CHANGES IN MACROELEMENT CONTENT OF RHIZOMES OF *NUPHAR LUTEA* (L.) Sibith. & Sm. DURING THE ANNUAL CYCLE

HENRYK TOMASZEWICZ

Department of Plant Ecology and Environmental Conservation,
Warsaw University
Al. Ujazdowskie 4, 00-478 Warszawa, Poland
e-mail: g.tomaszewicz@uw.edu.pl

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ABSTRACT

This study presents the results of studies on the chemical composition of rhizomes of *Nuphar lutea* which were carried out from July 2006 to November 2007 (the samples were collected at two-week intervals from March to November 2007). The first indication of the start of the growing season was the growth of leaves in the apical part of the rhizomes. Clearly visible signs that marked the beginning of the growing season were unfolding of leaves, which became arrow-shaped. The leaves had already unfolded and were arrow-shaped (saggitate) on April 10th when the temperature of the bottom water layer was 7°C. It may be assumed, therefore, that the growing season began between the 28th March and 10th April 2007. The phosphates, nitrates, sodium, calcium, total iron and sulphates levels in the rhizomes declined just after the growing season had started. The total nitrogen content remained at a constant level whereas the amount of dissolved silica increased. Considerable changes in the macroelement contents were noted when *Nuphar lutea* was in full bloom (10th-24th June). The macroelement contents presented in the tables and diagrams were expressed on a dry matter basis. An additional table illustrates the macroelement contents expressed on a fresh matter basis. The problem of collecting rhizomes of polycormic plants is discussed.

KEY WORDS: *Nuphar lutea*, macroelements, monitoring studies.

INTRODUCTION

The results of many years of studies (from March to November) on the chemical composition of *Nuphar lutea* in the phytocoenoses of Nupharo-Nymphaeetum albae occurring in different habitats (Tomaszewicz and Ciecierska 2009) indicated that the levels of some macroelements were found to be higher at the beginning (in April) than the end of the growing season (in November). Therefore studies were conducted to determine the changes in macroelement content of rhizomes of *Nuphar lutea* over one year period. The main objectives of the present work were to:

1) determine the changes in the macroelement contents during the winter months;

2) the first visible signs marking the beginning of the growing season;

3) determine the changes in the macroelement contents during the different phases of development of *Nuphar lutea* (the beginning of the growing season, flowering and fruiting periods, autumn – the appearance of young leaves and flower stalks).

MATERIAL AND METHODS

The rhizomes of *Nuphar lutea* were collected in the phytocoenosis of Nupharo-Nymphaeetum albae (see Tomaszewicz and Ciecierska 2009) growing in Lake Łaśmiady. The samples were taken from July 2006 to November 2007. From July 2006 to February 2007 the samples were collected in regular one month long intervals, and once every two weeks from March to November 2007. In addition the bottom water temperature was recorded starting in February 2007 as well as the thickness of ice (the first ice formed on the lake on 9th February) and the maximum length of the leaves in the apical part of the rhizomes. The time at which the floating leaves appeared and later disappeared, as well as the time of flowering and fructification were determined as well (Table 1). The winter of 2006-2007 was not a typical winter for this region. The ice did not form on the lake until February. It was about 16-20 cm thick and was broken up by waves in March. Usually the lake is covered by 50 cm thick ice until the beginning of April. Probably the 2007 growing season began 2-3 weeks earlier than normal.

TABLE 1. Phenology of *Nuphar lutea*.

Date	˚C	Description
10.02.07	2	The longest leaves in apical part of the rhizomes 7 cm long, folded, ice 6 cm thick
10.03.07	2	The longest leaves in apical part of the rhizomes 10 cm long, folded, ice 14 cm thick
28.03.07	2	The longest leaves in apical part of the rhizomes 10 cm long, folded, ice broken up by waves
10.04.07	7	The longest leaves in apical part of the rhizomes 12 cm long, unfolded, saggitate
24.04.07	12	The longest leaves in apical part of the rhizomes 16 cm long,
11.05.07	13	The longest leaves in apical part of the rhizomes 54 cm long,
24.05.07	17	Single leaves floating on the water surface, the longest flower stalks 27 cm long
10.06.07	23	Full bloom
24.06.07	24	Full bloom
10.07.07	17	Over 60% of the flowers have wilted
24.07.07	21	About 5% of the flowers are blooming
10.08.07	20	Flowers absent, about 20% of the floating leaves damaged
24.08.07	22	About 85% of the leaves damaged
10.09.07	15	Single leaves within a patch
24.09.07	13	Floating leaves absent
10.10.07	11	The longest leaves in apical part of the rhizomes 7 cm long, folded
24.10.07	10	The longest leaves in apical part of the rhizomes 7 cm long, folded
10.11.07	4	The longest leaves in apical part of the rhizomes 7 cm long, folded

Rhizomes were analyzed after cutting off 10 cm long apical parts. A detailed description of the methods used to prepare the rhizome material for chemical analysis and to determine the particular macroelements is given in Tomaszewicz and Ciecierska (2009). In addition the role of the particular macroelements in the life of a plant is discussed. The paper also provides a brief review of literature related to the problems addressed in the paper.

A comparison of the macroelements occurring in the rhizomes of *Nuphar lutea* at different phenological periods was performed using the STATISTICA package (StatSoft Inc. 2003; Stanisz 1998). This package was also used for PCA analysis (Principal Components Analysis) for comparison of the average values of all the macroelements occurring in the rhizomes of *Nuphar lutea*. A classification was also performed using PCA analysis (Principal Components Analysis and classification PCCA). The U Mann test (StatSoft Inc. 2003) was applied to show statistical differences between the groups obtained by classification.

A transformation of data was necessary for the purpose of statistical analysis. Since the distribution was not normal (the Shapiro-Wilk test) the original data were substituted by other values (Jongmann and others 1987). The Blis transformation was used for PCA analysis (ln+1 – ter Braak, Šmilauer 2002).

RESULTS

Phosphates

The phosphates levels in the rhizomes of *Nuphar lutea* varied greatly during the study period (Table 2, Fig. 1).

TABLE 2. The content of macroelements in the dry matter of rhizomes (g/kg), hydration (%), dry mineral and organic matter contents (%).

Date	PO_4	NO_3	Total N	K	Na	Ca	Total Fe	SO_4	SiO_2	Hydration	Min. matter	Org. matter
10.07.06	3.35	0.137	3.85	20.17	1.64	1.57	3.63	4.1	0.68	86.59	5.8	94.2
10.08.06	4.8	0.07	2.1	21.19	1.42	1.18	1.08	2.45	0.5	85.03	6	94
10.09.06	4.6	0.042	7.42	18.64	1.35	1.31	0.45	1.74	0.51	84.73	4.8	95.2
10.10.06	6.8	0.051	8.82	22.7	1.27	1.44	0.5	2.55	0.66	86.86	6.6	93.4
10.11.06	7.35	0.049	8.12	21.69	2.24	1.71	0.73	2.62	0.61	86.59	5.8	94.2
10.12.06	5.75	0.08	5.67	23.21	1.27	1.31	0.35	2.92	0.76	86.29	6.6	93.4
10.01.07	7.15	0.086	5.25	31.84	3.14	1.84	0.53	3.29	0.99	91.79	9.6	90.4
10.02.07	7.3	0.062	10.22	22.2	1.35	1.44	0.75	2.26	0.94	85.5	6.4	93.6
10.03.07	7.1	0.055	10.22	21.69	1.12	1.44	1.83	1.52	0.54	86.74	5.2	94.8
28.03.07	5.75	0.116	8.12	23.21	1.35	1.44	2.28	1.28	0.51	86.98	5.4	94.6
10.04.07	7.85	0.101	6.65	27.78	3.06	1.84	1.75	2.37	0.74	88.39	7.6	92.4
24.04.07	6.7	0.091	6.65	26.26	1.2	1.71	0.28	1.56	0.99	87.74	6.4	93.6
11.05.07	6.75	0.078	9.52	23.21	1.05	1.44	2.6	1.42	0.69	86.46	5.4	94.6
24.05.07	7.3	0.105	14.7	27.78	1.49	1.71	2.23	2.14	1.06	89.89	7.4	92.6
10.06.07	4.75	0.122	10.85	30.32	3.58	2.24	2.9	2.81	0.98	91.86	8.2	91.8
24.06.07	5.65	0.086	4.9	29.81	3.58	2.51	2.2	2.85	0.64	91.21	8.4	91.6
10.07.07	5.85	0.102	4.9	27.78	2.24	1.57	1.9	3.02	0.89	89.65	7.2	92.8
24.07.07	4.85	0.103	4.9	25.75	2.02	1.84	2.35	1.43	0.63	89.38	6.6	93.4
10.08.07	5.85	0.042	9.1	26.26	1.49	1.57	2.23	1.58	0.74	88.51	6.6	93.4
24.08.07	7.4	0.063	7.7	28.29	3.06	1.57	2	1.66	0.83	89.72	7.6	92.4
10.09.07	8.1	0.08	10.5	31.33	2.47	1.98	2.93	1.66	0.64	91.46	9.2	90.8
24.09.07	7.1	0.053	8.75	25.75	3.06	1.57	2.48	1.63	0.55	88.19	7.2	92.8
10.10.07	8.25	0.08	9.1	30.83	4.11	1.84	1.9	1.56	0.7	90	8.2	91.8
24.10.07	8.05	0.074	9.1	25.75	2.54	1.57	2.08	1.84	0.61	88.16	6.4	93.6
10.11.07	8.6	0.094	6.3	30.83	4.78	1.98	1.8	2.09	1.06	90.55	9	91

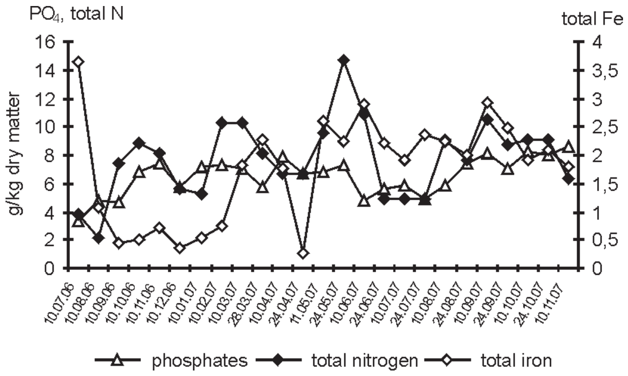
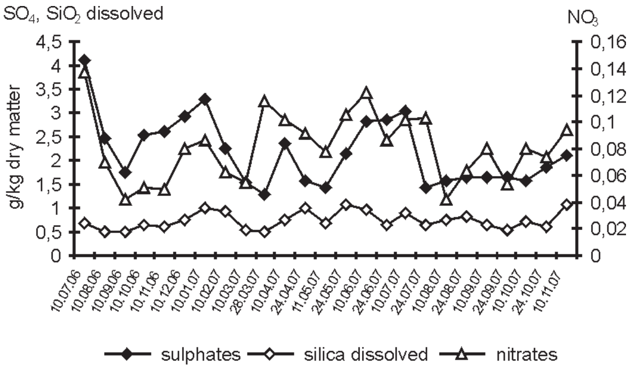


Fig. 1.



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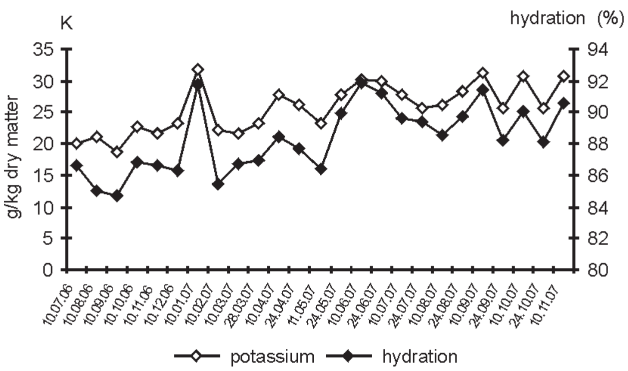

Fig. 3.

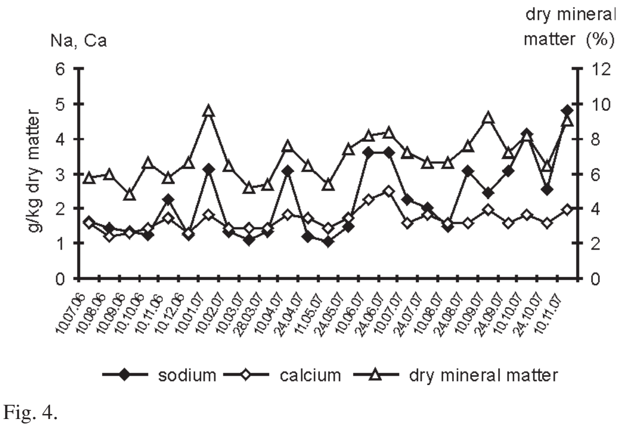

Fig. 4.

Figs 1-4. Changes in the content of macroelements (g/kg dry matter), hydration (%) and dry mineral matter (%)

There was more than a two-fold difference between the lowest and highest values of this parameter. The lowest PO_4 content of rhizomes was noted during the flowering and fruiting periods (10.06-10.08.2007). In 2006, when the growing season started more than two weeks later than in 2007, low PO_4 levels were still detected at the beginning of September. In the remaining months the PO_4 content increased considerably. The reasons for the slight variations are difficult to explain. They may be ascribed to the age of the rhizomes. Further studies are, therefore, needed to properly interpret these results. At the beginning of the growing season when the leaves in the apical part of the rhizomes unfolded and became arrow-shaped (between 28.03 and 10.04) the PO_4 levels remained fairly constant. The PO_4 content declined sharply during the period between the appearance of floating leaves and intensive growth of flower stalks (24.05-10.06). A slow increase in PO_4 was noted in the second half of August (phosphate translocation from the old leaves and uptake from the sediments). At the end of the growing season, the PO_4 levels were higher than those recorded at the beginning of the growing season.

Nitrates

Considerable variations in nitrates levels were observed during the course of the study (Table 2, Fig. 2). The sharpest decline was noted starting in August and the lowest values of this parameter occurred when *Nuphar lutea* stopped blooming and produced fruit. Low values of the above parameter were recorded in autumn and winter. The NO_3 levels increased at the beginning of the growing season (the end of March and the beginning of April). During this time the leaves expanded from the apical part of the rhizomes. At this stage the reasons for the low levels of NO_3 in some of the samples are difficult to interpret.

Total nitrogen

The highest total nitrogen contents in the rhizomes of *Nuphar lutea* were noted just before and during intensive flowering period (24.05-10.06). The lowest values of this parameter were detected after intensive flowering and during the fruiting period (at the end of June, July). The total N levels increased towards autumn (Table 2, Fig. 1). Greater amounts of total N were observed in February and at the beginning of March. These findings are difficult to interpret. During this time the lake was covered by ice. The total N levels were not related to the water content of the rhizomes. The values of this parameter increased considerably after the growing season had started.

Potassium

The potassium content did not change greatly during the growing season. Lower values of this parameter were found in autumn and winter (Table 2, Fig. 3). Greater amounts of K were detected in the case of rhizomes collected in 2007 than in those from 2006.

There was a clear relationship between the K levels and the water content of rhizomes, dry mineral and organic matter contents. Higher values of K were determined in more highly hydrated rhizomes which had higher dry mineral matter content and lower content of dry organic matter. The difference between the lowest and the highest value of K was over 40%. The content of potassium, and other cations like Na, Ca and Mg, determine the water content of the cytoplasm (Mengel and Kirkby 1983).

When *Nuphar lutea* was in full bloom the rhizomes had higher water content as well as higher content of dry mineral matter, including potassium. The K levels were lower before intensive flowering and during the fruiting period.

Sodium

The sodium levels varied during the study period. A three-fold difference was noted between the highest and lowest value of this element (Table 2, Fig. 4). The highest Na content was detected when *Nuphar lutea* was in full bloom (10-24th June). As in the case of potassium the Na levels increased considerably at the beginning of the growing season when the leaves in the apical part of the rhizome became saggitate (10th April). The Na content of rhizomes was not closely related to their water and mineral matter contents as opposed to K and Ca. The Na levels were higher at the end than the beginning of the growing season.

Calcium

The calcium levels in the rhizomes were strongly related to the water content, as well as dry mineral and organic matter contents (Table 2, Fig. 4). At least a two-fold difference was detected between the highest and lowest values of Ca. As in the case of potassium and sodium the Ca levels increased at the beginning of the growing season and the highest values of this element were noted when the plant was in full bloom. During this time the rhizomes had high water content. Higher Ca contents in some autumn months coincided with higher water and mineral matter contents. The Ca values were similar in the remaining months. Comparable Ca levels were also noted at the beginning and end of the growing season.

Total iron

The content of total iron in the rhizomes of *Nuphar lutea* varied during the study period. There was an almost thirteen-fold difference between the highest and lowest values of this parameter. The lowest amount of total Fe was noted just after the growing season had started (Table 2, Fig. 1). At the same time the levels of PO_4, NO_3, Na, SO_4, total N, K and Ca declined. The lowest total Fe content was observed in winter months, whereas the highest values of this element were noted in the summer. The total Fe levels were not related to the water content of rhizomes or dry mineral matter. Comparable levels of this parameter were found at the beginning and end of the growing season.

Sulphates

The sulphates levels changed during the course of the study. A three-fold difference was found between the highest and lowest values of this parameter. The lowest SO_4 content was noted at the beginning of the growing season (Table 2, Fig. 2). The highest levels were detected in the summer months. Similar variations occurred in the case of SO_4 and NO_3 contents. Comparable levels of SO_4 were detected at the beginning and end of the growing season. The water content of the rhizomes and dry mineral matter were related to SO_4 levels.

Silica dissolved

The dissolved silica concentrations did not change significantly during the whole study period. In extreme cases, the observed differences were no greater than two-fold (Table 2, Fig. 2). The lowest values of dissolved SiO_2 were noted in the summer during the fruiting period and in the autumn months. Similar levels were found at the beginning and end of the growing season. No close relationship was found between the water content of rhizomes, mineral matter content and dissolved SO_2 concentrations.

DISCUSSION

A detailed analysis of the results of 17 months of monitoring studies concerning changes in the macroelement content of rhizomes of *Nuphar lutea* enabled us to answer the objectives set out earlier in this paper. Some problems related to the methods of collecting rhizomes of polycormic plants for chemical analysis were resolved.

In the present study as well as in other works dealing with similar issues the contents of macrolements and compounds were given per gram or kilogram of dry plant matter. The analysis of the results and diagrams from the whole study period indicated that in many cases there was a sudden increase or decline in the amount of a given parameter in the rhizomes of *Nuphar lutea*. After two weeks, when the next samples were collected, the levels of this element decreased or increased suddenly. These results were quite difficult to interpret, especially that the data were obtained in autumn and winter months, when the growing season had practically ended.

A slight decrease in the levels of some macroelements in rhizomes of *Nuphar lutea* may be attributed to the fact that the above nutrients were used by plants to stimulate the development of young leaves and flower stalks, which would start to grow in spring when the bottom water temperature reached ≈ 5°C.

It was found that sudden changes in the macroelement contents during winter were closely related to the water content of rhizomes as well as dry organic and mineral matter contents. It is evident that the water content of rhizomes cannot decrease or increase by several or even by a dozen or so percent within a two week period, particularly during the autumn and winter months. *Nuphar lutea* is a perennial polycormic plant. Usually one individual plant in the phytocoenosis occupies an area of dozens of square meters. Therefore the particular parts of the rhizomes are different ages. In the present study samples were taken randomly at several locations. Thus older perennial rhizome parts which had lower water content as well as the younger parts of the rhizomes which had higher water content could

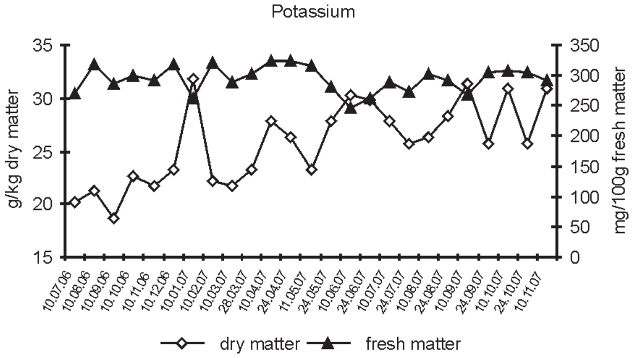

Fig. 5. Changes in the K content in terms of dry matter content (g/kg), and fresh matter content (mg/100 g).

TABLE 3. The content of macroelements in the fresh matter of rhizomes (mg/100 g).

Date	PO_4	NO_3	Total N	K	Na	Ca	Total Fe	SO_4	SiO_2
10.07.06	44.92	1.837	51.63	270.48	21.99	21.05	48.68	54.98	9.11
10.08.06	71.86	1.05	31.44	317.21	21.26	17.66	16.17	36.68	7.49
10.09.06	70.24	0.641	113.3	284.63	20.61	20.01	6.87	26.57	7.79
10.10.06	89.35	0.67	115.89	298.28	16.69	18.92	6.57	33.51	8.67
10.11.06	98.56	0.657	108.89	290.86	30.04	22.93	9.79	35.13	8.18
10.12.06	78.83	1.097	77.74	318.21	17.41	17.96	4.8	40.03	10.42
10.01.07	58.7	0.706	43.1	261.41	25.78	15.11	4.35	27.01	8.13
10.02.07	105.85	0.899	148.19	321.9	19.58	20.88	10.88	32.77	13.63
10.03.07	94.15	0.729	135.52	287.61	14.85	19.09	24.27	20.16	7.16
28.03.07	74.87	1.51	105.72	302.19	17.58	18.75	29.69	16.67	6.64
10.04.07	91.14	1.173	77.47	322.53	35.53	21.36	20.32	27.52	8.59
24.04.07	82.14	1.116	81.53	321.95	14.71	20.96	3.43	19.13	12.14
11.05.07	91.4	1.056	128.9	314.26	14.22	19.5	35.2	19.23	9.34
24.05.07	73.8	1.062	148.62	280.86	15.06	17.29	22.55	21.64	10.72
10.06.07	38.67	0.993	88.32	246.8	29.14	18.23	23.61	22.87	7.98
24.06.07	49.66	0.756	43.07	262.03	31.47	22.06	19.34	20.05	5.63
10.07.07	60.55	1.056	50.72	287.52	23.18	16.25	19.67	31.26	9.21
24.07.07	51.51	1.094	52.04	273.47	21.45	19.54	24.96	15.19	6.96
10.08.07	67.22	0.485	104.56	301.73	17.12	18.04	25.62	18.15	8.5
24.08.07	76.07	0.648	79.16	290.82	31.46	16.14	20.56	17.06	8.53
10.09.07	69.17	0.683	89.67	267.56	21.09	16.91	25.02	14.18	5.47
24.09.07	83.85	0.626	103.34	304.11	36.14	18.54	29.29	19.25	6.5
10.10.07	82.5	0.8	91	308.3	41.1	18.4	19	15.6	7
24.10.07	95.31	0.876	107.74	304.88	30.07	18.59	24.63	21.79	7.22
10.11.07	81.27	0.888	59.54	291.34	45.17	18.71	17.01	19.75	10.02

have been collected for chemical analysis. Dykyjova (1979) indicated that various authors determined different levels of certain elements for the same water species. Slavíková (1973), Dykyjova (1979), Mengel and Kirkby (1983) also pointed out that the age of the plants may play an important role here.

In the present study the results were given in grams per kilogram of dry mass of rhizomes in *Nuphar lutea* (g/kg). For comparison, some results of temporal changes in the contents of the particular macroelements were given in terms of fresh matter (Table 3). In addition changes in K levels expressed in terms of dry matter content and fresh matter were presented in Figure 5. It was demonstrated that the observed temporal changes in the macroelement contents in the rhizomes expressed in terms of fresh matter were less considerable in comparison with that expressed in terms of dry matter.

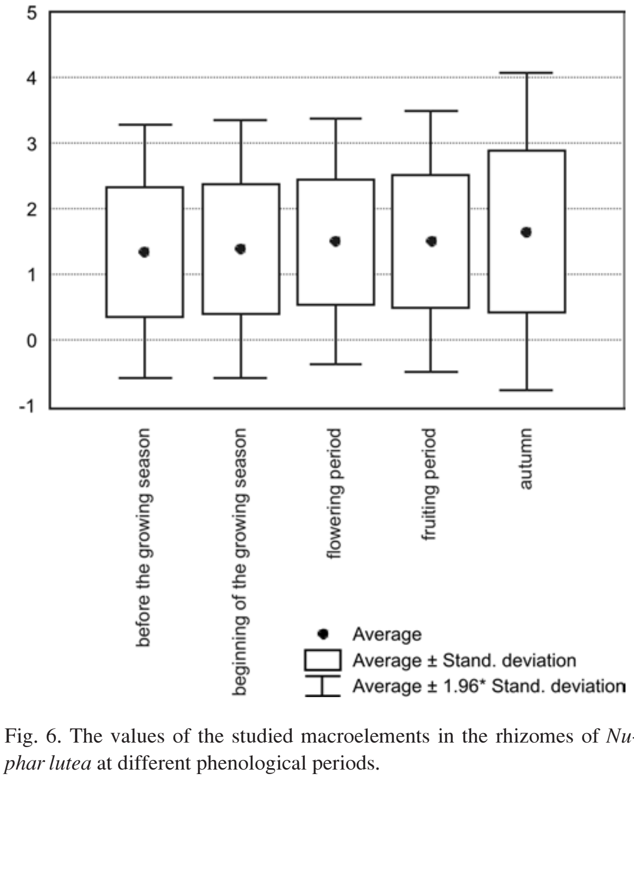

Fig. 6. The values of the studied macroelements in the rhizomes of *Nuphar lutea* at different phenological periods.

The analysis of the tables and diagrams showed variations in the content of the particular macroelements, especially at the beginning of the growing season (the beginning of April), during intensive flowering (June) and fruiting period (July). However these differences were not significant (Fig. 6). The separateness of phenological periods was obtained. Flowering period, fruiting period, autumn, the period before the growing season and the beginning of the growing season were separated (Fig. 7) but their differences were not confirmed statistically (U Mann test). This may be attributed to the fact that in some polycormic plants (e.g. *Nuphar lutea*) with strongly developed rhizome systems the flower stalks and leaf mass are very small compared to the rhizome mass. The great amounts of macroelements which have been accumulated in rhizomes are taken up intensively during the growing season. However the loss of elements is not considerable when one considers the total amount of compounds accumulated in rhizomes. The rhizomes of *Nuphar lutea* grow on the bottom of the lake and are green in the upper part (about 40%). After the growing season has begun (when small leaves which emerged in autumn and flower stalks start to grow and develop) the normal assimilation and production of various compounds takes place, which are later translocated to young leaves and flower stalks. In early spring the water is clear and no algal blooms are observed, so the assimilation process should be quite intensive. Therefore the loss of elements in rhizomes is reduced during the growth of young leaves and flower stalks. Later, *Nuphar lutea* develops large submer-

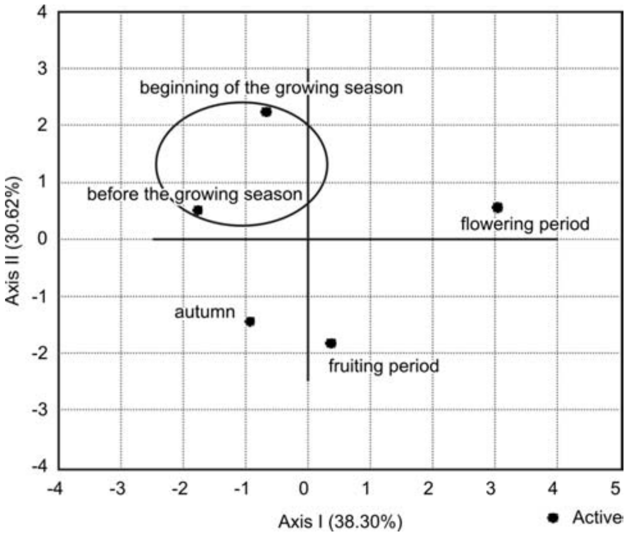

Fig. 7. Principal Components Analysis. Classification of the average values of all the studied macroelements in the rhizomes of *Nuphar lutea* at different phenological periods: before the growing season, beginning of the growing season, flowering, fruiting and autumn. Two main components contain 68.92 % of the range of studied parameters.

ged leaves which also provide many assimilation products, and their intensive development is observed during the flowering and fruiting periods. Therefore *Nuphar lutea* does not accumulate excessive amounts of various compounds in rhizomes during the summer. Thus the levels of some elements at the beginning of the growing season are comparable to those detected at the end of the growing season. In the case of samples taken after the growing season had started the rhizomes could have higher amounts of elements in early spring than the rhizomes collected at the end of the growing season. Similar observations regarding the development of leaves and flowers of *Nuphar lutea* were made by Šuchová (1976).

It is often difficult to establish the onset of the growing season as the ice covering the lake melts at different times. In the case of north-eastern Poland the beginning of the growing season usually starts at the end of March as well as in the middle of April. Only regular studies conducted over several years can illustrate changes in the content of the particular macroelements in plants. The most reliable results are obtained when monitoring studies are carried out during an annual cycle. Sample collection, especially during the growing season, should be done at short intervals (e.g. every two weeks).

To sum up, the content of macroelements does not change considerably in polycormic plants with strongly developed rhizome-root systems. The most noticeable variations occur at the beginning of the growing season as well as during flowering and fruiting periods. Less profound changes are witnessed in autumn during leaf and flower stalk formation (which start to grow in early spring). In the case of *Nuphar lutea*, the rhizomes which are green in the upper part start photosynthesis when the bottom water temperature reaches ≈ 5°C. Therefore the loss of macroelements is reduced during leaf and flower development.

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