Macrophytes and Charophytes in Ecological Assessment of the Protected Lakes in Donetsk Region, Ukraine

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Abstract First study of macrophytes and Charophytes of three protected lakes in Donetsk region of Ukraine has been implemented for ecological assessment of the lakes environment by bio-indication methods. Altogether five species of macrophytes and three species of macroalgae were revealed. Rare species *Chara dominii* Vilh. was found in massive grow in the Flora Lake. The lakes Flora and Molodezhnyy are mesotrophic and low-to middle organic polluted, while the Azotnyy Lake is oligotrophic, low organically polluted. Statistical methods show that organic pollution come mostly from ground waters enriched by mine waters, and together with soluble sodium from surface runoff they are stimulated species growth. Lake Molodezhnyy can be used as referent site for monitoring, whereas the Azotnyy Lake needs more attention in study of toxic substances, and the Flora Lake for organic matter influence. Bio-indication methods together with statistic approaches can be used as sensitive instrument for ecosystem state assessment and watershed protection in the technogenically impacted area such as Donetsk region of Ukraine.

Keywords Macrophytes, Charophytes, Ecology, Bio-indication, CCA, Lakes, Ukraine

of the ecological status of water bodies and requires an evaluation of different methods. The main waterway flowing through the center of the city of Donetsk is the river Kalmius. Its river basin in the territory of Donetsk is regulated by plenty of water reservoirs and ponds and is a convenient model for monitoring studies of surface waters in the region that is under long-term anthropogenic press [6]. Studies of aquatic vegetation ponds Donetsk held for more than 30 years ago [7-10] and do not reflect the current state of the flora. Bio-indication methods of Donetsk water bodies' assessment was not performed [11]. Our research of macrophytes was partially covered in the paper [12] but still remain in initial stage.

In recent, bio-indication approach in the Eastern Mediterranean and Black Sea basins is rather developed [2-4,13] not only with microscopic algae but also with charophytes macroalgae [14,15]. We implemented of summarized information about species autecology [16,17] for macrophytes and macroalgal community from the three different lakes in purpose to characterize the environment in each lake that are important as recreation and wildlife objects.

The aim of present study is to identify of Charophytes that studied firstly for this lakes locality in Ukraine, to characterize the lake environment by bio-indication methods on the base of revealed diversity of algae and macrophytes.

1. Introduction

Freshwater algae are widely used as bio-indicators of aquatic environment [1] but ecological assessment with algal, charophytes, and macrophyte aquatic plants is in Ukraine in initial step only [2-4]. Avoid species ecology and its habitats, in Ukraine mostly give attention to some biological activities of this species with detailed description of species morphology and floristic aspects [5].

Center industry of South-East Ukraine is Donetsk, where a small area centered metallurgical, machine-building, chemical plants and coal mines that discharge large amounts of wastewater [6]. Such intense exposure leads to disruption

2. Materials and Methods

2.1. Sampling and Laboratory Studies

The macrophytes samples were collected by Andrew Fatyukha during 2012-2014 monthly field trips from 0-70 cm depth of each studied lake. Charophyte samples were taken in each lake by scooping. Both, macrophyte and charophyte samples were herbarized and transported to the Donetsk Botanical Garden for microscope studies. Part of the charophyte samples were sent to the Institute of

Evolution, University of Haifa and studied under Nikon light microscope and Leica stereomicroscope with digital cameras under magnification $\times 100$ -1000. Part of charophyte samples was sent to the Central Siberian Botanical Garden and studied under stereomicroscope Carl Zeiss Stereo Discovery V12 and micrographs were taken with the digital camera AxioCam MRS-S and Axiovision 4.8 software.

Charophytes were treated with HCl to remove calcium carbonate. The structure elements were observed with Nikon with digital camera, DinoLight camera, and light microscopes (LM) in the Institute of Evolution, University of Haifa and the Central Siberian Botanical Garden SB RAS with help of international handbooks [18-20]. Macrophyte and charophyte abundance were assessed as percentage scale in each lake.

Elemental analysis of water samples was carried out in the research laboratory of "Lux", Donetsk, Ukraine. A large suite (33 total) of elements (Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Sr, Ti, V, W, Zn, Zr) were quantified by inductively coupled plasma atomic emission spectrophotometer (ICP-OES) Shimadzu ICPE-9000 according to DSTU ISO 11885:2005.

Statistical analysis of the correlation between species diversity and major water condition variables was calculated by distance-weighted least squares using the Statistica 7.1 Program.

Statistical significance of variables was assessed using the Pearson correlation method with wessa.net.

Canonical Correspondence Analysis (CCA) with CANOCO for Windows 4.5 package [21] was used to determine the relationships of species diversity in algal

communities and their environmental variables.

2.2. Description of the Study Site

Three lakes selected for study are located in the city of Donetsk and its industrial zone (Figure 1). Lakes are seasonally fluctuated in water transparency and biomass of macrophytes. Lakes are used for purpose of recreation and wildlife. Area and length of the shoreline counted using the site cadastral maps of Ukraine (<http://www.map.land.gov.ua/kadastruva-karta>), coordinates with cards Google (<https://www.google.com.ua/maps>).

The Azotnyy Lake (Figure 2) is placed in the Pushkin Park of Donetsk, in the upper right tributary Durnaya of the Kalmius River with $48^{\circ}00'36.4''\text{N}$ $37^{\circ}44'47.8''\text{E}$. Surface area is about 1.7 hectares, the shoreline 700 m, shallow, muddy, along the causeway passes a busy highway, the upper part overgrown with macrophytes. Near the lake is located the Donetsk plant of chemicals, but direct discharges are not exist. Lake is used for purpose of wildlife. Sample was collected in the silty sand bottom 2 meters from the shore at a depth of 50 cm.

The Molodezhnyy Lake (Figure 3) of Donetsk Botanical Garden is located in the arboretum botanical garden, in the upper left tributary Bogodukhov of the Kalmius River coordinates with $48^{\circ}00'10.0''\text{N}$ $37^{\circ}53'04.7''\text{E}$. Surface area is about 3.9 hectares, the shoreline 890 m, deep, muddy, direct discharges are absent, in personal observations with transparent water throughout the year that is non-typical for Donetsk ponds. Samples were collected in the silty bottom, about 2 meters from the shore at a depth of 50 cm, where macrophytes form dense thickets.

Figure 1. Three studied water bodies in the Donetsk region, Ukraine.

Figure 2. The Azotnyy Lake area in the Donetsk region, Ukraine.

Figure 3. The Molodezhnyy Lake area in the Donetsk region, Ukraine.

Figure 4. The Flora Lake area in the Donetsk region, Ukraine.

The Flora Lake (Figure 4) is located in a forested area in the upper right tributary Durnaya of the Kalmius River with coordinates 47°59'04.5"N 37°43'45.9"E. Surface area is about 4 hectares, the shoreline 1200 m, deep, muddy, located near two mine dump, direct discharges are not exist. Samples were collected in the silty sand bottom, about 3 meters from the shore at a depth of 50 cm, where macrophytes form dense thickets.

3. Results and Discussion

3.1. Chemical Composition of the Lakes Water

Chemical variables were measured in three studied lakes between April 2012 and October 2013. Can be seen (Table 1) that chemical variables reflected low polluted waters [13, 16] which enriched by calcium, magnesium, and mostly sodium and sulfates. Water Quality Class has been assessed when it possible for elements according [16]. The Azotnyy Lake water was saturated round the year with iron, potassium and manganese, while calcium, lithium, magnesium, sodium, sulfate, silicon and strontium were significantly lower than in the lakes Flora and Molodezhnyy. That show environmental differences between the Azotnyy Lake that can be classified as under anthropogenic impact, and lakes Flora and Molodezhnyy, which looks like more natural because are located in a forested area (Figures 3-4).

Table 1. Chemical variables in the studied lakes water (mg l⁻¹). Standard according to [16], Table 22. Carbonate rock: Pristine streams draining most common carbonate rock types; DWNC: Global Discharge-Weighted Natural Concentration. Water Quality Class: according to [16], Table 5.

Water body	Sampling date	B	Ba	Ca	Fe	K	Li	Mg	Mn	Na	P	S	Si	Sr	Zn
Flora	Apr-12	0.77	0.03	160	0.005	3.56	0.10	126.0	0.004	567	0.01	635	2.46	2.78	0.001
Flora	May-12	0.86	0.03	199	0.003	4.46	0.11	135.0	0.001	575	0.01	618	1.22	5.70	0.001
Flora	June-12	0.74	0.04	184	0.001	4.43	0.11	141.0	0.003	524	0.02	418	2.80	5.12	0.002
Flora	July-12	0.78	0.04	152	0.001	4.64	0.13	143.0	0.001	549	0.35	404	3.20	5.51	0.002
Flora	Aug-12	0.58	0.04	206	0.001	3.83	0.11	142.0	0.001	675	0.05	467	5.73	4.39	0.002
Flora	Sep-12	0.77	0.03	172	0.002	2.06	0.10	152.0	0.002	332	0.00	450	4.05	4.49	0.003
Flora	Oct-12	0.79	0.03	156	0.004	4.61	0.13	154.0	0.006	611	0.17	480	4.16	6.16	0.004
Flora	Nov-12	0.69	0.03	136	0.005	3.64	0.15	141.0	0.007	624	0.03	352	3.52	6.96	0.004
Flora	Apr-13	0.69	0.04	174	0.003	4.22	0.13	134.0	0.004	506	0.01	506	4.53	5.89	0.003
Flora	July-13	0.71	0.04	168	0.003	4.36	0.12	140.0	0.007	578	0.14	485	4.29	6.73	0.002
Flora	Oct-13	0.71	0.04	175	0.004	4.74	0.11	156.0	0.007	603	0.17	439	5.75	5.88	0.004
Molodezhnyy	Apr-12	0.55	0.03	133	0.008	4.31	0.04	75.5	0.004	288	0.00	456	2.57	1.75	0.003
Molodezhnyy	May-12	0.55	0.04	155	0.006	5.70	0.05	85.6	0.003	330	0.01	450	0.54	3.66	0.004
Molodezhnyy	June-12	0.44	0.03	146	0.002	5.94	0.06	108.0	0.002	372	0.02	350	0.39	3.56	0.002
Molodezhnyy	July-12	0.51	0.04	107	0.002	5.48	0.06	96.1	0.002	352	0.34	326	0.72	3.62	0.003
Molodezhnyy	Aug-12	0.40	0.03	164	0.002	5.55	0.06	98.7	0.002	470	0.05	401	1.79	3.09	0.004
Molodezhnyy	Sep-12	0.52	0.03	136	0.003	2.96	0.06	89.3	0.006	232	0.00	335	1.47	3.22	0.005
Molodezhnyy	Oct-12	0.56	0.03	109	0.005	6.11	0.07	97.5	0.006	389	0.17	424	1.47	4.20	0.005
Molodezhnyy	Nov-12	0.64	0.05	189	0.005	6.06	0.11	127.0	0.009	565	0.03	387	2.08	5.98	0.005
Molodezhnyy	Apr-13	0.58	0.02	127	0.007	5.44	0.07	83.0	0.004	357	0.01	421	1.62	2.55	0.002
Molodezhnyy	July-13	0.62	0.04	132	0.008	5.94	0.07	101.0	0.002	364	0.29	504	1.51	3.51	0.003
Molodezhnyy	Oct-13	0.59	0.04	136	0.008	5.73	0.07	107.0	0.006	376	0.12	474	1.34	4.62	0.004
Azotnyy	Apr-13	0.42	0.03	67	0.055	10.40	0.03	63.8	0.301	168	0.00	144	1.05	0.98	0.003
Azotnyy	July-13	0.44	0.03	70	0.068	11.90	0.02	67.8	0.317	180	0.03	159	1.20	1.22	0.002
Azotnyy	Oct-13	0.42	0.03	75	0.062	12.80	0.04	70.1	0.348	191	0.04	162	1.53	1.44	0.003
Carbonate rock		n.a.	n.a.	2560	n.a.	13	n.a.	640	n.a.	34	n.a.	39	47	n.a.	n.a.
DWNC		n.a.	n.a.	670	n.a.	32	n.a.	259	n.a.	159	n.a.	54	136	n.a.	n.a.
Water Quality Class		n.a.	n.a.	n.a.	I	n.a.	n.a.	n.a.	I	n.a.	I	n.a.	n.a.	n.a.	n.a.

3.2. Diversity and Ecology of Macrophytes and Algae

We identified five species of macrophytes (Table 2) and three species of charophytes in studied lakes: *Chara dominii* Vilh. (Figure 5), *Chara tomentosa* L. (Figure 6), and *Chara intermedia* A. Braun in A. Braun, Rabenh. et Stitzenb. (Figure 7). *Chara dominii* was reported from single locality in Volyn Polissya [18, 22-24] and this record was the single for Europe. Unfortunately specimens of *C. dominii* were lost from Ukrainian botanical collection (according personal communication of Prof. P.M. Tsarenko, Botanical Institute, National Academy of Science of Ukraine) and this record couldn't be checked. So our collection is a checkable record for this species in Europe and a new record for Steppe in Ukraine. *Chara tomentosa* and *C. intermedia* are known

from Steppe. Species *Chara tomentosa* is extremely rare in Ukraine. Species *Chara intermedia* was reported mainly for bays of Black Sea [18, 22-24] and therefore can be marked as more tolerant species.

Studied lakes surface was covered by five species of macrophytes (Table 2), diversity and percent of coverage of which are fluctuated from lake to lake and stay richer in the Flora Lake. Charophytes also were diverse in this lake and represent by two species like in the Azotnyy Lake, while only single species was found in the lake Molodezhnyy. The Flora Lake inhabits by rare species of charophytes – *Chara dominii* and now we only have Ukrainian specimens of this species for comparative studies.

Table 2. Diversity and ecological preferences of macrophyte and charophyte species in the studied lakes with percentage of the lake surface coverage and species autecology according [16]

Taxa	Code	Flora	Molodezhnyy	Azotnyy	Species-specific index s	Saprobity
<i>Potamogeton pectinatus</i> L.	POTPEC	2.9	2.5	18.5	1.70	b-o
<i>Potamogeton perfoliatus</i> L.	POTPER	2.9	2.5	3.7	2.30	b
<i>Potamogeton crispus</i> L.	POTCRI	2.9	0.0	0.0	2.60	a-b
<i>Ceratophyllum demersum</i> L.	CERATO	14.7	0.0	3.7	2.20	b
<i>Myriophyllum verticillatum</i> L.	MYRVER	2.9	0.0	0.0	1.80	o-a
<i>Chara tomentosa</i> L.	CHATOM	0.0	0.0	54.1	1.20	o
<i>Chara dominii</i> Vilh.	CHADOM	14.7	0.0	0.0	-	-
<i>Chara intermedia</i> A. Braun in A. Braun, Rabenh. et Stitzenb.	CHAIINT	58.8	94.9	20.0	-	-
Total species		7	3	5		
Index S		2.16	2.00	1.41

Figure 5. *Chara dominii*: 1 – view of thallus, 2 – axis with axial cortex, stipulodes and base of whorl

Figure 6. *Chara tomentosa*: 1 – view of thallus, 2 – branchlets with axial cortex and oogonia, 3 axis with axial cortex, stipulodes, base of whorl, and branchlets

Figure 7. *Chara intermedia*: 1 – whorl of branchlets, 2 – axis with branchlets, axial cortex, stipulodes, and base of whorl

3.3. Bio-indication of the Studied Lakes Environment

We use bio-indication and statistical methods on the base of algal and macrophyte species which inhabiting the lakes in purpose to characterize of the lakes water quality and ecosystem sustainable.

Table 2 shows Index saprobity S value that we calculated on the base of coverage percent and species-specific index s. Water in the Flora (S=2.16) and Molodezhnyy (S=2.0) lakes was organically more polluted than in the Azotnyy lake (S=1.41). Water quality in first two lakes can be assessed according to [16] as Class III, whereas Azotnyy lake water corresponds to Class II. It correlates with representation of abundant beta-mesosaprobic macrophyte *Ceratophyllum demersum* with index s = 2.2 in the Flora Lake, and oligosaprobic *Chara tomentosa* with s = 1.2 in

the Azotnyy Lake. Therefore, the Flora and Molodezhnyy lakes can be characterized as mesotrophic state and low to middle organic pollution, while the Azotnyy Lake was oligotrophic with low organic pollution [16].

Environmental variables and species diversity relationships were calculated with Statistica 7.1, wessa.net, and CANOCO Programs.

Water variables as a whole in the in the studied lakes are fluctuated in small range (Table 1). Nevertheless, we can see differences in respect of biological variables such as Species richness and Index Saprobity S. So, algal and plant growth in the lakes have positive correlation of species richness with Lithium (Pearson 0.75, $p < .01$), Magnesium (Pearson 0.58, $p < .04$), Sodium (Pearson 0.72, $p < .01$), Silica (Pearson 0.81, $p < .0001$), and Strontium (Pearson 0.72, $p < .01$). Index Saprobity S correlated positively with

Barium (Pearson 0.65, $p < .02$), Lithium (Pearson 0.91, $p < .001$), Magnesium (Pearson 0.87, $p < .001$), Sodium (Pearson 0.57, $p < .01$), Silica (Pearson 0.81, $p < .003$), and Strontium (Pearson 0.72, $p < .01$).

Macrophyte and charophyte species show its own preferences of environmental variables. So, *Potamogeton perfoliatus* abundance is negatively correlated with organic pollution which reflect Index saprobity (Pearson -0.86 , $p < .001$), while *Potamogeton crispus* and *Myriophyllum verticillatum* have positive correlation (Pearson 0.66, $p < .02$ in both cases). *Chara dominii* and *C. intermedia* show positive correlation of its abundance with organic enrichments (Pearson 0.66, $p < .02$ and 0.77, $p < .007$ respectively), but *C. tomentosa* don't show any correlation.

It was important to reveal from which sources come organic pollution that stimulated algal and macrophyte species richness and growth. We hypothesized that calcium comes from an underground supply, while easily soluble sodium from surface runoff. Figure 8 shows that Index of organic pollution has trend to increase with calcium increasing when sodium is low. In the same time organic pollution is slightly stimulated by sodium concentration also. Species richness has negative trend and increase with calcium and sodium increasing. By this can be concluded that pollution come mostly from ground source and stimulated species growth.

Figure 9 show that macrophyte *Potamogeton pectinatus* (and other macrophytes, as we calculated) prefer calcium and sodium enriched water, while macroalga *Chara intermedia* have negative preferences with low concentration of calcium and sodium. This conclusion correlated with chemical data in Table 1, and data of

species representation in Table 2.

Canonical Correspondence Analysis (CCA) shows (Figure 10) that water variables are separated into two different groups. Group of manganese, potassium and iron have negative influence on most of species excluding *Chara tomentosa* which is stimulated in the Azotnyy Lake. All other variables including of organic pollution Index S are grouped in second set and stimulated growth of macrophyte *Ceratophyllum demersum* and charophytes *Chara dominii* and *Chara intermedia* which inhabited lakes Flora and Molodezhnyy. Interesting that this set of variables and influenced species are well growing under organic pollution increasing with groundwater input enriched by mine water as well as with surface runoff [25]. This situation looks like natural, in the initial stage of ecosystem trophic state, when species growth is stimulated by organic enrichments. The lakes with high pH and calcium enrichments like in England are representing especially group and has been recommended more studies be undertaken on eutrophication responses of macrophytes. In lakes where macrophyte species composition varies with water depth, eutrophication is apparent through reductions in productivity especially of Characeae and Potamogetonaceae [26] like in our lakes.

Therefore, the Molodezhnyy Lake can be used as reference site for anthropogenic impact assessment. The Flora Lake needs to more attention in research of complex influence of organic enriched and mine waters flowing into the lake from runoff or ground waters of the catchment area, whereas the Azotnyy Lake can be exposed to toxic substances, as we assume.

Figure 8. Water calcium, sodium, Index saprobity S, and number of algal species relationships in aquatic communities of the Donetsk lakes.

Figure 9. Water calcium, sodium, macrophyte *Potamogeton pectinatus*, and charophyte macroalga *Chara intermedia* relationships in aquatic communities of the Donetsk lakes.

Figure 10. Canonical Correspondence Analysis (CCA) plot of species and environmental variables relationships in aquatic communities of the Donetsk lakes.

4. Conclusions

Three studied lakes ecosystem in the Donetsk area were in first time analyzed by bio-indication methods and statistics. As a result, we revealed five macrophyte species and three species of charophyte macroalgae. Lakes Flora and Molodezhnyy is mesotrophic and low-to middle polluted by organic matters, while the Azotnyy Lake show oligotrophic state and low organically polluted waters. Statistics pointed us that organic pollution comes mostly from groundwater enriched by mine waters and stimulated species growth together with soluble sodium from surface runoff. Whereas macrophyte species are resistant to inorganic concentration, charophytes are preferred calcium enriched waters, especially *Chara intermedia* is sensitive.

We can to conclude that the Molodezhnyy Lake can be marked as reference site of anthropogenic impact, the Flora Lake with inhabiting of rare species, such as *Chara dominii* is best object for organic pollution monitoring, and the Azotnyy Lake can be given more attention in following monitoring of toxic substances impact. Bio-indication methods implemented together with statistic approaches can be used as sensitive instrument for ecosystem state assessment and watershed protection in the technogenically impacted area such as Donetsk region of Ukraine.

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