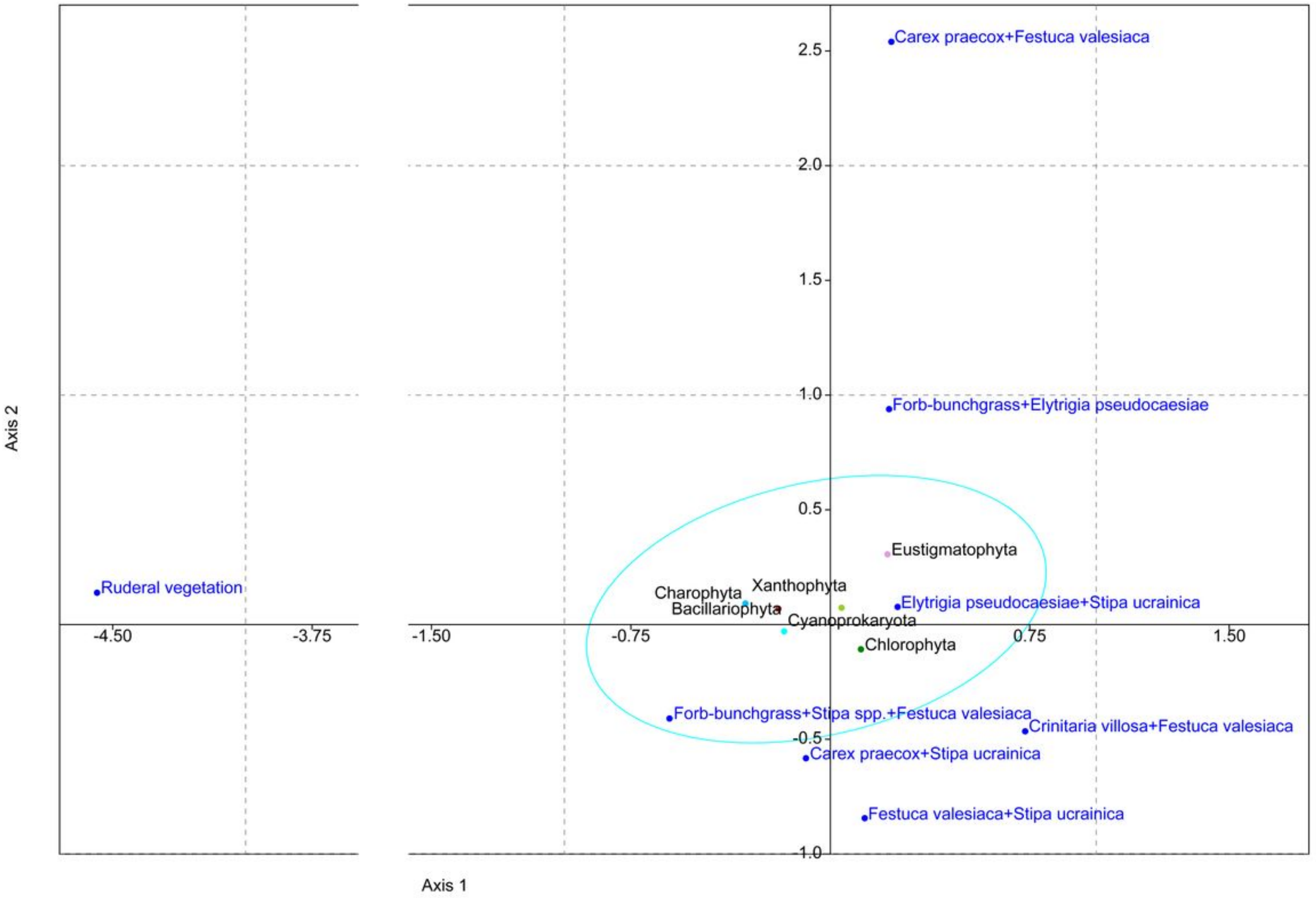

Figure 4

Scatter plot of the correspondence analysis between the species richness of cyanoprokaryotes and microalgae at the phyla level and associations of the higher vegetation where this species were registered

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Appendix.docx