A NEW LOCALITY OF THE FRESHWATER POPULATION OF Ulva flexuosa SUBSP. Pilifera (Chlorophyta, Ulvophyceae) IN POZNAŃ (WIELKOPOLSKA)¹

Andrzej Rybak, Beata Messyasz, Lidia Szendzina, Marta Pikosz, Maciej Koperski

Department of Hydrobiology, Institute of Environmental Biology, Adam Mickiewicz University Umultowska str. 89, 61-614 Poznań, rybak@amu.edu.pl

Summary. In the latter part of the April of 2011 green algae thalli from the genus *Ulva* were found at the new, until now not known site in the Wielkopolska region. Thalli of *Ulva* (syn. *Enteromorpha*) *flexuosa* subsp. *pilifera* (Kützing) Bliding 1963 were collected to a further morphological research from the small, nameless, artificial dam reservoir located in the north part of Poznań. It was the second site, after the Malta Lake, of its taxon in Poznań and the sixth site in Poland. At the newly described site of *Ulva flexuosa* subsp. *pilifera* located near the west shore of the reservoir free-floating thalli were appeared in the sparseness (5–10 ind./m²). Moreover, individual thalli of *Ulva* strongly were covered by filamentous algae which formed dense mats on the water surface. Thalli of *Ulva* at the new site were observed from the end of April until the end of May of 2011.

Key words: Ulva flexuosa, macroalgae, green algae, habitat conditions, Poznań

INTRODUCTION

Localities of taxa from the genera *Ulva* (syn. *Enteromorpha*) were recorded in freshwater ecosystems of Poland already from 1849 (*Ulva intestinalis* L. – in Miękinia and Duszniki Zdrój) [Göppert and Cohn 1850]. From the 19th century on the area of our country 90 sites of 9 taxa of *Ulva* were found.

Ulva flexuosa (syn. *Enteromorpha flexuosa* (Wulfen ex Roth) J. Agardh 1883) subsp. *pilifera* (Kütz.) Bliding 1963 (= *E. pilifera* Kützing 1845) – can reach lengths up to 1 m [Starmach 1972]. Young thalli are weakly branched but mature ones have numerous prolifications. Cells have the square or rectangular

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shape with dimension from 22 to 30 μ m of the length and to 12 μ m of the width and arrange into longitudinal and crosswise rows. Chloroplasts being inside cells contain from 2 to 4 and even to 6 pyrenoids. In addition, at marine populations of *Ulva flexuosa* subsp. *pilifera* a regular isomorphic alternation at generations is observed. Mature sporophytes produce zoospores of 10 × 5 μ m size. However, gametophytes produce male gametes reaching dimension of 6.3 × 2.7 μ m and a little bit bigger female gametes of 6.7 × 3.4 μ m [Starmach 1972, Pliński 1988].

Recently, freshwater U. flexuosa subsp. pilifera were identified at 5 inland sites on the territory of Poland. The response of biotic part of ecosystem to the dynamically changing habitat conditions (eutrophication) in the various water reservoirs is often manifested by the highly developed macroscopic algae e.g. filamentous green algae (Oedogonium, Mougeotia, Cladophora, Voucheria) and thalli of Ulva. Algae from the genera Ulva are restricted to the marine waters wherever the substratum is favourable and sufficient concentration of chlorides in the water is available. Areas with inland waters in Poland where species of Ulva occurs at lower anthropogenic salinity is rather small. This may be misleading, however, because it is such a small green alga (in the first stages of its development) and is present only in localized pockets of the water reservoir where creates dense patches of mature thalli flowing on the surface of water. Thus, important is to pay attention for occurrence freshwater populations of Ulva in all types of reservoirs. The aim of the paper is presentation the new freshwater site of U. flexuosa subsp. pilifera in the Wielkopolska area, hence also in Europe. Particular attention was paid to the morphological features describing this species and to characterise its favoured habitat conditions to development in freshwaters.

MATERIAL AND METHODS

Thalli of macroalgae were sampled from the surface of the examined site in the reservoir in the spring of 2011 (mainly 17.05.2011). Additionally, before preserving samples, tube-shaped thalli of *Ulva* were separated mechanically from filamentous green algae from the genera *Oedogonium* (Chlorophyta) into which they were woven. The cleaned samples were, then, put in plastic containers of 100 ml volume and fixed with 4% solution of formalin. Morphometric analysis, necessary for the species level identification were carried on conserved material. A light microscope (Axioskop 2 MOT Zeiss) was used for microscopic analysis of collected material and cells of *Ulva* were observed at ×20 and ×40 magnification. Measurements of the length and width of cells including its shape were made as well as cells arranging in the thallus were determined. Moreover, prolifications attendance at *Ulva* thalli were checked. Get scores of distinctive features were next compared with morphometric data for *Ulva* taxa recorded in the area of the country, included in the Starmach key [1972] and in publications: Kowalski [1975], Sitkowska [1999], Rybak and Messyasz [2011].

In the present study water temperature (°C), conductivity (μ S·cm⁻¹), pH, oxygen concentration (mg·dm⁻³), water oxygenation (%) and TDS (mg·dm⁻³) from the site containing *Ulva* were measured using YSI Professional Plus protocols. Water samples (500 ml) were collected for chemical analyses and preserved with 0.5 ml of chloroform(CHCl₃) and then stored in refrigerators at -10° C. Chemical analyses for the following variables: ammonium, nitrate, phosphate, total iron and sodium chloride were determined using standard methods for a Hach DR 2800 spectrophotometer.

Well-known from literature sites and the new freshwater site of *U. flexuosa* subsp. *pilifera* were marked in the map of Poland in the arrangement of ATPOL squares [Zając and Zając 2001].

RESULTS AND DISCUSSION

Distribution of Ulva flexuosa subsp. pilifera in Europe

Ulva flexuosa subsp. *pilifera* is a dominating taxon from the genera *Ulva* in freshwater ecosystems of Europe [Mareš 2009]. As yet, till 2011 *Ulva flexuosa* subsp. *pilifera* was found at the 16th sites in freshwaters of Europe taking into consideration eight sites in the Czech Republic, five in Poland, two in Sweden and the one site in Slovakia (Tab. 1). In Poland this taxon was recorded from 1970 [Kowalski 1975] in the channel on Międzyodrze (Szczecin, surroundings of the A2 motorway) where appeared since June by the October. Sitkowska [1999] observed a presence of this taxon of *Ulva* in the pond in Piotrowice (Łódź province) in years 1984–1987. A few years later (1994–1995) *U. flexuosa* subsp. *pilifera* was found in the pond in Kuciny. Interestingly, in 2008 the taxon developed on a large scale in a fish pond in Arturówko (Łódź province) [Sitkowska 2008 – unpublished], and a year later its appearing was also noticed in the Wielkopolska area in the Malta Lake [Rybak and Messyasz 2011].

In Sweden for the first time *U. flexuosa* subsp. *pilifera* at the freshwater site was found in 1994 in the Mälaren Lake where again was observed by Leskinen in 1996, and then in the August of 1997 in the Saxån river [Mareš 2009]. In Slovakia Palaticka noted the presence of *U. flexuosa* subsp. *pilifera* in the Žitavie river in May of 2008 [Mareš 2009]. However, in Czech Republic Mareš collected samples of this green algae species in 2007 from the Hradec Králové stream and from a Zámecký fish pond in Lednice, and in the July of 2008 from the outflow of a fish pond located in Oleksovic surroundings, and next from a fish pond Hlohovecký in Hlohovec and the water reservoir in Nemilkov. Added to which, in June 2009 Skácelová registered *Ulva* in two fish ponds in Hlohovec (ponds: Prostřední and Mlýnský) and in August Kaštovský monitored appearing of its in a pond in the Staré Hrady town [Mareš 2009].

T	Time	Туре	Thallus	Thallus	Dreashas	Source
Location	of occurrence	of ecosystem	length, cm	width, cm	Branches	
Poland: Międzyodrze, Szczecin	VI–X, 1970	canal	15.0-25.0	1.5-3.0	present	Kowalski [1975]
Poland: Piotrowice, Łódź	1984-1987	pond	20.0-30.0	1.0-2.0	present	Sitkowska [1999]
Poland: Kuciny, Łódź	1994-1995	pond	20.0-30.0	1.0-2.0	absent	Sitkowska [1999]
Sweden: near Mälaren	1994 (Holm)	lake	n.d.	n.d.	n.d.	Mareš [2009]
	1996					
	(Krusenberg)					
Sweden: Skåne	VIII, 1997	river	n.d.	0.05	present	Mareš [2009]
Czechy: Zámecký, Lednice	IX, 2007	fish pond	5.0-10.0	1.0	present	Mareš [2009]
Czech Republic: Hradec, Králové	VIII 2007, 2008	stream	5.0-20.0	0.5-3.0	n.d.	Mareš [2009]
Slovakia: near Žitava	V, 2008	river	5.0	0.5	n.d.	Mareš [2009]
Poland: Arturówek, Łódź	2008	pond	20.0-30.0	1.0-3.0	present	Sitkowska [2008]
Czech Republic: Nemilkov	VII, 2008	water tank	10.0-30.0	3.0-15.0	absent	Mareš [2009]
Czech Republic: Hlohovecý	VII, 2008	fish pond	>10.0	0.25-1.0	present	Mareš [2009]
Hlohovec						
Czech Republic: near Oleksovice	VII, 2008	artificial stream	10.0-30.0	0.1-1.0	n.d.	Mareš [2009
Poland: Poznań	VI, 2009	dam reservoir	15.0-41.0	0.4-4.2	present	Rybak and Messyasz
						[2011]
Czech Republic: Prostředn,	VII, 2009	fish pond	n.d.	n.d.	n.d.	Mareš [2009]
Hlohovec						
Czech Republic: Mlýnský,	VII, 2009	fish pond	n.d.	n.d.	n.d.	Mareš [2009]
Hlohovec						
Czech Republic: Staré Hrady	IX, 2009	fish pond	n.d.	n.d.	n.d.	Mareš [2009]
Poland: Morasko, Poznań	IV–V, 2011	retention	2.0-8.70	0.2-1.2	present	own research
		reservoir				

Table 1. The occurrence of freshwater Ulva flexuosa subsp. pilifera in Europe

n.d. – no data

Macroalga *Ulva flexuosa* subsp. *pilifera* recorded at the freshwater sites in Poland was connected mainly with artificial, small reservoirs (ponds) and with the considerable anthropogenic influence (e.g. fish-culture). In the instance of *Ulva* sites in others countries there was the one site of the artificial origin (the Czech Republic, surroundings of Oleksowic, the stream flowing from a fish pond) and in the case of remaining sites they were half-natural ecosystems staying under the great influence of the man on the account of the close neighbourhood of urban agglomerations.

Table. 2. Number of Ulva sites recorded in freshwater ecosystems on territory of Poland

Taxon	Number of sites	Precentage
Ulva compressa L.	5	5.5
Ulva paradoxa C. Agardh	2	2.2
Ulva prolifera O.F. Müller	6	6.6
Ulva flexuosa Wulfen	10	10.9
Ulva flexuosa subsp. pilifera (Kützing) M.J. Wynne	6	6.6
<i>Ulva intestinalis</i> L.	36	39.7
<i>Ulva prolifera</i> O.F. Müller	5	5.5
Ulva tubulosa L.	2	2.2
Ulva plumosa Hudson	1	1.0
<i>Ulva</i> sp.	18	19.8
Total	91	100%

Sources: Göppert i Cohn 1850, Preuschoff 1883, Kozłowski 1890, Nitrady 1898, 1904, Torka 1910, Raciborski 1888, 1910, Rouppert 1913, Liebetanz 1925, Namysłowski 1927, Wysocka 1952, Marczek 1954, Piotrowska 1961, Wilkoń-Michalska 1963, Podbielkowski 1969, Pliński 1971, 1973, Kowalski 1975, Dambska 1976, Sitkowska 1999, Messyasz 2009, Messyasz and Rybak 2008, 2009; 2011, Gołdyn 2000, Endler *et al.* 2006, Kuczyńska-Kippen 2009, Rybak and Messyasz 2011.

Oral information: Borysiak 2005, Bielecki 2006, Sitkowska 2008, Staniszewski 2008, Burchardt 2009, Pelechaty 2009, Dembowska 2009, Stachnowicz 2009, Szajda 2009, Szyper 2009, Borowiec 2010, Brzeg 2010, Rybak 2010, Gąbka 2011, Gołdyn 2011.

On the ground of carried out examinations and collected references, as yet (state for October 2011), a presence of 9 taxa from the genera *Ulva* was confirmed at the 91st freshwater sites in Poland, among them *U. flexuosa* subsp. *pilifera* thalli which were observed at six sites (Tab. 2, Fig. 1).

During the taxa identification from the genera *Ulva* such features as the thallus structure, the shape and dimensions of cells, the structure of chloroplasts, number of pyrenoids and formation of grains of starch [Bliding 1968] are taken into consideration but how it turns out they are demonstrated the considerable phenotypic changeability [Hofmann *et al.* 2010]. In general, the effect of the great morphological plasticity of these macroalgae can be their incorrect determination to the species. Basing on the carried out analyses, contained only the morphological structure which were not confirmed with genetic studies, most often identified a representative of the genera *Ulva* in inland waters of Poland is *U. intestinalis* (L.) [Göppert and Cohn 1850, Preuschoff 1883, Kozłowski 1891, Nitrady 1898, 1904, Torka 1910, Raciborski (Wójcicki) 1910, Rouppert 1913,



Fig. 1. Distribution of sites of the freshwater *Ulva flexuosa* subsp. *pilifera* in Poland on the map, with usage the ATPOL grids: 1 – known in the literature as an inland localities, 2 – new locality in Poznań

Liebetanz 1925, Namysłowski 1927, Wysocka 1952, 1954, Marczek 1954, Jagielski and Zabawski 1954, Piotrowska 1961, Wilkoń-Michalska 1963, Podbielkowski 1969, Pliński 1971, 1973, Kowalski 1975, Messyasz and Rybak 2008, Endler *et al.* 2006, Messyasz 2008, Stachnowicz (unpublished), Kuczyńska-Kippen 2009]. Its presence was confirmed almost on the half from all of examined sites. Distribution of *U. intestinalis* at freshwater sites were explained by the wide tolerance range for changes of water salinity, the temperature and light conditions [Wysocka 1952, Woodheed and Mass 1975, Reed and Russel 1979, Messyasz 2009]. It is pointing that other taxa from this genera are characterized by a clearly narrower tolerance for changes of these habitat factors. Messyasz and Rybak [2011] are listed a few most important environmental factors which were determined the presence and numbers of *Ulva flexuosa* subsp. *pilifera* in the Malta Lake (Poznań, Poland). The freely occurrence of this green alga species was connected with the following environmental conditions:

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(1) atmospheric (the great solar exposure, the small amount of rainfall), (2) habitat (small waving of water and high contents of nutrients). Thus, on account of diversity of its needs taxa from this genera it is possible to found in all sorts of aqueous ecosystems, both stagnant waters: ponds [Sitkowska 1999], dam reservoirs [Gołdyn 2000], lakes [Messyasz and Rybak 2009], as well as flowing waters: streams [Messyasz and Rybak 2009], a drainage ditch [Liebetanz 1925] and rivers [Kowalski 1975].

A new locality of Ulva flexuosa subsp. pilifera in Poland

A new, unknown site of species from the genera *Ulva* was located in the Wielkopolska province, in the north part of Poznań city (N 52°28'3.58'', E 16°55'57.31'', ATPOL BD09). Thalli of *Ulva* were sampled from the nameless dam reservoir situated in the area of Adam Mickiewicz University Campus in Poznań, between Morasko and Umultowo districts (Fig. 2). The found site of *Ulva* was localised in a small water body with the anthropogenic genesis which is classified as shallow as its depth is not exceeding 1.5 m. The surface area of the pond is about 1.5 ha, at the length not exceed 200 m and average width of ± 100 m. The reservoir is supply with waters from three little streams, two from



Fig. 2. The location of the new site of freshwater *Ulva flexuosa* subsp. *pilifera* in the northern part of Poznań (Wielkopolska Region): 1 – station, 2 – retention tank, 3 – watercourses, 4 – roads, 5 – forest with pines, 6 –forest with alders, 7 – green areas, 8 – wetlands, 9 – buildings, 10 – direction of water runoff

them inflow in the north part of the pond and one in the western part. Moreover, this reservoir is connected by culvert with the another reservoir placed below the investigated one. The bottom of the pond is sludgy and with shores strongly covered with rush vegetation, dominated by *Phragmitetum communis* (W. Koch 1926) Schmale 1939 community. From the western side, the reservoir is surrounded by the alder forest which overgrows a boggy area, while from eastern side is covered with fix of pine.

Analysis of thalli and cells morphology

Ulva thalli and its cells were subjected to morphological analysis. Thalli of *Ulva flexuosa* subsp. *pilifera* from the new site, with respect to specimens observed in different places, were achieved smallish size $(2.00-8.70 \times 0.20-1.20 \text{ cm})$. Our results show that maximum value of the thalli length and width were not correspond with dimensions of thalli from sites obtained earlier. The same as well as characterised earlier by Kowalski [1975], Sitkowska [1999] and Messyasz and Rybak [2011] thalli of *Ulva* also from the new site had numerous but little branches and were strongly creased on the entire surface and moreover, were characterised by a intestinally-fold shape [Marczek 1954]. However, they differed in colouring because in contrast with most often met dark-green thalli, examined *Ulva flexuosa* subsp. *pilifera* thalli were assumed the colour's shade from yellow to brightly green. Interestingly, on that score they were similar to *Ulva* thalli gathered by Mareš [2009] from the area of the Czech Republic.

Cells of *Ulva* were arranged in distinct oblong rows and clearly assumed the rectangular shape. Dimensions of the cells size were fluctuated from 38.21 to 71.02 μ m in the length and 25.02 to 42.72 μ m in the width. Inside cells pyrenoids, usually 2–3 or rarely 1 or 4, with the size from 6.32 to 11.67 μ m in the diameter were observed. On the ground of our findings (Tab. 3, Fig. 3, 4), basing on Starmach [1972] and Pliński [1988] keys to determining filamentous green algae examined material was identified as *Ulva flexuosa* subsp. *pilifera*.

Size	Length of thalli	Width of thalli	Length of cells	Width of cells	Diameter of pyrenoids	Number of pyrenoids
	cm	cm	μm	μm	μm	
Min.	2.00	0.20	38.21	25.02	6.32	1
Max.	8.70	1.20	71.02	42.72	11.67	4
Mean	4.38	0.49	50.74	34.73	8.63	-
SD^*	1.91	0.22	7.00	4.24	1.31	-

Table. 3. Size of Ulva flexuosa subsp. pilifera thalli and cells

*standard deviation

n = 50



Fig. 3. Thalli fabric of freshwater Ulva flexuosa subsp. pilifera from the new site in Poznań



Fig. 4. The arrangement of cells in Ulva flexuosa subsp. pilifera thalli with visible pyrenoids

In general, thalli from the genera *Ulva* are characterised by a great morphological plasticity connected mainly with the rank of water salinity [Reed and Russell 1978, Young *et al.* 1987], with the seasonal changeability and the individual's ontogenesis [Blomster *et al.* 1999]. Analysed thalli were differed in

the length in comparing with individuals of the same taxon coming from other known sites [Kowalski 1975, Sitkowska 1999, Sitkowska 2008 – unpublished, Mareš 2009, Rybak and Messyasz 2011], except thalli recorded from the Žitavy river in Slovakia in May of 2008 [Mareš 2009].

Thalli of *Ulva flexuosa* subsp. *pilifera* at the examined site were strongly overgrown by filamentous algae from the genera *Oedogonium*. Generally, a phenomenon of a growth and a development of one macroalgae on the surface of the other macroalgae is observed in the aqueous environment [Rindi and Guiry 2004]. Filamentous epiphytes can reduce the access of the light, to compete with the host for nutrients and gasses dissolved in the water, what in the significant way can have an negatively influence on their growth [Buschmann and Gómez 1993]. And so it is probable, that small thalli sizes of *Ulva flexuosa* subsp. *pilifera* found in the pond in Poznań is resulted perhaps from the limiting *Ulva* development by filamentous algae.

Results of physico-chemical analysis

Thalli of macroalga were found in water in which the conductivity amounted the value of 824 μ S·cm⁻¹, the oxygenation of the 68.2% and pH 8. The content of nitrates in water collected directly from under the macroalgae mat amounted to 0.6 mg·dm⁻³ and in the case of ortophosphates 0.11 mg·dm⁻³ and chlorides 80 mg·dm⁻³. However, sample of water collected in the same time but from the place where mats of macroalgae were not noted smaller value of nitrates (0.3 mg·dm⁻³) and chlorides (55 mg·dm⁻³) were indicated while, the others parameters were not differ significantly. It was observed, that presence of strongly developed mats of *Ulva*, particularly if they occupy over the 25% of the entire surface of the reservoir, is changed physicochemical conditions prevalent in the water [Romano *et al.* 2003].

Factors Locations	рН	cond. µS·cm ⁻¹	Cl⁻ mg·dm ⁻³	N-NO ₃ mg·dm ⁻³	N-NH₄ mg∙dm ⁻³	P-PO ₄ mg·dm ⁻³	Source
Piotrowice	7.5–8.0	n.d.	20	0.04	0.5	0.23	Sitkowska
Kuciny	7.5–8.5	n.d.	20	0.08	0.5	n.d.	[1999] Sitkowska [1999]
Malta	7.98	682	107.5	0.05	0.54	0.07	Rybak and Messyasz
							[2011]
Morasko	8.0	824	80	0.60	n.d.	0.11	own study
Hradec	8.02	830	48.80	0.70	0.07	0.20	Mareš [2009]
Králové							
Hlohovec	8.75	1550	122.00	< 0.01	0.07	0.20	Mareš [2009]
Oleksovice	7.57	1005	84.00	0.05	0.16	0.03	Mareš [2009]
Nemilkov	7.24	2705	270.00	< 0.01	0.92	0.09	Mareš [2009]

Table. 4. Habitat conditions of water where freshwater Ulva flexuosa subsp. plifera were reported

n.d. – no data

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It is possible to describe habitat requirements of *Ulva flexuosa* subsp. *pilifera* on the basis of comparison the information about sites condition of studied alga from the area of Poland and the Czech Republic (Tab. 4). Comparing habitat data it was stated that water in which alga thalli appear is characterized by a lightly alkaline pH (7.2–8.0), never acid [Rybak and Messyasz 2011]. *Ulva flexuosa* subsp. *pilifera* in inland reservoirs was observed in waters in which concentrations of chlorides fluctuated from 20 to 270 mg·dm⁻³. Thus, this taxon is able to the free development in poorly salted waters. In the case of other species of *Ulva (U. intestinals)* and of its marine populations the low salinity can create the rise of thalli prolifications [Blomster *et al.* 1998]. However, in inland ecosystems it was found that level of the *Ulva* branching is also dependent on the temperature as well as different and not precisely determined environmental factors [Messyasz and Rybak 2011].

CONCLUSIONS

To sum up, the results indicate that *Ulva flexuosa* subsp. *pilifera* development in small water reservoirs may directly relate to its habitat conditions (e.g. value of nutrients, filamentous green algae density) and this in turn influences the morphometric features of individual specimens of thalli. The work is also documented the new *Ulva flexuosa* subsp. *pilifera* locality relating to the current research on this alga taxon in Poland and Europe.

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NOWE STANOWISKO SŁODKOWODNEJ POPULACJI Ulva flexuosa subsp. pilifera (Chlorophyta, Ulvophyceae) W POZNANIU (WIELKOPOLSKA)

Streszczenie. Pod koniec kwietnia 2011 r. znaleziono plechy zielenicy z rodzaju *Ulva* na nowym, dotąd nieznanym stanowisku w Wielkopolsce. Plechy błonicy oszczepowatej *Ulva* (syn. *Enteromorpha*) *flexuosa* subsp. *pilifera* (Kützing) Bliding 1963 pobrano do dalszych badań morfologicznych z niewielkiego, bezimiennego, sztucznego zbiornika retencyjnego zlokalizowanego w północnej części Poznania. Było to drugie po Zbiorniku Maltańskim stanowisko tego taksonu w Poznaniu, a szóste w Polsce. Na nowo opisanym stanowisku błonicy oszczepowatej, zlokalizowanym przy zachodnim brzegu zbiornika, wolno pływające plechy występowały w niewielkiej ilości (5–10 os./m²). Pojedyncze plechy błonicy w nowym stanowisku występowały od końca kwietnia do końca maja 2011 r.

Słowa kluczowe: Ulva flexuosa, błonica, makroglony, zielenice, warunki siedliskowe, Poznań