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EFFECT OF GROWTH RETARDANT ON SOME MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS OF CHRYSANTHEMUM

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K e y w o r d s: pot chrysanthemum, flurprimidol, growth retardation, transpiration, stomatal conductance.

A b s t r a c t

The aim of this work was to evaluate the effectiveness of a single flurprimidol foliar spray on growth, flowering, transpiration rate and stomatal conductance of medium height chrysanthemums "Kodiak" and "Jewel Time", grown in pots. Flurprimidol applied once, as a foliar spray, at concentrations of 7.5, 15 or 22.5 mg dm^{-3} was effective in reducing stem extension without adverse side-effects. The degree of growth inhibition varied by cultivar and flurprimidol concentration. The desirable heights of "Jewel Time" and "Kodiak" chrysanthemums were obtained with flurprimidol at 7.5 or 22.5 mg dm^{-3}, respectively. Growth retardant slightly delayed anthesis, reduced the canopy width, the leaf area, plant dry weight and the number of inflorescences but had no effect on inflorescence diameter. There was no significant effect of flurprimidol on leaf stomatal conductance to water vapor and transpiration rate per unit leaf area of both chrysanthemum cultivars. Chemical name used: α-(1-methylethyl)-α-[4-(trifluromethoxy)phenyl]-5-pyrimidinemethanol (flurprimidol).

WPŁYW FLUROPRIMIDOLU NA NIEKTÓRE MORFOLOGICZNE I FIZJOLOGICZNE CECHY CHRYZANTEM

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S ł o w a k l u c z o w e: chryzantema doniczkowa, fluroprimidol, retardant wzrostu, transpiracja, przewodność szparkowa.

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A b s t r a k t

Celem pracy była ocena skuteczności fluroprimidolu, stosowanego dolistnie, jednokrotnie, na wzrost, kwitnienie, transpirację i przewodność szparkową średnio silnie rosnących chryzantem "Kodiak" i "Jewel Time;, uprawianych w doniczkach. Fluroprimidol stosowany jednokrotnie, dolistnie, w stężeniach 7.5, 15 lub 22.5 mg dm^{-3} skutecznie hamował wydłużanie się pędów, nie powodując niepożądanych skutków ubocznych. Stopień zahamowania wzrostu zależał od odmiany i stężenia fluroprimidolu. Pożądane wysokości chryzantem "Jewel Time" i "Kodiak" uzyskano stosując fluroprimidol, odpowiednio w stężeniach 7.5 lub 22.5 mg dm^{-3}. Retardant wzrostu nieznacznie opóźnił kwitnienie, zmniejszył średnicę roślin, powierzchnię liścia, suchą masę roślin i liczbę kwiatostanów ale nie miał wpływu na średnicę kwiatostanu. Nie stwierdzono istotnego wpływu fluroprimidolu na przewodność szparkową liści i transpirację na jednostkę powierzchni liścia obu odmian chryzantem. Nazwa chemiczna: α-(1-metyloetylo)-α-[4-(trifluorometoksyfenylo]-5-pirymidynometanol (fluroprimidol).

Introduction

Flurprimidol (a pyrimidine) belongs to a group of growth retardants which inhibit the *ent*-kaurene oxidase in the biosynthetic pathway of gibberellins. The elongation of cells mainly depends on the level of gibberellins in the plant tissues (GRAEBE 1987). The *ent*-kaurene oxidase inhibitors cause inhibition of gibberellin biosynthesis (REED et al. 1989), thereby inhibiting the elongation of plant cells. Thanks to shorter cells, growth retardant treated plants also have shorter internodes, but the number of internodes and leaves on the plant remains unchanged. These compounds can also influence leaf size, flower size, pedicel length, number of flowers, flower longevity, number of days to anthesis and the aging process of plants. Flurprimidol has a long duration of effectiveness in the plant tissues and can be used once, at very low doses, in order to receive short and well compact plants. Flurprimidol applied as a single application, at very low doses, has proved very effective in restricting extension growth of *Globba winitii* Siam (POBUDKIEWICZ and PODWYSZYŃSKA 1999), *Pelargonium* x *hortorum* L.H. Bailey (POBUDKIEWICZ 2000a), *Cuphea ignea* (POBUDKIEWICZ 2000b), *Streptocarpus hybridus* (POBUDKIEWICZ 2000c), dwarf alstroemeria (POBUDKIEWICZ et al. 2000), *Helianthus annuus* (WHIPKER et al. 2004), oriental hybrid lily (POBUDKIEWICZ and TREDER 2006), *Euphorbia pulcherrima* (CURREY and LOPEZ 2011) and many other flowering plants cultivated in pots.

Chrysanthemum, one of the most important ornamental crops worldwide can be produced both as pot plant or cut flower. A major problem with chrysanthemums grown as pot plants is plant height greater than desired and an irregular plant habit. The fastest and the cheapest way to improve compactness and to reduce the height of chrysanthemums is application of growth retardants. Many researchers have shown that there is a great variation in sensitivity of chrysanthemum cultivars to application of growth

retardants. For example, TAYAMA and CARVER (1992) have demonstrated that uniconazole at 20 mg dm^{-3} applied as a foliar spray to *Dendranthema grandiflora* Tzvelev reduced the height of "Bright Golden Anne" plants by 27% as compared to the control plants. On the other hand, STARMAN (1990) applying uniconazole at this same concentration (20 mg dm^{-3}) to *Dendranthema grandiflora* Tzvelev, "Puritan" and "Favor" obtained very little growth inhibition (8%). Due to different responses of chrysanthemums to growth retardant treatments, doses of these compounds, required to inhibit stem elongation, should be determined individually for each variety or a group of varieties. For retardation of chrysanthemum growth, have been used various growth retardants, including daminozide (PAPAFOTIOU and VAGENA 2012), chlormequat chloride (HAGUE et al. 2007), paclobutrazol (PASIAN 1999) but there is very little information on the influence of flurprimidol on height suppression and flowering of chrysanthemums. Flurprimidol, applied as a foliar spray, has been reported to suppress stem elongation of tall-growing *Dendranthema grandiflora* Tzvelev "Altis" and "Surf" POBUDKIEWICZ and NOWAK 1997) but it has not been used to control the plant growth of medium height chrysanthemums "Kodiak" and "Jewel Time".

Chrysanthemums have very high demand for water and they transpire a lot of water. A quantity of water transpired by plants might depend on the number and size of their leaves. Chrysanthemums with small leaves require smaller quantities of water and they transpire less intensively compared to cultivars with large, densely spaced leaves. There are reports which indicate that growth retardants might also reduce transpiration rate in some plants (JALEEL et al. 2007) but there is no information concerning the influence of flurprimidol on transpiration not only in chrysanthemums but also in other flowering pot plants. Due to the lack of such information an attempt was made to examine the effect of flurprimidol on chrysanthemum transpiration. This study was undertaken to evaluate the effectiveness of a single flurprimidol application on growth, flowering and transpiration of medium height chrysanthemums (*Dendranthema grandiflora* Tzvelev) "Kodiak" and "Jewel Time;, grown in pots.

Materials and Methods

The experiment was repeated twice, in growing seasons 2010 and 2011, from August till November. Medium height "Kodiak" and "Jewel Time" chrysanthemums (both cultivars: 7-week photoperiodic response) were used in this study. The chrysanthemums were obtained as rooted cuttings from a commercial source and planted into 12 cm pots (3 cuttings per pot). The pots

were filled with commercial substrate TS2 (Klasmann Deilmann GmbH, Geeste, Germany) based on blend white sphagnum peat. Plants were grown in a greenhouse with temperature ranging from 17°C to 25°C during the day and 14°C to 18°C during the night. After the cuttings were established, the upper nodes were removed leaving 4 leaves on the remaining plant. The plants were grown in the greenhouse, in conditions of controlled short photoperiod (black-out from 5 p.m. until 7 a.m.) since the day of potting. Composition of nutrient for chrysanthemum fertilisation was different for vegetative phase: (mg dm^{-3}) N-217, P-62, K-285, Ca-160, Mg-24, S-SO_4 <50, Fe-0.84, Mn-0.27, Zn-0.20, B-0.11, Cu-0.032 and Mo-0.048 and generative phase: (mg dm^{-3}) N-140, P-46, K-254, Ca-100, Mg-18, S-SO4 <50, Fe-0.84, Mn-0.27, Zn-0.20, B-0.11, Cu-0.032 and Mo-0.048. Electrical conductivities (EC) for vegetative phase and generative one were 2.2 and 1.6 mS cm^{-1}, respectively.

The plants were selected for uniformity prior to growth retardant application. Chrysanthemums were treated with flurprimidol (Topflor 015 SL) following pinching, when lateral shots were 3–5 cm in length. Flurprimidol was applied as a single foliar spray, at concentrations of 7.5, 15 and 22.5 mg dm^{-3}. The control plants were treated with a tap water at the same time. Chrysanthemums were sprayed with a hand sprayer (1 liter) until whole plants were thoroughly covered with spray solution but the solution was not allowed to drop off. No surfactants were added to the flurprimidol spray solutions. Environmental conditions on the day of growth retardant application were as follows: air temperature – 18°C, relative humidity – 75%, sky cloudy, time of application – early in the morning.

Data recorded at the beginning of flowering included the following measurements: shoot length (was the distance from substrate surface to the top of the inflorescence), canopy width (width was determined from the average of two canopy diameter measurements), number of inflorescences (including all visible buds) and leaf area (determined for fully expanded leaves, developed after flurprimidol application). Leaf area was measured using stationary planimeter (Delta-T Devices, LTD., Cambridge, UK). When plants were fully flowering, the inflorescence diameter was measured. Time to anthesis was evaluated on the day when the ray florets of the first inflorescence per plant were completely unfolded. Fully expanded leaves that developed after flurprimidol application were used for measurements of stomatal conductance to water vapor and transpiration rate (per unit leaf area). The measurements of transpiration rate and stomatal conductance were performed at midday on attached leaves using the LI-1600 Steady Porometer from Li-COR. At the end of the experimental period, the whole plants were cut off at the base (just above the soil surface). Then, the fresh weight of each plant was recorded. After that the plants were dried at 70°C until they reached a constant mass to determine dry weights.

The experiment was arranged as a randomized complete block design with three replications (each one included 5 plants per treatment). The data were averaged over two growing seasons. The experimental data were subjected to an analysis of variance. The Duncan's multiple range test at 5% was used for mean separation. Values of $p = 0.05$ were considered to be statistically significant. All statistical analyses were performed with Statistica package, version 10 (2011).

Results

The influence of growth retardant concentration on "Kodiak" and "Jewel Time" chrysanthemums was similar in two growing seasons. Flurprimidol appeared to be very effective for controlling plant height of tested cultivars and a single application was sufficient to achieve short and good quality plants (Figure 1a). The degree of growth inhibition varied by cultivar and retardant concentration. In both cultivars, shoots were shorter when plants received higher flurprimidol applications. Compared to the control, retardant at concentration of 7.5 mg dm^{-3}, applied to "Jewel Time" and "Kodiak" resulted in 41% and 25% shorter shoots, respectively. Higher flurprimidol doses resulted in further inhibition of stem elongation of both cultivars. The shoots of "Jewel Time" and "Kodiak" plants sprayed with the highest retardant concentration were 54% and 42% shorter, respectively relative to the untreated plants. The desirable plant height (16–18 cm) for "Jewel Time" was obtained with the lowest flurprimidol concentration (7.5 mg dm^{-3}) but for "Kodiak" the highest growth retardant concentration (22.5 mg dm^{-3}) was needed in order to achieve the proper height. There were also differences in shoot length between tested cultivars. Shoots of flurprimidol treated "Jewel Time" were about 25% shorter than shoots of similar plants of "Kodiak".

Flurprimidol also had an apparent effect on canopy width of both cultivars at both growing seasons. At the time of flowering, the canopies of "Jewel Time" and "Kodiak" plants treated with flurprimidol at all tested doses were much narrower compared to the control and those differences were statistically significant (Figure 1b). Relative to the untreated plants, flurprimidol at 7.5 mg dm^{-3} reduced canopy widths of "Jewel Time" and "Kodiak" by 20% and 12%, respectively. Higher growth retardant doses resulted in further reduction of canopy widths of both cultivars. Compared to the control the canopies of "Jewel Time" and "Kodiak" plants treated with the highest flurprimidol concentration were 26% and 19% narrower, respectively. Growth retardant treated chrysanthemums were smaller in size and more compact. Shoots of flurprimidol treated chrysanthemums were not only shorter, more rigid but

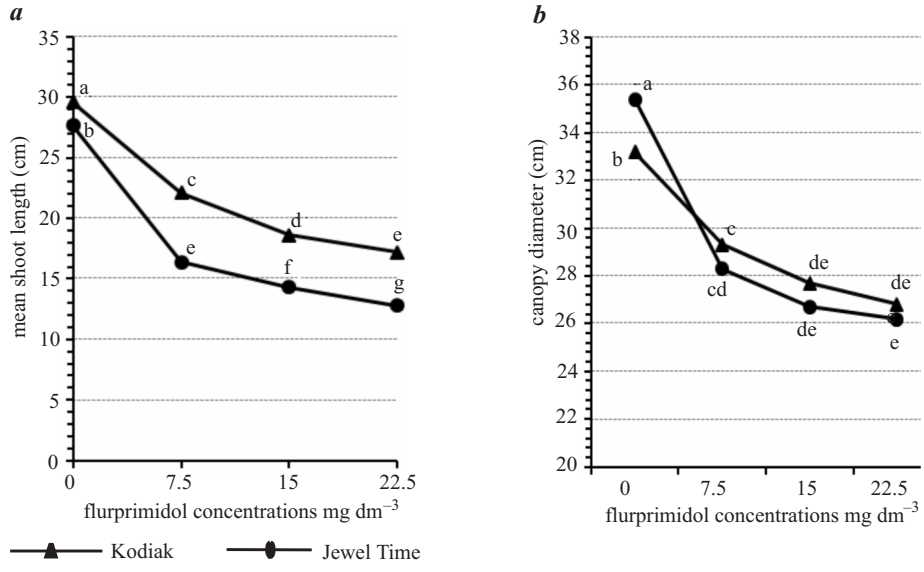

Fig. 1. Mean shoot length (*a*) and canopy diameter (*b*) of "Kodiak" and "Jewel Time" chrysanthemums as affected by flurprimidol applied as single, foliar spray. Data averaged over two growing seasons. The means indicated by the same letter do not differ significantly at $p = 0.05$, according to Duncan's multiple range test

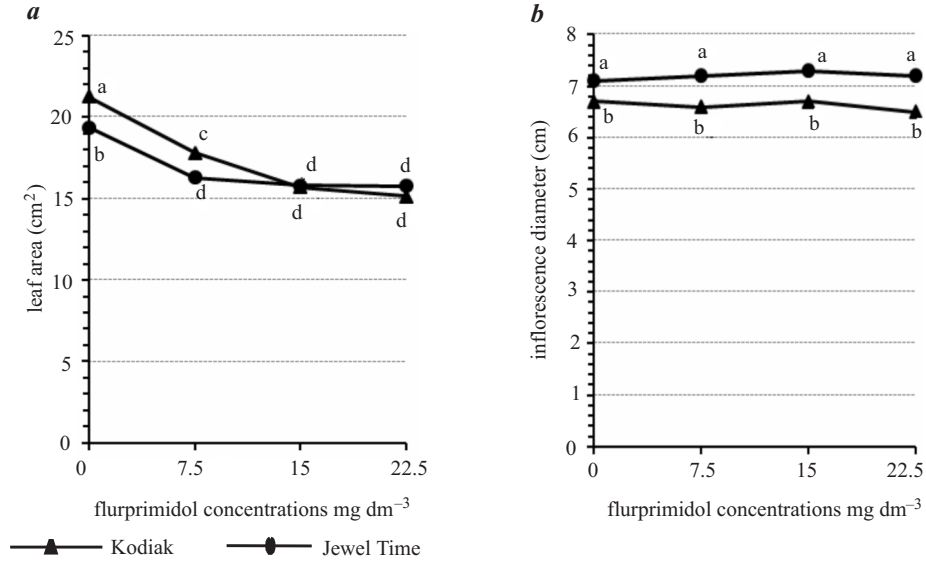

Fig. 2. Effect of flurprimidol applied as single, foliar spray on leaf area (*a*) and inflorescence diameter (*b*) of "Kodiak" and "Jewel Time" chrysanthemums. Data averaged over two growing seasons. The means indicated by the same letter do not differ significantly at $p = 0.05$, according to Duncan's multiple range test

also aligned relative to each other thus the inflorescences on the plant were placed on the same level, which greatly increased the plant quality. Plants exposed to flurprimidol also had intensified green leaf pigmentation. There was almost no abscission of the oldest leaves in low portions of growth retardant treated plants, relative to the control. No phytotoxicity was observed on flurprimidol treated chrysanthemums.

Growth parameters other than shoot length and canopy width were also affected by flurprimidol. The reactions of tested cultivars to increasing retardant concentrations were similar at both growing seasons. There were significant differences in leaf area between control plants and chrysanthemums treated with flurprimidol at 7.5–22.5 mg dm^{-3} (Figure 2a). The leaves of "Kodiak" and "Jewel Time" chrysanthemums treated with the lowest flurprimidol concentration (7.5 mg dm^{-3}) were 16% smaller as compared to the control. In "Jewel Time", higher retardant doses did not result in further leaf area reduction. In "Kodiak" leaf area was retarded with increasing flurprimidol concentrations up to 15 dm^{-3} but the higher concentration (22.5 mg dm^{-3}) caused no additional, statistically significant retarding effect, compared to flurprimidol at 15 mg dm^{-3}.

There was no effect of flurprimidol on inflorescence diameter of both cultivars but inflorescences of "Kodiak" were slightly smaller compared to those of "Jewel Time" (Figure 2b). Inflorescences of "Kodiak" plants treated with the highest retardant concentration (22.5 mg dm^{-3}) were 10% smaller than those of similar plants of the "Jewel Time".

Each chrysanthemum cultivar responded differently to growth retardant application, but the trend of reaction was the same at two growing seasons. On the day of taking data, there were significant differences between the number of inflorescences on the control plants and those of plants treated with flurprimidol at all tested doses (Figure 3a). Compared to the control, flurprimidol at 7.5 mg dm^{-3} reduced the number of inflorescences of "Kodiak" by 13% but the higher concentrations (15–22.5 mg dm^{-3}) caused no additional reduction. Relative to the control, "Jewel Time" plants sprayed with flurprimidol at 7.5 mg dm^{-3} and 15 mg dm^{-3} had 18% and 29% less inflorescences, respectively, but the higher concentration (22.5 mg dm^{-3}) resulted in plants of approximately the same number of inflorescences as those treated with retardant at 15 mg dm^{-3}.

Compared to the control flurprimidol slightly delayed anthesis of both tested cultivars when applied at concentration of 7.5 mg dm^{-3} and above (Figure 3b). Anthesis occurred within a few days in all growth retardant treated "Jewel Time" and "Kodiak" plants. There were differences in the number of days to anthesis between cultivars. "Kodiak" reached anthesis four days earlier than "Jewel Time".

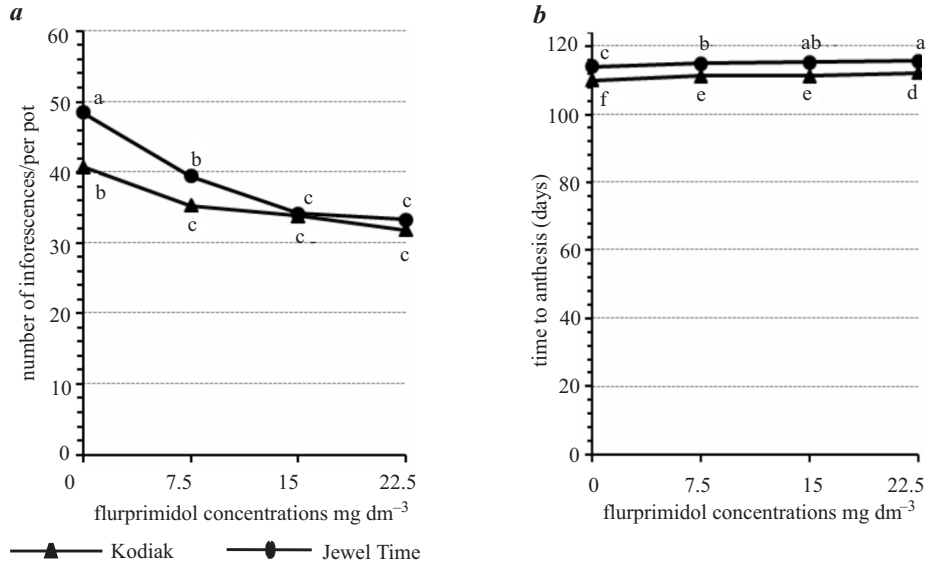

Fig. 3. Number of inflorescences per pot (*a*) and number of days to anthesis (*b*) of "Kodiak" and "Jewel Time" chrysanthemums as affected by flurprimidol applied as single, foliar spray. Data averaged over two growing seasons. The means indicated by the same letter do not differ significantly at $p = 0.05$, according to Duncan's multiple range test

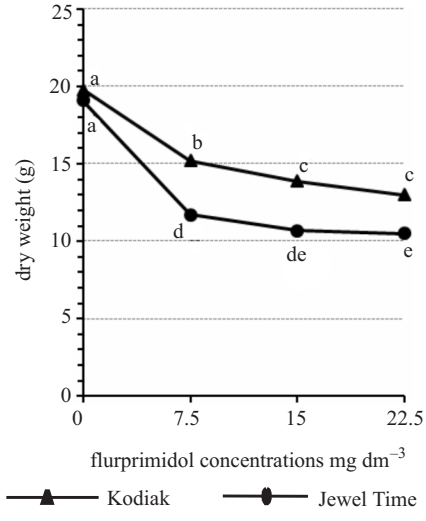

Fig. 4. Dry weights of "Kodiak" and "Jewel Time" chrysanthemums as affected by flurprimidol applied as single, foliar spray. Data averaged over two growing seasons. The means indicated by the same letter do not differ significantly at $p = 0.05$, according to Duncan's multiple range test

Growth retardant influenced the dry and fresh weights of both cultivars similarly at two growing seasons. The dry weight (Figure 4) and fresh weight (data not shown) of "Kodiak" and "Jewel Time" treated with flurprimidol at 7.5–22.5 mg dm^{-3} were significantly reduced compared to the control. The dry weights were smaller with higher flurprimidol doses. Relative to the untreated plants, retardant at the highest concentration reduced dry weights of "Kodiak" and "Jewel Time" up to 34% and 47%, respectively. There were also differences in dry matter between tested cultivars. The dry weights of "Jewel Time" treated with flurprimidol at 7.5, 15 and 22.5 mg dm^{-3} were 23%, 23.5% and 20% smaller, respectively than those of "Kodiak".

Compared to the control, flurprimidol at 7.5, 15 and 22.5 mg dm^{-3} did not influence leaf stomatal conductance to water vapor of "Kodiak" and "Jewel Time" chrysanthemums (Figure 5a). Single growth retardant applications at all concentrations also had no effect on transpiration rate per unit leaf area of both cultivars although there was a trend of increasing transpiration rate for plants treated with increasing concentrations of flurprimidol (Figure 5b). "Jewel Time" chrysanthemums carried out the transpiration per unit leaf area at a similar level compared to "Kodiak".

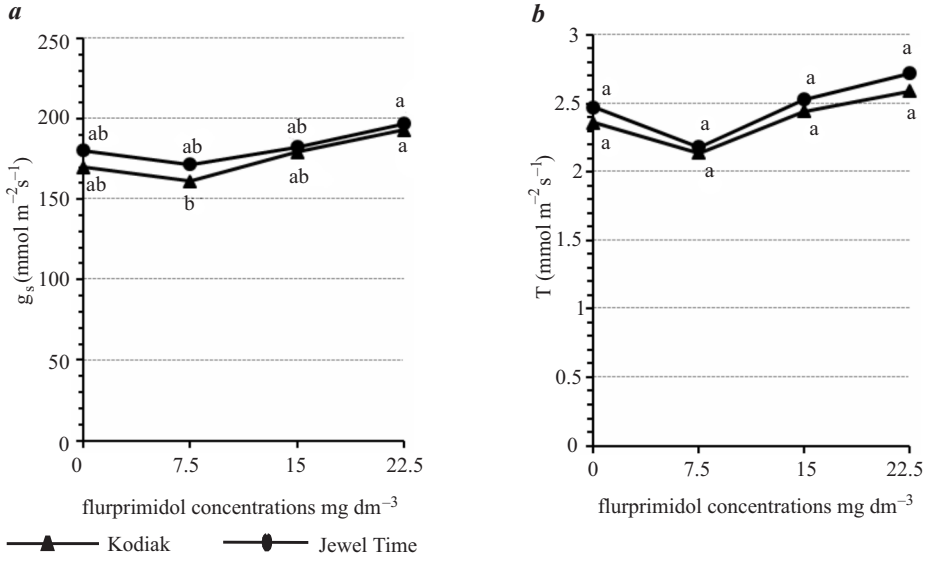

Fig. 5. Effect of flurprimidol applied as single, foliar spray on stomatal conductance to water vapor (g_s) (*a*) and transpiration rate (T) per unit leaf area (*b*) of "Kodiak" and "Jewel Time" chrysanthemums. Data averaged over two growing seasons. The means indicated by the same letter do not differ significantly at $p = 0.05$, according to Duncan's multiple range test

Discussion

In the present experiment a single flurprimidol treatment was sufficient to inhibit stem elongation of medium height "Kodiak" and "Jewel Time" chrysanthemums. This study shows that flurprimidol doses should be differentiated depending on chrysanthemum variety. "Jewel Time" plants were very sensitive to applied chemical and required the lowest flurprimidol concentration (7.5 mg dm^{-3}) in contrast to "Kodiak" which needed the highest growth retardant concentration (22.5 mg dm^{-3}) in order to achieve the desirable shoot length (16–18 cm). This concurs with our previous results, which showed that tall-growing chrysanthemums "Altis" required higher doses of flurprimidol for height suppression compared to "Surf" (POBUDKIEWICZ and NOWAK 1997). Much higher flurprimidol concentration (75 mg dm^{-3}) was used to suppress stem elongation of *Dendranthema grandiflora* Tzvelev "Nob Hill" grown in different climate conditions (BARRETT et al. 1987). HICKLENTON (1990), using another growth retardant,uniconazole, reported that the magnitude of *Dendranthema grandiflora* Tzvelev response to that growth retardant treatment was cultivar dependent. Uniconazole applied as a post-plant spray, at 10 mg dm^{-3} reduced plant heights of "Deep Luv", "Tip" and "Tara" by 19%, 39% and 35%, respectively relative to the control. Other authors also reported that chemical doses should be varied according to chrysanthemum cultivars (GARDNER and METZGER 2005, PASIAN 1999). The data obtained in this experiment demonstrate, that the efficacy of flurprimidol is superior to daminozide which is commonly used in commercial chrysanthemum production. Flurprimidol applied just once, at very low concentrations (7.5–22.5 mg dm^{-3}) was sufficient for proper height suppression of "Jewel Time" and "Kodiak" while daminozide had to be applied several times at very high concentrations (2000–3500 mg dm^{-3}) in order to achieve short chrysanthemums (PAPAFOTIOU and VAGENA 2012). Single flurprimidol application (compared to multiple daminozide treatment) in chrysanthemum cultivation has the economic advantage to producers due to reduced labor costs. The use of flurprimidol at very low concentrations (compared to very high daminozide doses) might also have environmental benefits. We have also observed good regulating activity of flurprimidol, applied once, at very small doses, in a variety of other plant species, including dwarf carnation (POBUDKIEWICZ and NOWAK 1994), seed propagated geranium (POBUDKIEWICZ 2000a), dwarf alstroemeria (POBUDKIEWICZ et al. 2000), oriental hybrid lily (POBUDKIEWICZ and TREDER 2006) and a variety of other plant species cultivated in pots.

In the study reported here chrysanthemums were sprayed with flurprimidol when lateral shoots were very short, so from the very beginning those shoots were developing under the influence of retardant. Thanks to it the first

internodes on such plants were kept very short. In pot chrysanthemums the length of the first internode plays very important role. Plant having short first internodes, develops leaves very low and this is why there is no empty space between the pot rim and the first leaves on the growth retardant treated plant. These leaves, in the low part of plants, also do not fall off until the end of the production cycle. In contrast, the oldest leaves of the untreated plants often turn yellow and fall off in the low parts of chrysanthemums. This results in an empty space between the pot rim and the first leaves on the plant which greatly diminish the plant quality. In the present study, abscission of the oldest leaves was only observed at the low parts of the control plants but it was not noted in flurprimidol treated ones. No leaf abscission in growth retardant treated plants might be associated with higher cytokinins (GROSSMANN 1990) and polyamines (GROSSMANN et al. 1987) contents. Cytokinins and polyamines such as spermine and spermidine delay aging of plants and this might be the reason why leaves in growth retardant treated plants do not fall off and remain green for a longer time compared to the untreated plants.

In the present study, due to reduced lengths of all internodes, the shoots were not only shorter but also aligned relative to each other. As a result all the inflorescences on the plant were placed on this same level, which greatly increased the plant quality. In the study reported here, flurprimidol sprayed "Jewel Time" and "Kodiak" plants, were more densely foliated and more compact resulting in higher quality appearance. These chrysanthemums narrower in width might also be an economic advantage to commercial growers due to increased plant density on greenhouse benches. Improved shape of flurprimidol treated pot plants was observed in our previous studies with tall-growing "Altis" and "Surf" chrysanthemums (NOWAK 1997) and other plant species, including *Pelargonium x hortorum* L.H. Bailey (POBUDKIEWICZ 2000a), *Cuphea ignea* (POBUDKIEWICZ 2000b) and *Streptocarpus hybridus* (POBUDKIEWICZ 2000c).

In this research project the leaf area was diminished in flurprimidol treated "Kodiak" and "Jewel Time" but due to reduced size of the whole plant, the leaves were proportionate to the entire smaller chrysanthemum. The reduced leaf area of flurprimidol sprayed plants was also observed in other plants species, including *Globba vinniti* (Siam) (POBUDKIEWICZ and PODWYSZYŃSKA 1999), *Cuphea ignea* (POBUDKIEWICZ 2000b) and oriental hybrid lily (POBUDKIEWICZ and TREDER 2006). Intensified green leaf pigmentation was observed in the present study with medium height chrysanthemums and in the previous experiment with tall-growing chrysanthemums (POBUDKIEWICZ and NOWAK 1997), which might be associated with higher chlorophyll content. There is no information about the flurprimidol influence on the chlorophyll level in chrysanthemum leaves but there are reports which indicate that the chloro-

phyll content per unit leaf area was increased by: daminozide in *Chrysanthemum indicum* (MAHALLE et al. 2001), uniconazole in *Chrysanthemum zawadskii* ssp. *Naktongense* (YOO and KANG 1999) or by paclobutrazol in *Dendranthema grandiflora* (KUCHARSKA and ORLIKOWSKA 2008).

In ornamental plants, growth retardants may not affect the number of flowers (POBUDKIEWICZ and GOLDSBERRY 1989), can reduce (POBUDKIEWICZ 2000a, POBUDKIEWICZ et al. 2000) or sometimes even increase the number of flowers (JUNG et al. 2000, YOO and KANG 1999). In the work reported here flurprimidol reduced the number of inflorescences and buds in both cultivars. This may be due to the fact that the assessment of the number of inflorescences per plant was done once, at the beginning of flowering. Our observations carried out over many years, in different experiments, have shown that on the day of taking data (at the beginning of flowering) very often flurprimidol treated plants had less flowers than the control ones. We have also noted that flowering period of flurprimidol sprayed plants was much longer compared to the untreated ones and during that time, the number of flowers per plant was increasing. Very often at the end of the flowering period, the number of flowers on growth retardant treated plants was similar to that on the control plants. In this experiment fewer inflorescences and buds in flurprimidol treated chrysanthemums at the beginning of flowering, may be due to the fact that growth retardants indirectly delay the aging process of plants by increasing the cytokinin (GROSSMAN 1990) and polyamines (GROSSMAN et al. 1987) contents with the result that plants are aging slower and have longer period of time to produce the yield. At the beginning of flowering smaller number of inflorescences in flurprimidol treated plants was also observed in our previous study with tall-growing chrysanthemums (POBUDKIEWICZ and NOWAK 1997). Reduced number of inflorescences, in chrysanthemums treated with other growth retardants, have also been reported by other workers (SCHUCH 1994).

The influence of flurprimidol on flower size of ornamental plants may depend on plant cultivar (POBUDKIEWICZET al. 2000), method of flurprimidol application (POBUDKIEWICZ and TREDER 2006) and the dose of this growth retardant (POBUDKIEWICZ 2000c). Flurprimidol may not influence the flower size, when used at concentrations optimum for height suppression (POBUDKIEWICZ and NOWAK 1994) but used at too high doses it often slightly diminish flower diameter (POBUDKIEWICZ 2000b). In this study even the highest flurprimidol concentration has not affected inflorescence size of medium height chrysanthemums which is consistent with our previous observations in tall-growing chrysanthemums (POBUDKIEWICZ and NOWAK 1997). In contrast other growth retardants e.g. uniconazole (STARMAN 1990) and daminozide (HICKLENTON 1990) influenced inflorescence size depending on chrysanthemum cultivar.

Variable effects of flurprimidol treatments on time to anthesis of ornamental plants have been reported. Flurprimidol induced early flowering in geranium (POBUDKIEWICZ 2000a), delayed anthesis of oriental hybrid lily (POBUDKIEWICZ and TREDER 2006) or had no effect on number of days to flowering of *Cuphea* (POBUDKIEWICZ 2000b) or *Streptocarpus* (POBUDKIEWICZ 2000c). Delayed anthesis is usually observed when flurprimidol is applied at very high doses (POBUDKIEWICZET al. 2000). Chrysanthemum appears to be very sensitive to flurprimidol application as compared to other species. Flurprimidol at a very low concentration (7.5 mg dm^{-3}) applied to "Kodiak" and "Jewel Time" in the present study delayed anthesis, but in dwarf carnations "Snowmass" anthesis was unaffected even by double flurprimidol treatment, at very high concentration, 45 mg dm^{-3} (POBUDKIEWICZ and NOWAK 1994). Other growth retardants, applied at very low doses, have also been reported to increase the number of days to flowering of chrysanthemums (STARMAN 1990, TAYAMA and CARVER 1992).

In the study reported here flurprimidol has not affected stomatal conductance to water vapor and transpiration rate per unit leaf area of "Kodiak" and "Jewel Time" chrysanthemums. The present findings on the influence of flurprimidol on transpiration are in accordance with some earlier findings with flurprimidol treatments. There was no effect of flurprimidol on transpiration rates in *Forsythia spectabilis* (VAIGRO-WOLF and WARMUND 1987), *Acer rubrum*, *Juglans nigra* and *Quercus palustris* (STERRETT et al. 1989). There are reports which indicate that other growth retardants usually decrease transpiration. Uniconazole was reported to reduce transpiration on a per leaf area basis of *Dendranthema grandiflora* Tzvelev "Dalvina" (SCHUCH 1994) and paclobutrazol to decrease transpiration rate in *Catharanthus roseus* (L.) G. Don. (JALEEL et al. 2007). In the present study no effect of flurprimidol on the chrysanthemum transpiration rate might be due to the fact that measurements of transpiration and stomatal conductance were carried out in a relatively long time (5 weeks) following retardant application. Some workers have shown that transpiration was reduced if measurements were performed in a short time following retardant treatment. For example, 24 hours after daminozide application to tomato plants, transpiration was reduced up to 34%, but after 5 days transpiration was only reduced 19% (MISHRA and PRADHAN 1971). NORCINI (1991) has shown that flurprimidol reduced transpiration rate and stomatal conductance of pruned *Euonymus fortunei* 3 days after treatment. Eighteen days following treatment transpiration rate and stomatal conductance were not lower in growth retardant treated plants compared to control ones. The author has also demonstrated that values of transpiration and stomatal conductance of flurprimidol treated plants were even slightly higher compared to values of the untreated plants if those measurements were made 18 and 21 days after flurprimidol application to *Euonymus* and pruned *Ligustrum x vicaryi*, respectively. This is consistent with results obtained in

the present study. Flurprimidol applied to "Kodiak" and "Jewel Time" chrysanthemums at higher concentrations caused even slight increase in transpiration rate and stomatal conductance relative to the control. In the experiment reported here no effect of flurprimidol on chrysanthemum transpiration might also be connected with abscisic acid (ABA) level in plants. ABA is the hormone that triggers closing of the stomata when soil water is insufficient to keep up with transpiration. Flurprimidol was reported to reduce the ABA contents in *Foeniculum* sp. (HOFMAN et al. 1992) and *Pseudotsuga menziesii* (GRAHAM et al. 1994) while other growth retardants, which resulted in stomata closure, increased the levels of abscisic acid in apple seedlings (Sutthiwal Setha Kondo 2009) and olive trees (ULGER et al. 2010). Perhaps in "Kodiak" and „Jewel Time" chrysanthemums flurprimidol has not influenced the endogenous abscisic acid content, which is responsible for closing of stomata and thus that growth retardant could not affect the transpiration.

Conclusions

Flurprimidol was highly effective for height control of "Kodiak" and "Jewel Time" chrysanthemums and a single treatment was sufficient to achieve short and very high quality plants. Flurprimidol doses should be varied according to chrysanthemum cultivar. Growth retardant at concentration of 7.5 mg dm^{-3} is recommended for "Jewel Time" but much higher concentration – 22.5 mg dm^{-3} is required for "Kodiak" in order to produce short and well compact chrysanthemums. Flurprimidol reduced the leaf area and had minimum effect on the time to anthesis. Growth retardant applications had no influence on inflorescence diameter, stomatal conductance and transpiration rate per unit leaf area of medium height chrysanthemums. Narrower in width plants treated with retardant might also be an economic advantage to commercial growers due to increased plant density on greenhouse benches. Thanks to intensified green leaf pigmentation, inflorescences placed on this same level, no leaf abscission and improved compactness, the flurprimidol treated chrysanthemums were more decorative and of much higher quality.

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ZOOBENTHOS IN POST-EXPLOITATION RESERVOIRS OF MARLS AND LIMESTONE IN OPOLE SILESIA

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K e y w o r d s: anthropogenic water bodies, macroinvertebrate communities, diversity.

A b s t r a c t

Large layers of carbonate rocks in Opole region for years serve as an exploitation material for the cement-lime industry. The mining results in numerous post-exploitation reservoirs, which biocenosis is poorly known. The objective of the study was to determine the effect of the environmental features on the distribution of macroinvertebrates and the community structure, and to present the significance of these water bodies for regional biodiversity. The research was carried out between June and November 2010 at eight reservoirs. Altogether 66 taxa were found, although only from 12 to 38 were recorded in particular reservoirs. The widespread and abundant were dipterans Chironomidae, dragonflies *Ishnura* sp. and *Coenagrion* sp., and mayflies *Caenis* sp. and *Cloeon* sp., especially numerous in charales meadows. Based on faunistic similarity, three groups of reservoirs were distinguished, which differed in size, character of a littoral zone and origin of waters (underground vs surface).

FAUNA DENNA W ZBIORNIKACH POEKSPLOATACYJNYCH MARGLI I WAPIENIA W REGIONIE ŚLĄSKA OPOLSKIEGO

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Abstrakt

Duże pokłady skał węglanowych, występujące w regionie Opola, stanowią od lat materiał eksploatacyjny dla rozwijającego się tu przemysłu cementowo-wapienniczego. Wynikiem prowadzonych prac wydobywczych są liczne zbiorniki poeksploatacyjne margli i wapieni, których biocenoza jest słabo poznana. Głównym celem badań było poznanie fauny dennej oraz określenie wpływu cech środowiskowych na rozmieszczenie organizmów i strukturę zespołów, a także ukazanie znaczenia zbiorników antropogenicznych w zachowani bioróżnorodności w regionie. Badania prowadzono w okresie czerwiec-listopad 2010 r. Objęto nimi osiem zbiorników. Ogółem w faunie dennej odnotowano przedstawicieli 66 taksonów, przy czym w poszczególnych zbiornikach reprezentowanych było od 12 do 38. Największą stałość występowania, a jednocześnie największy udział w zespołach, miały muchówki Chironomidae, ważki *Ishnura* sp. i *Coenagrion* sp. oraz jętki *Caenis* sp. i *Cloeon* sp., szczególnie liczne w siedliskach łąk ramienicowych. Na podstawie analizy podobieństwa faunistycznego wyodrębnione zostały trzy grupy zbiorników, które różniły się pod względem wielkości, charakteru strefy brzegowej i pochodzenia wód (podziemne vs powierzchniowe).

Introduction

Anthropogenic reservoirs have been objects of environmental studies for years. This refers to both big impoundments (PRUS et al. 1999) as well as to small fish and irrigation ponds (ABELLON et al. 2006), and reservoirs created through exploitation of mineral resources (BOLIER and VAN BREEMEN 1976). In Poland, the subject of the post-exploitation reservoirs study is very broad. It takes into consideration water chemistry (GALAS 2003, CZOP et al. 2011), a specific ecological group, like zooplankton (EJSMONT-KARABIN 1995) and zoobenthos (DUMNICKA and GALAS 2006), or only a single taxonomic group (KRODKIEWSKA 2003, STRZELEC and SERAFIŃSKI 2004). Nevertheless, the biocoenosis of these reservoirs remains not fully discovered, which is primarily a result of a huge variety of the reservoirs.

Considering the progressive degradation of natural aquatic ecosystems, man-made reservoirs often serve as substitute habitats for many organisms, including rare species (LEWIN and SMOLINSKI 2006, KOPERSKI 2010). Their presence in the environment and, often enough, the specificity of their habitat conditions, favor not only dispersion of organisms, but also spreading of various animal and plant species (BUCZYŃSKI and PAKULNICKA 2000, BARYŁA et al. 2005). During the last decade more and more often the significance of anthropogenic water bodies has been stressed in relation to biodiversity protection and conservation (LE VIOL et al. 2009, GIORIA et al. 2010).

Anthropogenic reservoirs in Opole Silesia have been formed mainly as a result of natural aggregate mining of sands and gravels, which may be found in the Odra river's fluvial terrace. Due to the geological structure, the formation of many reservoirs in the central part of the region is related with an exploitation of carbonate materials (marls and limestone). So far only vegetation of these reservoirs has been known and described (NOWAK et al. 2007).

The aim of the present study was to identify the occurrence and distribution of macroinvertebrates inhabiting the post-exploitation reservoirs of carbonate rocks, as well as to determine the impact of environmental features on benthic community structure.

Study area

The study was carried out in eight reservoirs located in the central part of Opole Plain. Two of them, classified as big (i.e. Bolko and Piast), have been created after flooding of excavations formed after marls exploitation in Opole. Other reservoirs are related to exploitation of limestone, including one medium-size (i.e. Szym) which was formed after flooding of a part of quarry near Szymiszów, as well as five small ponds (i.e. G1-G5) created in the 90' of the 20th century as a part of reclamation of the quarry's floor in Gorażdże. The reservoirs' characteristics has been presented in Table 1.

Table 1

Basic morphometric parameters and biotope characteristics of the water bodies

Reservior	Area [ha]	Depth [m]		Individual characteristics
		max.	mean	
Bolko	40	16	6	Flooded by Odra river during the flood of 1997. The littoral zone in 80% consist of steep bank with no vegetation.
Piast	22	12	8	Created in the 70' of the 20th century. A narrow littoral zone with rush-plants dominated by *Phragmites australis*, partially overshadowed by willows (*Salix* sp.) growing at the shoreline.
Szym	5	2	1	Created in the 70' of the 20th century. Frequent fluctuations of the water level, depending on precipitation intensity. Consequently, a large part of the littoral zone is formed by flooded grass and clumps of trees (*Salix* sp., *Alnus* sp).
G1*	0.13	1	0.3	Open exposure, a thick layer of clay sediments (up to 15 cm). During a vegetation period *Phragmites laxmanii* overgrow on more than half of the reservoir's surface.
G2*	0.56	0.6	0.4	A shallow pool area supplied mainly by rainwater, overgrown by clumps of careces, grass and willows.
G3 G4 G5	0.44 0.52 0.30	1.2 1.5 1.8	0.5 0.8 0.6	Reservoirs of a stabilized shoreline, with a narrow zone of rush-plants dominated by *Phragmites australis*, outside of which nearly all area of the bottom is covered by charales meadows. G5 open exposition, G3-G4 partially overshadowed by alders (*Alnus* sp).

* no fish

Materials and Methods

The research was carried out in 2010, through monthly collection of samples between June and November. At each of the big reservoirs the samples were collected only in a littoral zone (to a depth of 2 m), at four sites evenly distributed around the reservoir. At small reservoirs the samples were collected in littoral and central zones, according to a variation of biotope features. Sampling was done by means of a standard D-frame net (mesh-size: 0.3 mm), through kick-sampling technique with three replicates at every site. This semiquantitative hand-net sampling is known as an efficient method for bioassessment of the macroinvertebrate communities of ponds or the littoral zone of lakes (GARCIA-CRADO and TRIGAL 2005). The samples were then filtered using a 0.3 mm sieve and preserved in 70% alcohol. Taxonomic identification of mollusks and insects was done to the genus or species level, except for dipterans (to the family level). Other taxonomic groups (Oligochaeta, Hydrachnida) were only counted.

The zoocenological characteristic of macroinvertebrate communities was based on the following indicators: total number of taxa, frequency, domination, and diversity according to the Shannon-Wienner's index. The degree of similarity between macroinvertebrate communities and classification of reservoirs was defined on the basis of Ward's method and a hierarchical cluster analysis (DIGBY and KEMPTON 1987).

Simultaneously basic physical and chemical water properties were set. Field parameters taken on-site were: temperature, pH, electrical conductivity (EC), and dissolved oxygen content. The analysis was carried out according to the Polish Standards, using appropriate standard gauges.

Results and Discussion

Water chemistry

The results of water analysis were diverse to a large extent, indicating both seasonal variability of the basic water parameters, as well as an impact of a reservoir's size on the variabilities' intensity (Tab. 2). Particularly in reference to aerobic conditions. While big reservoirs Bolko and Piast provided larger stabilization, the small post-mine ponds demonstrated considerable fluctuations. The highest oxygen content (6.4–9.6 mg O_2 dm^{-3}) was obtained during the period of a full plant vegetation in July, while since September a gradual decline was observed down to the lowest content in November (2.0–3.1 mg O_2 dm^{-3}). The pH values ranged from 7.0 to 9.0, revealing

a tendency to water alkalization. Considering the geological basis, the higher values of pH and water alkalinity may be regarded as natural features of the studied reservoirs.

Table 2

Basic physico-chemical water properties

Water body	Temp. [°C]		pH		EC [μScm^{-1}]		DO [mg O_2 dm^3]	
	min.	max	min.	max	min.	max	min.	max
Bolko	9.4	20.6	8.3	9.0	506	522	7.7	9.9
Piast	10.5	20.5	8.3	8.7	545	620	8.7	10.0
Szym	9.0	21.0	7.5	8.0	495	630	8.5	9.4
GI	8.2	25.1	7.6	8.5	273	430	2.0	9.6
GII	7.8	21.1	7.4	8.2	372	502	2.4	7.2
GIII	8.6	21.7	7.5	8.0	481	653	2.3	8.9
GIV	8.4	21.4	7.0	8.2	491	634	3.1	6.4
GV	9.0	22.7	7.6	8.4	495	633	3.2	9.5

Macroinvertebrates

Altogether, 66 benthic taxa were found in the studied area (Tab. 3), albeit the total number of taxa in particular reservoirs varied from 12 (G1) to 38 (Szym) (Fig. 1). Regardless of the level of taxonomic identification, these values are most likely to be higher as they do not cover the spring season. A taxonomic richness of bottom fauna in Szym reservoir resulted mainly from a large variety of beetles (14 genera). Their occurrence was supported by diverse habitats of this sufficiently large and shallow water body. NILSSON (1984) has already described the increase in richness of aquatic beetles as a consequence of the increase in the number of habitats with increasing pond size. Despite a significant difference in taxonomic richness, the diversity of macroinvertebrates inhabiting particular reservoirs was high. Moreover, values of Shannon-Wienner's index calculated for benthic communities in big reservoirs (H' 1.84-2.22) and small ponds (H'1.69–2.50) were comparable. Although a positive correlation between reservoir's size and species diversity or abundance is widely accepted in relation to vascular plants, it is not so unambiguous in relation to fauna. Both similar trends (ALLEN et al. 1999) and lack of any significant connection between a size of a water body and a taxonomic diversity (OERTLI et al. 2002) or abundance of macroinvertebrates (GEE et al. 1997) have been shown. The size of a reservoir might play a secondary role in this case.

Table 3
List of taxa represented in benthic fauna in the investigated water bodies; Reservoirs: Bolko (Bol), Piast (Pia) and Szym (Szy). Górażdże ponds: G1 – G5

Taxa	Bol	Pia	Szy	G1	G2	G3	G4	G5
1	2	3	4	5	6	7	8	9
Ephemeroptera								
Cloeon dipterum (Linneaus, 1761)	+	+	+	+	+	+	+	+
Cloeon simile (Eaton, 1870)					+	+		
Caenis horaria (Linneaus, 1758)	+	+	+	+		+	+	+
Caenis robusta Eaton, 1884						+		
Trichoptera								
Limmephilus flavicornis (Fabricius, 1787)		+			+		+	+
Limnephilus nigriceps (Zetterstedt, 1840)						+		
Athripsodes aterrimus (Stephens, 1836)			+			+		
Oecetis furva (Rambur, 1842)						+		
Triaenodes bicolor (Curtis, 1834)							+	
Agrypnia varia (Fabricius, 1793)	+	+	+				+	
Cyrnus crenaticornis (Kolenati 1859)			+			+		
Cyrnus insolutus McLachlan, 1878	+	+	+				+	+
Odonata								
Aeschna cyanea (O.F. Müller, 1764)								+
Aeschna mixta Letreille, 1805							+	
Libellula quadrimaculata Linneaus, 1758								+
Sympetrum sanguineum (O.F. Müller, 1764)			+		+			
Coenagrion puella (Linneaus, 1758)	+	+	+	+	+	+	+	+
C. pulchellum (Vander Linden, 1825)	+	+	+	+	+	+	+	+
Ischnura elegans (Vander Linden, 1820)	+	+	+	+	+	+	+	+
Chalcolestes viridis (Vander Linden, 1825)		+			+			+
Lestes sponsa (Hansemann, 1823)					+			
Sympecma paedisca Brauer, 1877				+				
Heteroptera								
Corixa sp.					+			
Sigara sp.	+	+	+	+	+			
Mesovelia furcata Mulsant & Rey, 1852			+					
Nepa cinerea Linnaeus, 1758					+			
Ilycoris cimicoides (Linneaus, 1758)					+			+
Coleoptera								
Acilius sp.			+					
Coelambus sp.			+					
Dytiscus sp.			+		+			
Hydaticus sp.			+					
Hydroporus sp.			+		+			
Hygrotus sp.			+					
Hyphydrus sp.			+					
Ilybius sp.	+	+	+					
Laccophilus minutus (Linneaus, 1758)				+				+
Bidessus sp.					+			+
Hydrobius sp.			+					
Hydrophilus sp.			+					
Laccobius bipunctatus (Fabricius, 1775)			+	+	+			
Haliplus sp.			+				+	
Noterus clavicornis (De Geer, 1774)			+	+				
Curculionidae non det.			+					

cont. table 3

1	2	3	4	5	6	7	8	9
Megaloptera								
Sialis morio Klingstedt, 1932					+		+	
Diptera								
Ceratopogonidae		+	+					
Chaoboridae			+		+			
Chironomidae	+	+	+	+	+	+	+	+
Dixidae			+					
Limoniidae								+
Crustacea								
Asellus aquaticus (Linneaus, 1758)	+	+	+					
Dicerogammarus villosus (Sovinsky, 1894)	+							
Mollusca								
Bithynia tentaculata (Linnaeus, 1758)	+							
Lymnaea stagnalis (Linnaeus, 1758)			+					+
Radix auricularia (Linnaeus, 1758)			+					
Radix baltica (Linnaeus, 1758)	+			+		+	+	
Armiger crista (Linnaeus, 1758)			+		+			
Gyraulus albus (O.F. Müller, 1774)			+			+		
Menetus dilatatus (Gould, 1841)			+		+			
Physa fontinalis (Linnaeus, 1758)	+	+						
Dreissena polymorpha (Pallas, 1771)	+	+						
Sphaerium sp.			+					
Hirudinea								
Hemiclepsis marginata (O.F. Müller, 1774)					+			
Theromyzon tessulatum (O.F. Müller, 1774)					+			
Erpobdella occulata (Linnaeus, 1758)					+	+		
Oligochaeta	+	+	+					
Hydrachnida	+	+					+	

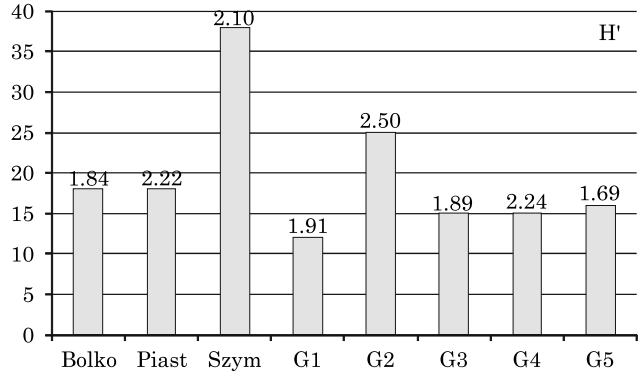

Fig. 1. Total number of taxa and values of Shannon-Wienner index (H') for macroinvertebrate communities in particular water bodies

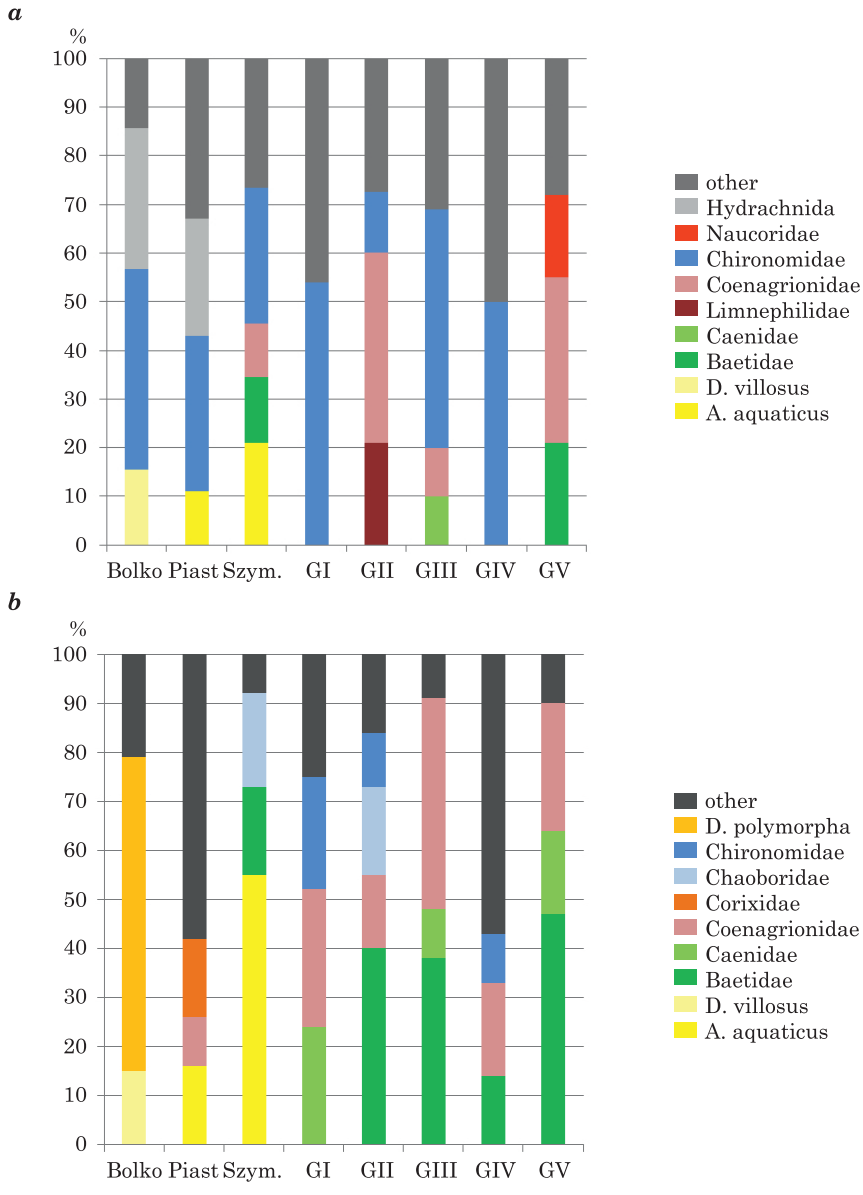

Fig. 2. The community structure of macroinvertebrates in particular water bodies during summer (A) and autumn (B)

Widespread dipterans Chironomidae, mayflies *Cloeon dipterum*, dragonflies *Ishnura elegans*, *Coenagrion puella* and *C. pulchellum* were present in all the investigated reservoirs. Moreover, organisms representing a further six

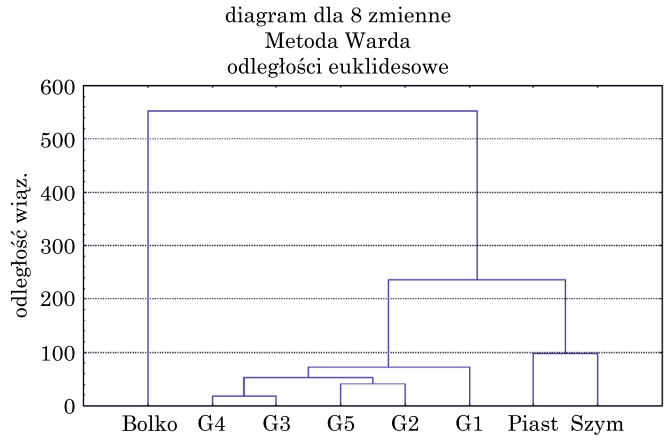

Fig. 3. Classification of water bodies based on similarities of macroinvertebrate communities. Ward's clustering method

families were frequently noted, including Caenidae (occurred in 88%), Dytiscidae and Lymnaeidae (in 75%), Limnephilidae, Phryganeidae and Corixidae (in 63%). The majority of them was also abundant and dominating in zoobenthos, which is typical for lowland ponds (PALIK et al. 2001, OERTLI et al. 2008). Nevertheless the structure of macroinvertebrate communities was different among particular reservoirs and changeable in time, due to the life-cycle of organisms (Fig. 2). Half of 12 dominating taxa were abundantly represented throughout the study period. During the summer in most of the reservoirs dominated Chironomidae, which formed 12.5–54% of the benthic community, while during the autumn Coenagrionidae (10–43%) and Baetidae (18–47%) were the most common predominant. Ubiquitous chironomids are a common dominant in ponds, showing high resistance to fluctuations of environmental conditions, including hydroperiod (BROOKS 2000, BOIX et al. 2001). Whereas the number of dragonflies and mayflies usually rises along with the rising biomass of macrophytes (WEATHERHER and JAMES 2001). Additionally, during the summer benthic communities were dominated by caddiesflies *Limnephilus flavicornis* (21% in G2), heteropterans *Naucornis cimicoides* (17% in G5) and Hydrachnidia (24–30% in Piast and Bolko). In the autumn also dominated heteropterans from the genus *Sigara* (16% in Piast), dipterans *Chaoborus* sp. (18–19% in Szym and G2) and mussel *Dreissena polymorpha* (64% in Bolko). Surprising might be relatively poor malacofauna, despite slightly alkaline environment favoring development of mollusc, especially in Górażdże ponds where only a few snail species were found, e.g.: *Radix baltica*, *R. auricularia*, *Lymnaea stagnalis*, *Armiger cristata*, *Gyraulus albus*, and *Menetus dilatatus*. Causes of this might be found in fish pressure as predators as well as too harsh

changes of oxygen conditions. Many species of molluscs might develop even in polluted waters (MICHALIK-KUCHARZ 2008), although their occurrence is to a large extent correlated with adequate aerobic conditions (SMITH et al. 2003).

On the basis of a faunist similarity three groups of reservoirs were distinguished (Fig. 3):

I – Bolko reservoir with benthic community distinctly different from any other. A few species, such as snail *Bithynia tentaculata* and crustacean *Dikerogammarus villosus*, were collected and observed only in this reservoir. Both species are known from their occurrence in Odra river in Opole (data not published). The alien *D. villosus*, recorded for the first time in Poland by JAŻDŻEWSKI et al. (2002) in the lower Odra River, spreads rapidly up the river. The presence of these species in Bolko reservoir might have been supported by the very close localization as well as by the fact of it being flooded by the river in the past. Furthermore, *Dreissena polymorpha* (found only in reservoirs in Opole) appeared here in large agglomerations. Rocky littoral zone favored not only the development of a numerous population of *D. polymorpha*, but also *B. tentaculata*, for which a positive correlation with rocky bottom was indicated in a study of Upper Silesia reservoirs (LEWIN and SMOLIŃSKI 2006).

II – Piast and Szym reservoirs, characteristic due to abundant *Asellus aquaticus* (about 10–55% of the benthic fauna). Such a big share of a detritivorous *A. aquaticus* was related with the supply of large quantities of organic matter (leaves from surrounding trees) and its accumulation in shallow zones of the reservoirs. Among habitat factors, sediment depth and canopy cover are regarded as affecting feeding groups rather than specific taxa (PALIK et al. 2001).

III – small ponds within the area of Górażdże mine. In macroinvertebrate communities there were no crustaceans (macro-), while Odonata and Ephemeroptera were numerous and various (16 and 7 species, respectively), although dominated by species of short life cycle, e.g. *Coenagrion puella*. Among dragonflies the appearance of *Sympecma paedisca*, represented by a few specimens only, is worth noting. It seems that the investigated ponds are isolated sites outside the main part of the range of this Siberian species (BERNARD et al. 2009).

Summing up, the investigated anthropogenic reservoirs were characterized by large diversity of environmental features and age. It was reflected by their benthic fauna which differed in both taxonomic richness and the community structure. Despite this and regardless a size of a water body, taxonomic diversity of macroinvertebrates, expressed as the values of Shannon-Wienner index, was high. In a regional conservation policy (restoration, management, protection) all these water bodies should be promoted as valuable freshwater ecosystems for macroinvertebrates development. However, further research is

suggested to give a better insight into the issue of spreading and development of the population of some species, including the invasive ones.

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LICHENS AND ALLIED NON-LICHENIZED FUNGI ON THE SPECIAL AREA OF CONSERVATION NATURA 2000 "SWAJNIE" PLH 280046 (NORTHERN POLAND)

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K e y w o r d s: Ascomycota, Basidiomycota, Mycetozoa, lichenized fungi, rare species, threatened and protected species.

A b s t r a c t

This paper presents the results of lichenological studies carried out during the XXV Meeting of Polish Lichenologists which took place in „Swajnie" – the Special Area of Conservation of NATURA 2000. The objective of the studies was to investigate species diversity and the habitat preferences of lichens and lichenicolous fungi. The studies resulted in the identification of 177 species, including 153 lichens and 23 non-lichenized, lichenicolous or saprobic fungi and 1 myxomycete. Of the identified taxa, 21 species are protected and 54 are listed on the Polish Red List of Endangered Lichens. The biota of the analysed area includes species that are rare in Poland and recorded on single sites e.g., *Absconditella sphagnorum*, *Bacidina delicata*, *Biatora mendax*, *Chaenothecopsis rubescens* and *Icmadophila ericetorum*. The lichenicolous fungus *Lichenostigma chlaroterae* was found for the first time in Poland.

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POROSTY I GRZYBY NAPOROSTOWE SPECJALNEGO OBSZARU OCHRONY SIEDLISK NATURA 2000 „SWAJNIE" PLH 280046 (POLSKA PÓŁNOCNA)

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K e y w o r d s: Ascomycota, Basidiomycota, Mycetozoa, gatunki zagrożone i chronione, rzadkie gatunki.

A b s t r a k t

Praca przedstawia wyniki badań lichenologicznych przeprowadzonych podczas XXV Zjazdu Lichenologów Polskich na terenie Specjalnego Obszaru Ochrony Siedlisk NATURA 2000 „Swajnie". Celem badań było poznanie zróżnicowania gatunkowego i preferencji siedliskowych porostów oraz grzybów naporostowych. W wyniku przeprowadzonych badań stwierdzono 177 gatunków, w tym 153 porostów, 23 grzybów niezlichenizowanych oraz 1 przedstawiciela Mycetozoa. Spośród odnotowanych taksonów 21 to gatunki prawnie chronione, a 54 znajduje się na czerwonej liście porostów zagrożonych w Polsce. Biota analizowanego terenu zawiera gatunki rzadkie w Polsce, m.in. *Absconditella sphagnorum*, *Bacidina delicata*, *Biatora mendax*, *Chaenothecopsis rubescens* i *Icmadophila ericetorum*. Grzyb naporostowy *Lichenostigma chlaroterae* został stwierdzony po raz pierwszy na terenie Polski.

Introduction

After the accession of Poland to the European Union, activities were undertaken aimed at creating a network of NATURA 2000 areas in Poland. Two European directives constitute the basis of this project, "Avian Directive" and "Habitats Directive", under which the Special Protection Areas (SPA) and Special Areas for Conservation (SAC) were established. The objective of the project is to protect and maintain certain types of habitats and species which are considered valuable and endangered in Europe. Currently, there are 145 "avian" areas and 849 "habitat" areas established in Poland (www.natura2000.gdos.gov.pl). In north-eastern Poland (KONDRACKI 2009) all

identified areas were investigated in terms of fauna and flora, but the biota of lichens and lichenicolous fungi of NATURA areas are still insufficiently recognized, among those also The Special Area of Conservation "Swajnie". This area is situated in the northern part of Pojezierze Olsztyńskie Lakeland, which is, comparing to southern part of that region, rather poorly lichenologicaly investigated. Although the first data originates from German publication by LETTAU (1919) and are related to the occurrence of a limited number of species near Lidzbark Warmiński, the accurate location of the localities in these reports is not given. Other lichenological resources that contain fragmented information from this area include also the publications by CIEŚLIŃSKI (2003a), KUBIAK et al. (2010), and KUKWA et al. (2013). Only the paper by KUBIAK et al. (2014) on the lichens of the Łyna river valley discusses the biota of lichens from this part of Pojezierze Olsztyńskie Lakeland. More data on the occurrence of lichens is available for the southern part of this region. It includes both papers that refer to single sites (LETTAU 1919, CIEŚLIŃSKI 2003a, KUBIAK et al. 2010, KUBIAK 2011a) and the compilations referring to specific areas: the nature reserves "Mszar", "Redykajny", "Dęby Napiwodzkie", "Koniuszanka II", and "Las Warmiński" (KUBIAK 2008, 2011b, KUBIAK, SUCHARZEWSKA 2012), the area of Olsztyn (KUBIAK 2005, KUBIAK, KUKWA 2008) and the forest arboretum in Kudypy (KUBIAK, BOBIŃSKA 2012). As the exceptional location and high diversity of forest communities of "Swajnie" area make it particularly interesting from a lichenological perspective, we undertaken the field studies to investigate the diversity of lichens and allied fungi there. The objective of this paper is to present results of our research.

Study area

The area of Wichrowskie Forests where "Swajnie" site is situated comprises the post-glacial plateau associated with the last Baltic glaciation. The consequences of this regressing glacier are numerous moraine belts with a variable direction and dense network of depressions after dead ice, which were transformed into peatlands. The highest prominence in this area amounts to 172.8 m a.s.l. (Range Kraszewo), whereas the lowest is 36.4 m (Łaniewo). The whole area of the Wichrowo Forest District is divided by several rivers flowing in the eroded beds forming gorges that sometimes have considerable relative heights (over 60 metres) and add typical mountain-like features to the landscape. The basic parent material includes loams and sands of glaciofluvial accumulation from which podzolic and brown soil is formed. Twelve percent of the surface is covered by post-bog hydrogenic soil generated

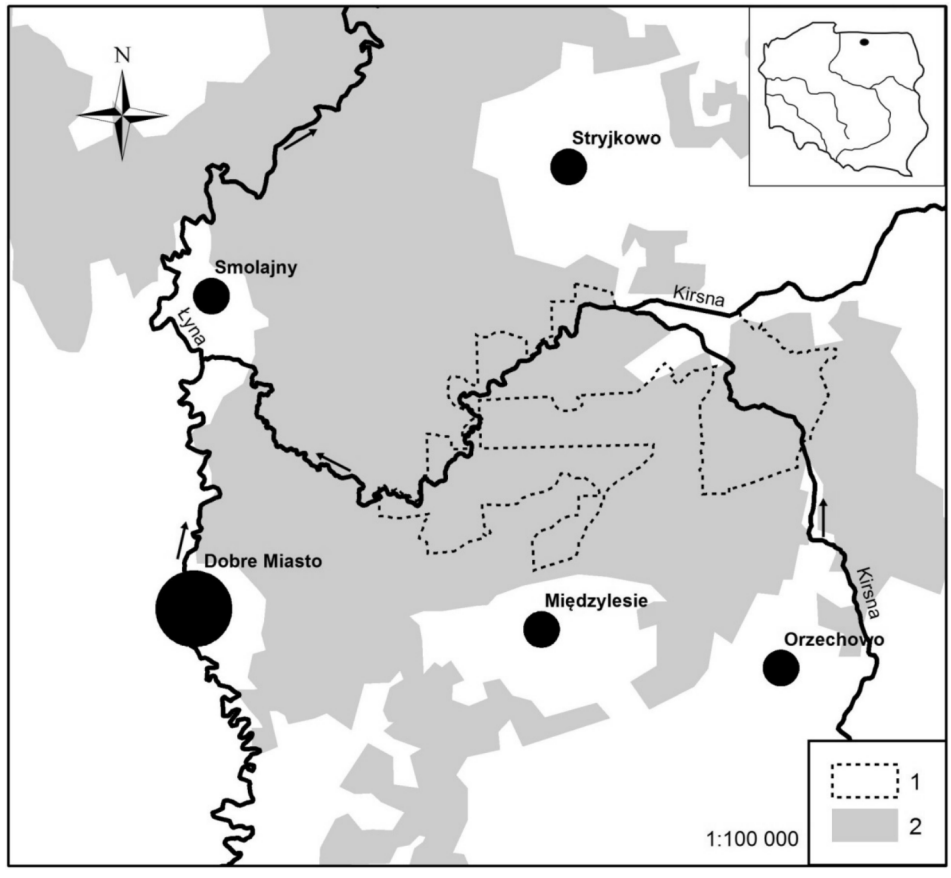

Fig. 1. Location of the research area: 1 – border of Special Area of Conservation NATURA 2000 "Swajnie", 2 – forests

from building-up peat (*Plan Urządzenia*... 2009). The "Swajnie" Special Area for Conservation was established in 2011. This area is situated within the administrative borders of Lidzbarski and Olsztyński counties, the east of Dobre Miasto city (Figure 1) and covers 1186.5 ha. Its range comprises almost the entire valley of the Kirsna river and adjacent forests of Wichrowo Forest District. In the valley of Kirsna river there are mainly communities of subcontinetal dry-ground forest *Tilio-Carpinetum*, ash-wood and alder river-side carr *Fraxino-Alnetum*, and small areas of alder spring forest *Cardamino-Alnetum glutinosae*. The other area is covered mainly by pine forests that in many places corresponds to fresh coniferous forest *Peucedano-Pinetum* and small surfaces of spruce and birch plantings. Pine, marshy coniferous forest *Vaccinio uliginosi-Pinetum*, boreal marshy spruce forest *Sphagno girgensoh-*

nii-Piceetum and numerous transitional and high peatbogs found in the depressions and around small water reservoirs are important components of the vegetation on this area. Non-forest lands, mainly meadows, cover a small area only. The "Swajnie" area was established to protect valuable habitats, mainly dry-ground forests and riverside carrs in the river valley and numerous peatbogs and marshy coniferous forests. This area is also very important for sustaining the population of organisms associated with aquatic and water-logged habitats (CZACHOROWSKI et al. 2009).

Materials and Methods

The field studies were conducted within the field session that accompanied the 25th Meeting of Polish Lichenologists entitled "Lichens in geographical, natural and cultural space" which took place in the Wichrowskie Forests (the Wichrowskie Forest District) on September 6–8, 2011. The examined object is located in the squares Be 22 and Be 23 in ATPOL grid square system (modified by CIEŚLIŃSKI, FAŁTYNOWICZ 1993). Due to the limited time for field research, more attention was given to the most valuable representative for the area habitats. The exploration for species was carried out in two working groups on 27 research sites. The species easily to identify in the field were recorded without collecting herbarium voucher. In the case of other taxa, specimens were collected for further detailed taxonomic analyses (anatomical or chemical). The collected material was stored in the herbarium of the Department of Botany and Environmental Protection, University of Warmia and Mazury in Olsztyn (OLS-L) and the duplicates of some species in UGDA and KRAM. The composition of secondary lichen metabolites was tested with thin layer chromatography (TLC) in B and/or C eluents (ORANGE et al. 2001). The nomenclature of the taxa was adopted mainly from DIEDERICH et al. (2015), FAŁTYNOWICZ (2003) and CZYŻEWSKA and KUKWA (2009) except for the selected representatives of the following taxa: the family *Teloschistaceae* Zahlbr. (ARUP et al. 2013), the genera *Alyxoria* Ach. (ERTZ, TEHLER 2011), *Epigloea* Zukal (DÖBBELER 1984, 1994), *Gyalecta* Ach. (BALOCH et al. 2013), *Lichenostigma* Hafellner (ERTZ et al. 2014), *Usnea* Dill. ex Adans. (ARTICUS et al. 2002, RANDLANE et al. 2009), *Varicellaria* Nyl. (SCHMITT et al. 2012), *Violella* T. Sprib. (SPRIBILLE et al. 2011), *Xylopsora* Bendiksby & Timdal (BENDIKSBY & TIMDAL 2013) and *Zwackhia* Korb. (ERTZ, TEHLER 2011), and species *Lepraria finkii* (LENDEMER 2011). In the list of localities, the ATPOL grid squares are given in square brackets. On the list of species, the types of substrates and numbers of sites are enumerated along each species. The lichens, which are indicators of lowland wild forests (CZYŻEWSKA, CIEŚLIŃSKI

2003) are indicated with I. The lichenicolous fungi are marked with an asterisk (*) and the saprobic fungi with a cross (+). The following abbreviations are used: Ag – bark of *Alnus glutinosa*, Ap – *Acer platanoides*, Bp – *Betula pendula*, Bpu – *Betula pubescens*, Ca – *Corylus avellana*, Cb – *Carpinus betulus*, Fe – *Fraxinus excelsior*, Fs – *Fagus sylvatica*, Lp – *Ledum palustre*, Pa – *Picea abies*, Ps – *Pinus sylvestris*, Pt – *Populus tremula*, Qr – *Quercus robur*, Sa – *Sorbus aucuparia*, Sc – *Salix cinerea*, Tc – *Tilia cordata*, Vm – *Vaccinium myrtillus*, c – concrete, p – peat, s – soil, st – stones, w – wood.

List of localities

1a – [Be 22], Dwa Stawy forest district, forest section 545; 54°0'17.35''N, 20°29'57.54''E, pine forest; **1b – [**Be 22], Dwa Stawy forest district, forest section 545; 54°0'17.35''N, 20°29'57.54''E, peatbog; **2** – [Be 22], Kochanówka forest district, forest section 412m; 54°1'35.35''N, 20°29'40.04''E, oak-linden-hornbeam forest; **3** – [Be 22], Kochanówka forest district, forest section 461c; 54°1'32.50''N, 20°29'14.43''E, oak-linden-hornbeam forest; **4** – [Be 22], Kochanówka forest district, forest section 463i; 54°1'16.95''N, 20°28'31.79''E, young birch forest; **5** – [Be 22], Kochanówka forest district, forest section 488g; 54°0'52.67''N, 20°28'53.63''E, boggy pine forest; **6** – [Be 23], Kochanówka forest district, forest section 477i; 54°0'54.30''N, 20°32'38.29''E, boggy pine forest; **7a** – [Be 23], Kochanówka forest district, forest section 481; 54°0'57.63''N, 20°31'38.24''E, oak-linden-hornbeam forest; **7b** – [Be 23], Kochanówka forest district, forest section 481; 54°0'57.63''N, 20°31'38.24''E, ash-alder stream-side forest; **8** – [Be 23], Gajnica forest district, forest section 508; 54°0'35.00''N, 20°32'11.90''E, oak-linden-hornbeam forest; **9a** – [Be 23], Kochanówka forest district, forest section 509; 54°0'37.44''N, 20°31'53.56''E, ash-alder stream-side forest; **9b** – [Be 23], Kochanówka forest district, forest section 509; 54°0'37.44''N, 20°31'53.56''E, oak-linden-hornbeam forest; **10** – [Be 23], Gajnica forest district, forest section 539b; 54°0'18.25''N, 20°32'13.39''E, oak-linden-hornbeam forest; **11** – [Be 23], Gajnica forest district, forest section 506; 54°0'32.25''N, 20°32'41.98''E, ash-alder stream-side forest; **12** – [Be 23], Dwa Stawy forest district, forest section 541; 54°0'21.62''N, 20°31'34.93''E, peatbog; **13** – [Be 23], Kochanówka forest district, forest section 509; 54°0'42.23''N, 20°31'43.03''E, oak-linden-hornbeam forest; **14a** – [Be 22], Dwa Stawy forest district, forest section 544; 54°0'11.76''N, 20°30'25.19''E, peatbog; **14b** – [Be 22], Dwa Stawy forest district, forest section 544; 54°0'11.76''N, 20°30'25.19''E, pine forest; **15a** – [Be 22], Dwa Stawy forest district, forest section 581; 53°59'38.05''N, 20°28'56.56''E, pine forest; **15b** – [Be 22], Dwa Stawy forest district, forest

section 581; 53°59'38.05''N, 20°28'56.56''E, young ash-alder stream-side forest; **16** – [Be 22], Dwa Stawy forest district, forest section 584b; 53°59'52.36''N, 20°28'10.55''E, peatbog; **17** – [Be 22], Kochanówka forest district, forest section 487g; 54°0'47.77''N, 20°29'15.58''E, boggy pine forest; **18** – [Be 22], Dwa Stawy forest district, forest section 554d; 53°59'50.25''N, 20°26'48.98''E, pine forest; **19** – [Be 22], Dwa Stawy forest district, forest section 550; 54°00'12.6''N, 20°28'28.39''E, glade, roadside tree; **20** – [Be 22], Dwa Stawy forest district, forest section 549; 54°00'19.19''N, 20°28'33.3''E, edge of the forest, roadside trees; **21** – [Be 22], Kochanówka forest district, forest section 516; 54°00'45.84''N, 20°29'14.37''E, oak-linden-hornbeam forest; **22** – [Be 22], Kochanówka forest district, forest section 458; 54°01'28.31''N, 20°30'17.86''E, edge of the forest, roadside trees.

Results

Within the area of "Swajnie", 153 lichens and 23 non lichennized fungi (including 16 lichenicolous and 8 saprophytic) were found and additionally one slime mould species, which is commonly reported in lichenological publications. The lichenicolous fungus *Lichenostigma chlaroterae* was found for the first time in Poland.

List of species

Absconditella sphagnorum Vězda & Poelt – on *Sphagnum* sp.; 1b, 16.

Alyxoria varia (Pers.) Ertz & Tehler (syn. *Opegrapha varia* Pers.) – Ap; 2.

Amandinea punctata (Hoffm.) Coppins & Scheid. – Ap, Bp; 9a, 19.

Arthonia radiata (Pers.) Ach. – Ca, Cb; 2, 7a, 8, 10, 9a, 13.

A. spadicea Leight. – Ag, Cb, Qr; 7a, 7b 8, 9a, 10.

A. vinosa Leight. – Ag, Qr; 2, 7b, 8, 9a; I.

Athallia pyracea (Ach.) Arup, Frödén & Szchting (syn. *Caloplaca pyracea* (Ach.) Th. Fr.) – Fs; 22.

**Athelia arachnoidea* (Berk.) Julich – on *Melanelixia glabratula* and *Zwackhia viridis*; 13, 15b.

Bacidia beckahausii Körb. – Ap; 2.

B. rubella (Hoffm.) A. Massal. – Ap, Qr; 2, 3.

Bacidina delicata (Leight.) V. Wirth & Vizda – Pa; 13.

B. phacodes (Korb.) Vězda – Ap; 11.

Biatora efflorescens (Hedl.) Erichsen – Cb; 13.

B. globulosa (Flörke) Fr. – Ap, Qr; 8, 11, 19.

B. mendax Anzi – Cb; 13.
B. ocelliformis (Nyl.) Arnold. – Ca; 9b; I.
Bilimbia sabuletorum (Schreb.) Arnold – c; 3.
Bryoria fuscescens (Gyeln.) Brodo & D. Hawksw. – Ag, w; 13, 15a.
Buellia griseovirens (Turner & Borrer ex Sm.) Almb. – Ag, Ap, Bp, Ca, Cb, Qr, Sc; 1a, 5, 7a, 9a, 10, 11, 13, 15b.
Calicium glaucellum Ach. – Pa, w; 11, 17.
C. salicinum Pers. – Qr; 3.
C. trabinellum (Ach.) Ach. – w; 12; I.
C. viride Pers. – Qr; 10; I.
Candelariella xanthostosigma (Ach.) Lettau – Ap; 19.
Catillaria nigroclavata (Nyl.) Schuler – Pt; 17.
Chaenotheca brunneola (Ach.) Müll. Arg. – w; 17; I.
C. chlorella (Ach.) Müll. Arg. – Bp; 2; I.
C. chrysocephala (Turner ex Ach.) Th.Fr. – Ag, Ap, Bp, Qr, Tc, w; 1a, 2, 3, 5, 6, 8, 10, 11, 15b, 17, 19.
C. ferruginea (Turner & Borrer) Mig. – Ag, Bp, Pa, Ps, Pt, Qr, w; 1a, 2, 5, 6, 7a, 9a, 11, 12, 13, 14a, 15a, 16, 17, 18.
C. furfuracea (L.) Tibell – Ag, Ap, Pa, w; 2, 11, 14a.
C. stemonea (Ach.) Müll. Arg. – Ap, w; 1b, 19.
C. trichialis (Ach.) Th. Fr. – Ap, Bp, Pa, Qr, w; 2, 3, 7a, 8, 14a, 19.
C. xyloxena Nádv. – w; 1b, 6.
+*Chaenothecopsis epithallina* Tibell – w; 8.
+*C. pusilla* (Ach.) A. F. W. Schmidt – w; 14b.
+*C. rubescens* Vain. – w; 17.
Cladonia arbuscula (Wallr.) Flot. em. Ruoss s. l. – s; 4.
C. botrytes (K.G. Hagen) Willd. – w; 17.
C. cenotea (Ach.) Schaer. – Ag, Pa, Ps, p, s, w; 1a, 6, 14a, 16, 17.
C. chlorophaea (Flörke) Spreng. – Qr, s, w; 1a, 4, 8.
C. ciliata Stirt. – s; 4.
C. coniocraea (Flörke) Spreng. – Ag, Bp, Cb, Pa, Ps, Pt, Qr, s, w; 1a, 2, 5, 6, 7a, 7b, 8, 9a, 10, 11, 12, 13, 14a, 14b, 15b, 16, 17, 21.
C. cornuta (L.) Hoffm. – p; 16.
C deformis (L.) Hoffm. – p, l; 16.
C. digitata (L.) Hoffm. – Ag, Bp, Pa, Ps, Qr, s, w; 1a, 2, 6, 8, 10, 13, 15a, 16, 17, 18.
C. fimbriata (L.) Fr. – Bp, Qr, s, w; 1a, 8, 11, 12, 13, 15b, 16, 17.
C. floerkeana (Fr.) Flörke – s; 4.
C. furcata (Huds.) Schrad. – Bp, s; 4, 12.
C. glauca Flörke – p, w; 16.
C. gracilis (L.) Willd. – s; 4.

C. grayi G**.** Merr. ex Sandst. – p, s; 16.

C. incrassata Flörke – p, w; 16, 17.

C. macilenta Hoffm – Ag, Bp, Pa, Ps, s, w; 1a, 2, 3, 5, 6, 11, 12, 13, 14ab, 15a, 16, 20.

C. phyllophora Hoffm – s; 4.

C. rangiferina (L.) F. H. Wigg. – s; 4.

C. squamosa (Scop.) Hoffm. – Bp, w; 1a, 2.

C. subulata (L.) F. H. Wigg. – Bp, Qr, s; 4, 5, 6.

C. sulphurina (Michx.) Fr. – w; 1a, 9a.

**Clypeococcum hypocenomycis* D. Hawksw. – on *Hypocenomyce scalaris*; 11, 12, 13, 15a, 16, 17.

Coenogonium pineti (Schrad. ex Ach.) Lücking & Lumbsh – Bp, Cb, Pa, Ps, Qr; 1a, 7a, 10, 13, 14a, 15a, 16, 17.

Cyphellium tigillare (Ach.) Ach – w; 12.

**Epicladonia sandstedei* (Zopf) D. Hawksw. – on *Cladonia conicraea*; 17.

**Epigloea urosperma* Döbbeler – on *Placynthiella dasaea*; 1b.

Evernia prunastri (L.) Ach. – Ap, Ag, Bp, Cb, Ca, Pa, Qr, Tc, w; 1a, 2, 3, 6, 7a, 8, 9a, 10, 11, 12, 13, 15b, 18, 19, 20, 21, 22.

Fellhanera subtilis (Vězda) Diederich & Sérus. – on Vm; 1a, 1b, 6.

Fuscidea pusilla Tønsberg – Ag, Bp, Bpu; 11, 14a.

Graphis scripta (L.) Ach. – Ca, Cb, Tc; 2, 7a, 8, 9a, 13.

Gyalecta fagicola (Hepp ex Arnold) Kremp. (syn. *Pachyphiale fagicola* (Hepp) Zwackh) – Ap; 11.

Hypocenomyce scalaris (Ach.) M. Choisy – Ag, Ap, Bp, Fs, Pa, Ps, w; 1a, 2, 3, 6, 7a, 8, 9a, 10, 11, 12, 13, 14a, 15a, 16, 17, 18, 20, 21.

Hypogymnia physodes (L.) Nyl. - Ag, Ap, Bp, Ca, Cb, Lp, Pa, Ps, Pt, Qr, Sc, Tc, w; 1a, 2, 3, 4, 5, 6, 7a, 7b, 8, 9a, 10, 11, 12, 13, 14a, 14b, 15a, 15b, 16, 17, 18, 19, 20, 21.

H. tubulosa (Schaer.) Hav. – Ag, Bp, Pa, Qr, Sc, w; 1a, 3, 4, 5, 6, 8, 9a, 11, 12, 13, 18.

Hypotrachyna revoluta (Flörke) Hale – Ag; 8; I.

Icmadophila ericetorum (L.) Zahlbr. – w; 6; I.

Imshaugia aleurites (Ach.) S.L.F.Meyer – Ap, Lp, Pa, Ps, w; 6, 11, 17.

Lecania cyrtella (Ach.) Th. Fr. – Ap; 11.

L. naegelii (Hepp) Diederich & P. Boom – Ap; 11.

Lecanora albellula (Nyl.) Th. Fr. – w; 17.

L. argentata (Ach.) Malme – Cb, Fs; 2, 9b, 22.

L. carpinea (L.) Vain. – Bp, Cb, Fs, Pt; 6, 9a, 13, 22.

L. chlarotera Nyl. – Cb; 13.

L. compallens van Herk & Aptroot – Ag, Cb; 9b, 11, 13.

L. conizaeoides Cromb. – Ag, Bp, Ca, Pa, Ps, Pt, Sa, w; 6, 8, 9a, 10, 11, 12, 13, 14a, 14b, 16, 17, 21.

L. expallens Ach. – Ca; 2, 11.

L. hagenii (Ach.) Ach. – twigs; 11.

L. intumescens (Rebent.) Rabenh. – Cb, Pt; 5, 13.

L. persimilis (Th. Fr.) Nyl. – Fs, twigs; 11, 22.

L. pulicaris (Pers.) Ach. – Ag, Ap, Bp, Cb, Pa, Ps, Qr, Sc, w; 1a, 1b, 8, 11, 12, 13, 14a, 14b, 15b, 16, 17.

L. saligna (Schrad.) Zahlbr. – Qr; 11.

L. subrugosa Nyl. – Fs, Pt; 5, 22.

L. symmicta (Ach.) Ach. – Ag, Ap, Bp, Pa, w; 5, 11, 15b, 16, 17.

L. varia (Hoffm.) Ach. – w; 13.

Lecidea nylanderi (Anzi) Th. Fr. – Ag, Bp, Pa, Ps, w; 9a, 11, 12, 14a, 17, 18.

L. turgidula Fr. – w; 17; I.

Lecidella elaeochroma (Ach.) M. Choisy – Cb, Pt, w; 2, 7a, 13, 17, 22.

L. subviridis Tønsberg – Ag; 11.

Lepraria elobata Tønsberg – Ag, Bp, Cb, w; 7b, 9b, 11, 15b.

L. finkii (B. de Lesd.) R. C. Harris – Ag, Ap, Bp, Cb, Ca, Pt, Qr, Tc, s, w; 1a, 2, 7a, 7b, 9a, 10, 11, 12, 13, 15b, 17.

L. incana (L.) Ach. – Ag, Bp, Cb, Pa, Ps, Qr, Tc, w; 1a, 2, 5, 6, 7a, 7b, 9b, 11, 13, 14a, 15a, 15b, 17, 18, 20, 21.

L. jackii Tønsberg – Ag, Bp, Cb, Pa, Ps; 2, 7a, 9b, 11, 12a, 13, 14b, 15a, 15b, 17, 18.

L. vouauxii (Hue) R. C. Harris – Ap; 19.

+*Leptorhaphis epidermidis* (Ach.) Th. Fr. – Bpu; 12.

+*Licea parasitica* (Zukal) G. W Martin – Ap; 19.

**Lichenoconium erodens* M. S. Christ. & D. Hawksw. – on *Hypocenomyce scalaris*; 15a, 16.

**L. lecanorae* (Jaap) D. Hawksw. – on *Lecanora* sp.; 12, 16.

**L. usneae* (Anzi) D. Hawksw. – on *Cladonia* sp.; 16.

Lichenomphalia umbellifera (L. Fr.) Redhead et al. – Ps, w; 1a, 17.

***Lichenostigma alpinum* (R. Sant., Alstrup & D. Hawksw.) Ertz & Diederich (syn. *Phaeosporobolus alpinus* R. Sant., Alstrup & D. Hawksw.) - on *Lecanora* sp.; 12.

**Lichenostigma chlaroterae* (F. Berger & Brackel) Ertz & Diederich (syn. *Phaeosporobolus chlaroterae* F. Berger & Brackel) – on *Lecanora* sp.; 15b.

***Lichenostigma maureri* Hafellner (syn. *Phaeosporobolus usneae* D. Hawksw. & Hafellner) – on *Usnea dasypoga*; 21.

Loxospora elatina (Ach.) A. Massal. – Bp, Tc; 2, 13; I.

Melanohalea exasperatula (Zahlbr) O. Blanco et al. - Ag, Tc; 11, 22.

Melanelixia glabratula (Lamy) Sandler & Arup – Ag, Ap, Bp, Ca, Cb, Fs, Pa, Qr, Sc, Tc, w; 1a, 2, 6, 7a, 8, 9a, 10, 11, 12, 13, 15b, 21, 22.

M. subaurifera (Fr. ex Duby) O. Blanco et al. – Ag, Ca, Cb, Bp, Pa, Qr; 10, 11, 12, 13, 15b, 22.

Micarea botryoides (Nyl.) Coppins – Ag; 9a.
M. denigrata (Fr.) Hedl. – twigs of Vm, w; 1b, 9b.
M. melaena (Nyl.) Hedl. – Bpu, Ps, p, w; 1b, 16, 17; I.
M. micrococca (Korb.) Coppins – Ag; 14a, 15b, 16.
M. misella (Nyl.) Hedl. – Qr, w; 12, 15b, 16.
M. prasina Fr. – Ps, w; 9a, 16, 17.
**Monodictis epilepraria* Kukwa & Diederich – on *Lepraria incana*; 9b, 13, 18, 20, 21.
+*Mycocalicium subtile* (Pers.) Szatala – w; 8, 12.
Ochrolechia arborea (Kreyer) Almb. – Ag; 11.
O. bahusiensis H. Magn. – Ag, Qr; 2, 7b, 11, 14a, 22.
O. microstictoides Räsänen – twigs of Pa, w; 16, 17.
Opegrapha niveoatra (Borrer) J. R. Laundon – Cb, Qr; 11, 13.
O. rufescens Pers. – Pt; 7a.
O. vulgata (Ach.) – Qr; 2, 18.
Parmelia sulcata Taylor – Ag, Ap, Bp, Ca, Cb, Pa, Qr, Sc, Tc, st, w; 1a, 2, 3, 5, 6, 7a, 7b, 8, 9a, 10, 11, 12, 13, 14a, 15b, 17, 18, 19, 21, 22.
Parmeliopsis ambigua (Wulfen) Nyl. – Ag, Bp, Cb, Ps, Sc, w; 1a, 6, 8, 9a, 11, 12, 13, 15b, 17.
Peltigera ponojensis Gyeln. – s; 6.
P. praetextata (Sommerf.) Zopf – Cb; 13.
Pertusaria amara (Ach.) Nyl. – Ag, Ap, Bp, Ca, Cb, Qr, Tc; 2, 3, 7a, 7b, 8, 9a, 10, 11, 13, 14a, 15b, 18, 19, 20, 22.
P. coccodes (Ach.) Nyl. – Ag, Bp, Cb Qr, Tc; 2, 3, 11, 13, 22.
P. leioplaca DC. – Cb; 2, 10, 13.
Phlyctis argena (Spreng.) Flot. – Ag, Ap, Bp, Ca, Cb, Fs, Pt, Qr, Sc, Tc; 1a, 2, 5, 7a, 7b, 8, 9a, 10, 11,13, 14a, 15a, 15b, 19, 21, 22.
Physcia adscendens (Fr.) H. Olivier – w; 17.
P. tenella (Scop.) DC. – Ag, Fs, Qr, st, w; 3, 6, 11, 15b, 21.
Physconia enteroxantha (Nyl.) Poelt – Ap; 19.
P. perisidiosa (Erichsen) Moberg – Ap; 19.
Placynthiella dasaea (Stirt.) Tønsberg – Bp, Bpu, Ca, p, w; 1a, 1b, 9a, 11, 12, 13, 14a, 16.
P. icmalea (Ach.) Coppins & P. James – Bp, s, w; 1b, 5, 6, 11, 16.
P. oligotropha (J. R. Laundon) Coppins & P. James – s; 11, 13.
Platismatia glauca W. L. Culb. & C. F. Culb - Ag, Ap, Bp, Ca, Cb, Pa, Ps, Qr, Tc, w; 1a, 2, 3, 4, 5, 6, 7a, 7b, 9a, 10, 11, 12, 13, 14a, 14b, 15b, 17, 18, 21, 22.
Pleurosticta acetabulum (Neck) Elix & Lumbsch. – Qr; 20.
Polycauliona polycarpa (Hoffm.) Frödén, Arup & Szchting (syn. *Xanthoria polycarpa* (Hoffm.) Rieber) – Ca, Qr, w; 3, 6, 8.
Porina aenea (Wallr.) Zahlbr. – Cb; 2, 7a, 9a, 13.

P. chlorotica (Ach.) Müll. Arg. – Cb, st; 9a, 10.

Protoparmelia hypotremella van Herk, Spier & V. Wirth – Ag; 11, 14a.

Pseudevernia furfuracea (L.) Zopf – Ag, Bp, Ca, Pa, Ps, w; 1a, 3, 6, 7a, 8, 9a, 10, 11, 12, 13, 14b, 15b, 16, 17, 18.

Pycnora sorophora (Vain.) Hafellner – Ag, Pa, Ps, w; 12, 17.

Ramalina farinacea (L.) Ach. – Ag, Ap, Bp, Ca, Cb, Fs, Qr, Tc, w; 2, 3, 8, 9a, 10, 11, 13, 17, 18, 19, 21, 22.

R. fraxinea (L.) Ach. – Ap; 19.

R. pollinaria (Westr.) Ach. – Ap, Bp, Tc; 9a, 19, 22.

Ropalospora viridis (Tønsberg) Tønsberg – Bp, Ca, Cb, Fs; 2, 10, 11, 13, 14a, 17, 21.

**Roselliniella cladoniae* (Anzi) Matzer & Hafellner – on *Cladonia* sp.; 15a, 16.

+*Sarea difformis* (Fr.) Fr. – resin of Pa; 12.

+*Sarea resinae* (Fr.) Kuntze – resin of Pa; 13, 17.

Scoliciosporum chlorococcum (Stenh.) Vězda – Ag, Bp; 11, 14b, 21.

S. sarothamni (Vain.) Vězda – twigs of Pa; 12.

**Taeniolella punctata* M. S. Christ. & D. Hawksw. – on *Graphis scripta*; 13.

Trapeliopsis flexuosa (Fr.) Coppins & P. James – Bp, s, w; 1a, 5, 6, 11, 13, 14a, 14b, 16.

T. glaucolepidea (Nyl.) Gotth. Schneid. – w; 13.

T. granulosa (Hoffm.) Lumbsch – Bp, p, s, w; 1a, 6, 12, 16, 17.

**Tremella cladoniae* Diederich & M. S Christ. – on *Cladonia coniocraea*; 11.

**T. lichenicola* Diederich – on *Violella fucata*; 12, 13, 17.

Tuckermannopsis chlorophylla (Willd.) Hale – Ag, Bp, Ca, Pa, Qr, w; 1a, 2, 4, 5, 6, 8, 9a, 12, 13, 15b, 18, 22.

T. sepincola (Ehrh.) Hale – Ag, Bp, Bpu, Pa w; 1a, 4, 5, 6, 11, 12, 13, 14a, 18.

Usnea dasopoga (Ach.) Röhl. (syn. *U. filipendula* Stirt.) – Bp, Qr; 2, 4, 17, 18, 21.

U. florida (L.) Weber ex F. H. Wigg. (syn. *U. subfloridana* Stirt.) – Bp, Pa; 2, 9a, 21. Note: All material was sterile with soralia.

U. hirta (L.) Weber ex F. H. Wigg. – Ag, Bp, Pa, Ps, w; 2, 3, 6, 10, 11, 12, 14b, 17.

Varicellaria hemisphaerica (Flörke) Schmitt & Lumbsch (syn. *Pertusaria* hemisphaerica (Flörke) Erichsen) – Cb, Tc; 13; I.

Violella fucata (Stirt.) T. Sprib. (syn. *Mycoblastus fucatus* (Stirt.) Zahlbr.) – Ag, Bp, Cb, Lp, Pa, Ps, Tc, w; 7a, 9a, 11, 12, 13, 14a, 15b, 17.

**Vouauxiomyces santessonii* D. Hawksw. – on *Platismatia glauca*; 17.

Vulpicida pinastri (Scop.) J. – E. Mattsson & M. J. Lai – Pa, w; 12, 13.

Xanthoria parietina (L.) Th. Fr – Ag, Ap, Bp, Cb, Fs; 9a, 11, 13, 19, 22.

Xylopsora caradocensis (Leighton ex Nylander) Bendiksby & Timdal (syn.

Hypocenomyce caradocensis (Leight. ex Nyl.) P. James & Gotth. Schneid.) – Pa, Ps; 9b, 13, 15a, 16, 18.

Zwackhia viridis (Ach.) Poetsch & Schied. (syn. *Opegrapha viridis* (Ach.) Behlen & Desberger) – Cb; 13; I.

Analysis of lichen biota

Within the area of "Swajnie" NATURA 2000, 54 species were identified which are featured on the Red List of Lichens in Poland (CIEŚLIŃSKI et al. 2006) (Table 1) and constitute over one-third of the whole lichen biota in the studied area. In accordance with the Regulation issued by the Minister of Environment on wild fungal species in Poland in 2014, 21 taxa are legally protected (Table 2). The presence of 11 species that are indicators of natural forests is an additional value of the analysed biota (CZYŻEWSKA, CIEŚLIŃSKI 2003) as they indicate well preserved forest communities.

Table 1

The lichen species of "Swajnie" area included in the red list of threatened lichens in Poland

Category of threat	Species	Number (precentage) of species
CR	*Chaenotheca chlorella*	1
EN	*Calicium trabinellum, Chaenotheca brunneola, C. stemonea, Cladonia botrytes, C. incrassata, Cyphellium tigillare, Hypotrachyna revoluta, Icmadophila ericetorum, Lecanora intumescens, Loxospora elatina, Physconia perisidiosa, Pleurosticta acetabulum, Ramalina fraxinea, Tuckermannopsis sepincola, Usnea florida*	15(10%)
VU	*Bacidia beckahausii, B. rubella, Biatora efflorescens, B. ocelliformis, Bryoria fuscescens, Calicium glaucellum, C. salicinum, C. viride, Chaenotheca xyloxena, Lecidea turgidula, Opegrapha niveoatra, O. rufescens, O. vulgata, Pachyphiale fagicola, Peltigera praetextata, Ramalina farinacea, R. pollinaria, Tuckermannopsis chlorophylla, Usnea dasypoga, U. hirta, Varicellaria hemisphaerica, Zwackhia viridis*	22(14%)
NT	*Alyxoria varia, Arthonia vinosa, Chaenotheca furfuracea, C. trichialis, Cladonia sulphurina, Evernia prunastri, Graphis scripta, Hypogymnia tubulosa, Lichenomphalia umbellifera, Micarea melaena, Pertusaria coccodes, P. leioplaca, Vulpicida pinastri*	13(8%)
LC	*Lecanora subrugosa*	1(<1%)
DD	*Lecanora persimilis, Trapeliopsis glaucolepidea*	2(1%)

Explanations: CR – Critically Endangered; EN – Endangered; VU – Vulnerable; NT – Near Threatened; LC – Least Concern; DD – Data Deficient

Table 2
The list of protected species occurring in "Swajnie" area (Regulation of the Minister of the Environment of 2014)

Status of protection	Species	Number of species
OS	*Cladonia incrassata*, *Hypotrachyna revoluta, Icmadophila ericetorum, Peltigera ponojensis, Ramalina fraxinea, Usnea florida*, *Tuckermannopsis sepincola*	7(5%)
OC	*Bryoria fuscescens, Cladonia arbuscula, C. ciliata, C. rangiferina, Hypogymnia tubulosa, Imshaugia aleurites, Melanelixia subaurifera, Pleurosticta acetabulum, Ramalina farinacea*, *R. pollinaria*, *Tuckermannopsis chlorophylla*, *Usnea dasopoga*, *U. hirta*, *Vulpicida pinastri*	14(9%)

Explanations: OS – strictly protected; OC – partially protected

Epiphytic lichens constitute the most numerous group and their dominance is directly related to high diversity of habitats (17 phorophyte species in different plant communities) and substrate. The richest lichenological biota was found on *Betula pendula* (54 species), *Alnus glutinosa* (52 species) and *Quercus robur* (40 species).

Among the epiphytes, *Bacidina delicata* and *Biatora mendax* are very valuable species. In Poland, the former one is found on several dispersed sites, e.g. in the region of Kujawy (CEYNOWA-GIEŁDON 2001), Gdańsk Pomerania (FAŁTYNOWICZ, KUKWA 2006, KUKWA et al. 2012), the Piska Primeval Forest (CIEŚLIŃSKI 2003a), Pojezierze Olsztyńskie Lakeland (KUBIAK 2002, 2005) and the Drawieńska Plane (SCHIEFELBEIN et al. 2012). The latter taxon had been for the first time reported by OHLERT (1870) as *Lecidea subflavida* Nyl. from the area near Ełk and Węgorzewo. Its contemporary sites have been so far identified only in the Białowieska Primeval Forest (PRINTZEN, PALICE 1999, KUKWA et al. 2008). *Biatora mendax* is found in shaded and humid places mainly on forested areas (PRINTZEN, PALICE 1999). Within the investigated region, one site of this species was found on bark of *Carpinus betulus* in a dry-ground forest in the valley of Kirsna. This is the second, presently known locality of this species in Poland.

Species growing on wood also constitute a large group in the area of "Swajnie". They are mainly reported on humid and marshy habitats with an abundance of dead wood. In this group, *Chaenothecopsis rubescens* and *Icmadophila ericetorum* are the rarest species. The first taxon is a non-lichenized fungus that has been so far found only in southern Poland (FAŁTYNOWICZ 2003) and the Białowieska Primeval Forest (KUKWA et al. 2008). This is the second known site in northern part of the country. *Icmadophila ericetorum* is a lichen that is becoming extinct in Poland (CIEŚLIŃSKI et al.

2006). In the northern part of the country, it has been classified as a critically endangered species (FAŁTYNOWICZ, KUKWA 2003, CIEŚLIŃSKI 2003b). The discovered site is most probably the fourth presently known in the lowland of Poland (comp. LIPNICKI 2003). On marshy habitats also *Absconditella sphagnorum*, a very rare species in Poland, was found. This taxon was first discovered on peat bogs in the Carpathians (BIELCZYK, KISZKA 2001). At present, there are also sites of this species located in the Tuchola Pinewoods (CEYNOWA-GIEŁDON 2003, CZARNOTA, KUKWA 2008, KUKWA et al. 2012), Sandomierz Basin and Pojezierze Kaszubskie Lakeland (CZARNOTA, KUKWA 2008). The presented sites are the first in the north-eastern part of Poland (comp. CIEŚLIŃSKI 2003a, CZARNOTA, KUKWA 2008).

Conclusions

A total of 177 lichens and non-lichenized fungi were identified in the "Swajnie" Special Area for Conservation. The biota of this area is rich and abundant in rare, protected and endangered species. The high diversity of habitats makes this region valuable not only in terms of the fauna and flora, but also lichenologically. The inclusion of this area in the NATURA 2000 programme has created a chance for protecting rare animal and plant species and will also contribute towards the protection of the valuable lichen biota. The NATURA 2000 programme has generated an opportunity for elaborating proper tools for forest management and simultaneously for protection of the precious components of the environment. Due to the limited extent of the conducted studies, it is justified to continue lichenological investigation of the area of "Swajnie" as well as the entire Wichrowskie Forest complex.

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VASCULAR PLANTS OF THE UPPER PART OF THE GRABIANKA RIVER VALLEY IN THE ELBLĄG PLATEAU LANDSCAPE PARK

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K e y w o r d s: protected and endangered taxa, rare species, erosion valley, northern Poland.

A b s t r a c t

The following list contains 292 taxa of vascular plants found in the upper part of the Grabianka river valley in the Elbląg Plateau Landscape Park. Among them, 12 species are protected by law and 6 species are considered to be endangered in Poland. Several interesting and rare taxa were recorded, including *Aconitum variegatum, Campanula latifolia*, *Epipactis purpurata, Gagea spathacea,* and *Centaurea nigra*.

FLORA ROŚLIN NACZYNIOWYCH GÓRNEJ CZĘŚCI DOLINY RZEKI GRABIANKI W PARKU KRAJOBRAZOWYM WYSOCZYZNY ELBLĄSKIEJ

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Abstrakt

Artykuł prezentuje wyniki badań flory naczyniowej w górnej części doliny rzeki Grabianki w Parku Krajobrazowym Wysoczyzny Elbląskiej. Stwierdzono występowanie 292 gatunków, w tym 12 objętych ochroną prawną oraz 6 taksonów umieszczonych na „Czerwonej liście roślin zagrożonych w Polsce". Do grupy gatunków rzadkich i interesujących można zaliczyć m.in.: *Aconitum variegatum, Campanula latifolia*, *Epipactis purpurata, Gagea spathacea* i *Centaurea nigra.*

Introduction

Natural river valleys serve as ecological corridors and are characterized by highly variable habitats and unique biodiversity of many groups of organisms (TOMIAŁOJĆ 1993). Programs and policy concerning the protection of the preserved parts of river valleys in Poland were presented in national concepts of ecological networks ECONET-PL and Natura 2000 (LIRO 1995, GACKA-GRZEŚKIEWICZ and CICHOCKI 2001, ZAJĄC 2003).

Valleys of small rivers are especially important in the northern Poland, in the areas of early post-glacial landscape (HERBICH and GÓRSKI 1993, BULIŃSKI 1995). Many of them, at least in their upper course, form deep erosion gorges. Very few data were published on the flora and vegetation in such environments (e.g. FALIŃSKI and FALIŃSKA 1965, BULIŃSKI 1979, PIOTROWSKA 1982, HERBICH 1994, JUTRZENKA-TRZEBIATOWSKI and DZIEDZIC 1994, BACIECZKO and WOŁEJKO 1997, BLOCH et al. 2000). Descriptions of the vegetation of similar, lowland river valleys from other regions were presented, e.g. by CZARNECKA (2005) or GIELNIAK and ZIELIŃSKA (2010).

The Elbląg Plateau (Wysoczyzna Elbląska) is one of the mesoregions of Gdańsk Seashore (Pobrzeże Gdańskie) where the forest-covered gorges of small watercourses are particularly important for biodiversity conservation (KONDRACKI 2009). The areas most valuable in terms of nature are protected within the Elbląg Plateau Landscape Park and belong to the Special Area of Conservation Natura 2000 "Erosion Valleys of the Elbląg Plateau" (PLH280029).

Reports indicating the occurrence of vascular plants in this mesoregion can be found in several, already historical works (KALMUSS 1884, 1890, ABROMEIT et al. 1898–1940), and in just a few more recent phytosociological (TOKARZ 1961, 1964, SZMEJA 1989) and ecological as well as floristic publications (SZMEJA 1985, GRZYBOWSKI et al. 2007, ŚLĘZAK 2012, GRZYBOWSKI and JUŚKIEWICZ-SWACZYNA 2013, GRZYBOWSKI and KRUK 2014). There are also unpublished data, contained in the protection plan of the Elbląg Plateau Landscape Park (BULIŃSKI 1997) and in the studies on nature reserves "Nowinka" and "Dolina Stradanki" (KRUK and TARATYKA 2000a, b, LEWCZUK et al. 2013).

Vascular flora of the Grabianka river valley located in the Elbląg Plateau has not been analysed so far. The aim of this study was a floristic assessment of the interesting and ecologically important upper course section of this river.

Study area

The Elbląg Plateau covers an area of approximately 450 km^2, forming a moraine plateau with a height of up to 197 m a.s.l., and steep slopes descending towards the Vistula Lagoon, alluvial delta of the Vistula (Żuławy Wiślane) and Warmia Plain (Równina Wamińska). Erosion valleys of over twenty small watercourses flowing radially from the central, highest part of the moraine plateau cut its edges (KONDRACKI 2009).

The valleys of the Elbląg Plateau are dominated by beech forests, mostly different forms of the fertile Pomeranian beech forest *Galio odorati-Fagetum,* or more rarely, a lowland acidophilous beech forest *Luzulo pilosae-Fagetum*. Smaller areas are covered by phytocoenoses of sub-Atlantic oak-hornbeam forest *Stellario-Carpinetum*. The bottoms of the valleys feature narrow strips of ash and alder riparian forests, mainly *Fraxino-Alnetum*, located along the watercourses. Moreover, scattered localities of submontane ash carrs *Carici remotae-Fraxinetum*, extremely rare in the lowland areas (TOKARZ 1961, 1964, MATUSZKIEWICZ 2005), and the stream-side *Stellario-Alnetum* association (A. Zalewska 2007 – pers. comm.) were recorded.

The Grabianka river, with the length of 10.8 km, is one of the largest watercourses within the Elbląg Plateau. It originates at an altitude of about 175 meters a.s.l., near the village of Pagórki (Figure 1), and enters the Vistula Lagoon, near the village of Kadyny. In its upland part the Grabianka is a stream of considerable slope, swift current and gravel bottom with many boulders, while outside the upland it runs slowly and in a wider sandy bed.

The studied area, covering about 150 hectares, is located in the upper part of the valley (Figure 1). Near its sources the Grabianka flows in a narrow ravine (valley sections Nos. 1–4). Then, the gorge becomes slightly wider and the stream winds gently in the bottom of a wooded erosion valley that cuts high moraine hills. Most of the slopes are about 60–80(100) m high and their inclination is 40°–60°. The hilltops and the slopes are covered mainly with mature beech stands. The slopes feature numerous seepage spring areas with tall-herb communities or patches of alder forests and active landslides. Oak-hornbeam forests are the most common in the lower parts of the slopes, and they are adjacent to riparian communities or watercourse beds. The banks of the stream at the base of the steepest slopes are covered by beech forests. There are also a few small surfaces of planted young spruces or pines and one

clearing. The bottom of the valley within the sections Nos. 7–13 is partially transformed by the presence of a soil-surfaced road with concrete culverts. Serveral stream bed edges have been artificially reinforced with concrete over a distance of about 200 m.

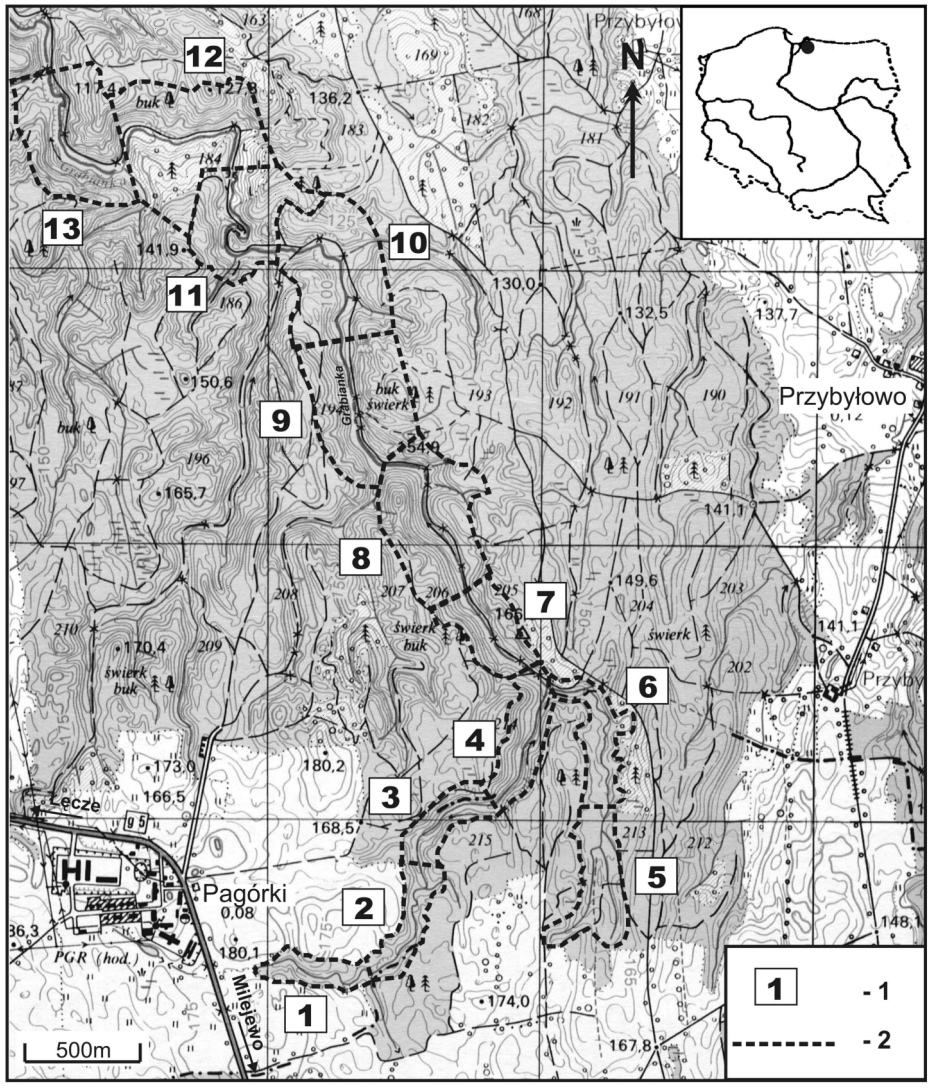

Fig. 1. Location of the study area and its division: 1 – numbers of the study sections of the river valley, 2 – border of the study sections of the river valley

The analyzed area is located within the main forest complex of the Elbląg Plateau Landscape Park, within the Górki Forest District (Elbląg Inspectorate). Using the Atlas of distribution of vascular plants in Poland (ATPOL) (ZAJĄC 1978) grid, the investigated area is situated in the square DA 96.

Materials and Methods

The study was carried out in the years 2008–2009. The studied valley has been divided into 13 sections, about 0.5 km long each (Figure 1). For each section an individual floristic inventory was taken for each different types of habitats. In this way 40 localities were set and examined. Such a division of the studied area was adopted in order to determine the frequency and local habitat preferences of species, especially the rare ones. The herbarium materials, containing the specimens of some rare or difficult to diagnose taxa, were deposited in the Herbarium of the Department of Botany and Nature Protection, University of Warmia and Mazury in Olsztyn (OLS). Species nomenclature followed MIREK et al. (2002). Species frequency was assessed in a five-point scale: very rare (1–2 localities), rare (3–6 localities), fairly frequent (7–12 localities), frequent (13–28 localities), and common (29–40 localities).

The list is presented below in an alphabetical order. The following characteristics were provided for each taxon: frequency, number (followed by "loc." – "locality/-ies") and list of localities (consisting of valley section number and a letter symbol of a habitat type: a – beech forest, b – oak-hornbeam forest, c – riparian forest, d – seepage spring area, e – roadside, f – clear-cut). Antropophytes are marked with an asterisk (ZAJĄC 1979, ZAJĄC et al. 1998, TOKARSKA-GUZIK et al. 2012).

Results and Discussion

As many as 292 species of vascular plants were recorded within the investigated area.

List of species

Acer platanoides L. – frequent; 22 loc.: 1b, 1c, 2b, 3b, 4a, 5a, 6b, 7c, 8a, 8c, 8e, 9a, 9c, 9e, 10a, 10c, 11a, 11c, 12a, 12c, 13a, 13e.

Acer pseudoplatanus L. – fairly frequent; 12 loc.: 1b, 1c, 10a, 10c, 10e, 11a, 11c, 11e, 12a, 12c-12e.

Abies alba Mill. – very rare; 1 loc.: 10a.

Achillea millefolium L. – rare; 6 loc.: 8e, 9e, 10e, 11e, 12e, 13e.

Achillea ptarmica L. – very rare; 1 loc.: 10e.

Aconitum variegatum L. – fairly frequent; 9 loc.: 1b, 2b, 4c, 6b, 7c, 9c, 10c, 11c, 12c.

Actaea spicata L. – fairly frequent; 10 loc.: 1b, 3b, 4b, 6b, 8a, 8c, 9a, 10a, 12a, 12c.

Adoxa moschatellina L. – fairly frequent; 7 loc.: 1b, 3a, 8a, 11b, 11c, 12c, 13c.

Aegopodium podagraria L. – frequent; 25 loc.: 1b, 1c, 2b, 3b, 4b, 4c, 5a, 5c, 6b, 6c, 7b, 7c, 8c, 8e, 9c, 9e, 10c, 10e, 10f, 11c, 11e, 12c-12e, 13e.

Agrimonia eupatoria L. – rare; 3 loc.: 10e-12e.

Agrostis stolonifera L. – rare; 4 loc.: 10e-12e, 13a.

Ajuga reptans L. – frequent; 26 loc.: 1b, 2b, 3a, 4a, 4c, 5a, 6b, 6c, 7c, 8d, 8e, 9a, 9c, 9e, 10a, 10c, 10e, 10f, 11c, 11e, 12a, 12c, 12e, 13a, 13c, 13e.

Alliaria petiolata (M. Bieb.) Cavara et Grande – rare; 3 loc.: 1b, 8e, 12c.

Allium ursinum L. – frequent; 14 loc.: 8a, 8c, 9a, 9c, 10a, 10c-10e, 11a, 11c, 12a, 12c, 13a, 13c.

Alnus glutinosa (L.) Gaertn. – frequent; 24 loc.: 1b, 2c, 3c, 4c, 6c, 7b, 7c, 8c, 8d, 9c, 9e, 10c-10f, 11c-11e, 12c-12e, 13a, 13c, 13e.

Alnus incana (L.) Moench – very rare; 2 loc.: 7c, 11c.

Alopecurus geniculatus L. – very rare; 1 loc.: 11c.

Anemone nemorosa L. – common; 31 loc.: 1b, 1c, 2b, 2c, 3a, 3b, 4a, 4b, 5a, 5c, 6b, 6c, 7a, 7c, 8a, 8c, 9a, 9c, 10a-10c, 11a-11d, 12a, 12c, 12d, 13a, 13c, 13e.

Anemone ranunculoides L. – frequent; 22 loc.: 1b, 2b, 2c, 3a, 4a, 5c, 6c, 7a, 7c, 8a, 8c, 9a, 9c, 10c, 11a-11c, 12a, 12c, 12d, 13a, 13c.

Angelica sylvestris L. – rare; 3loc.: 8c, 10c, 11e.

Anthoxanthum odoratum L. – rare; 3 loc.: 9e, 10e, 13e.

Anthriscus sylvestris (L.) Hoffm. – fairly frequent; 8 loc.: 1b, 6c, 10a, 10e, 11e, 12a, 12e, 13e.

Arctium lappa L. – very rare; 2 loc.: 5c, 12e.

Arctium minus Bernh – fairly frequent; 7 loc.: 8c, 8e, 9a, 11e, 13a, 13c, 13e.

Arctium nemorosum L. – fairly frequent; 8 loc.: 1c, 2b, 8a, 10a, 10f, 12a, 12d, 13a.

Arctium tomentosum L. – rare; 3 loc.: 1c, 2c, 13a.

Artemisia vulgaris L. – fairly frequent; 7 loc.: 1b, 1c, 9e, 10e, 11e, 12e, 13e.

Asarum europaeum L. – very rare; 1 loc.: 10f.

Astragalus glycyphyllos L. – very rare; 1 loc.: 2b.

Athyrium filix-femina (L.) Roth – common; 30 loc.: 1c, 2c, 3c, 4c, 5a, 5c, 6b, 6c,7b, 7c, 8a, 8c, 9a, 9c, 10a, 10c, 10e, 10f, 11a, 11c-11f, 12a, 12c-12e, 13a, 13c, 13e.

Bellis perennis L. – rare; 3 loc.: 8e, 10e, 12e.

Betula pendula Roth. – frequent; 18 loc.: 1b, 4b, 5a, 5c, 7b, 8a, 8e, 9e, 10a, 10e, 10f, 11a, 11d-11f, 12a, 12d, 13a.

Brachypodium sylvaticum (Huds.) P. Beauv. – frequent; 25 loc.: 1b, 3b, 4c, 4b, 6b, 7b, 7c, 8c-8e, 9a, 9e, 10a, 10c, 10e, 10f, 11c-11e, 12a, 12d, 12e, 13a, 13c, 13e.

Bromus inermis Leyss. – very rare; 1 loc.: 11e.

Calamagrostis arundinacea (L.) Roth. – frequent; 24 loc.: 1c, 2b, 3b, 4b, 6b, 7b, 8a, 8c, 9a, 9c, 10a, 10c-10 f, 11a, 11c, 11e, 11f, 12a, 12c, 13a, 13c, 13e.

Calamagrostis canescens (Weber) Roth. – very rare; 1 loc.: 13a.

Calluna vulgaris (L.) Hull. – rare; 4 loc.: 9a, 10e, 10f, 11f.

Caltha palustris L. – very rare; 1 loc.: 5c.

Campanula latifolia L. – rare; 4 loc.: 1b, 2b, 4c, 9c.

Campanula persicifolia L. – fairly frequent; 11 loc.: 2b, 3b, 4b, 7b, 8a, 9a, 10a, 11a, 12a, 12e, 13e.

Cardamine amara L. – frequent; 21 loc.: 1c, 2b, 2c, 3a, 3c, 4a, 4c, 5c, 6c, 7c, 8c, 9c, 10a, 10c-10e, 11c, 11d, 12c, 12e, 13c.

Cardamine flexuosa With. – fairly frequent; 9 loc.: 8a, 9a, 9c, 10a, 10c, 11c, 11f, 12c, 13a.

**Carduus acanthoides* L. – very rare; 1 loc.: 4b.

Carex digitata L. – rare; 6 loc.: 3a, 4b, 7b, 8a, 11f, 13a.

Carex hirta L. – rare; 5 loc.: 7b, 8e, 10e, 11e, 12e.

Carex ovalis Gooden. – rare; 3 loc.: 9e-11e.

Carex paire F. W. Schultz. – very rare; 2 loc.: 8c, 10f.

Carex pallescens L. – fairly frequent; 10 loc.: 2c, 3b, 4b, 5a, 6b, 9e, 10a, 10e, 12a, 12e.

Carex pilulifera L. – rare; 4 loc.: 8a, 10a, 12a, 13a.

Carex remota L. – frequent; 29 loc.: 2c, 3b, 3c, 4b, 4c, 5a, 5c, 6b, 6c, 7b, 7c, 8c-8e, 9a, 9c, 10a, 10c-10e, 11c-11e, 12a, 12c, 12d, 13a, 13c, 13e.

Carex sylvatica L. – common; 31 loc.: 1b, 2b, 3b, 4a-4c, 5a, 5c, 6b, 6c, 7b, 7c, 8c-8e, 9a, 9c, 9e, 10a, 10c, 10f, 11a, 11c-11e, 12a, 12c-12e, 13a, 13c.

Carpinus betulus L. – frequent; 27 loc.: 1b, 2b, 3b, 4b, 4c, 5a, 6b, 6c, 7b, 7c, 8a, 8c, 9a, 9c, 9e, 10a, 10c, 10e, 10f, 11a, 11c, 12a, 12c, 12e, 13a, 13c, 13e.

**Centaurea nigra* L. – very rare; 2 loc.: 8e, 9e.

Cerastium holosteoides Fr. em. Hyl. – fairly frequent; 8 loc.: 6b, 7b, 8e-13e.

Cerastium semidecandrum L. – rare; 3 loc.: 8e, 9a, 11e.

Cerasus avium (L.) Moench – rare; 4 loc.: 1b, 3b, 5a, 8e.

Chaerophyllum aromaticum L. – frequent; 14 loc.: 1b, 2b, 2c, 4c, 5a, 7b, 7c, 8c, 10c, 10e, 11c, 11e, 12c, 13c.

Chamaenerion angustifolium (L.) Scop. – rare; 5 loc.: 9a, 10e, 10f, 11e, 11f.

Chelidonium majus L. – very rare; 2 loc.: 10e, 12e.

Chrysosplenium alternifolium L. – common; 29 loc.: 1b, 1c, 2b, 2c, 3a, 3c, 4a, 4c, 5c, 6c, 7a, 7c, 8a, 8c, 8d, 9a, 9c, 10a, 10c-10e, 11a-11c, 12a, 12c, 12d, 13a, 13c.

Circaea alpina L. – frequent; 17 loc.: 4a, 4c, 5c, 7c, 8c, 8e, 9a, 9c, 9e, 10a, 10c, 11c, 12a, 12c, 13a, 13c, 13e.

Circaea intermedia Ehrh. – rare; 6 loc.: 3b, 10a, 10c, 11a, 11d, 11e.

Circaea lutetiana L. – frequent; 20 loc.: 1c, 2b, 2c, 3b, 3c, 4b, 4c, 5a, 6b, 6c, 7b, 7c, 8c, 9a, 10a, 10c, 12a, 12e, 13a, 13c.

Cirsium arvense (L.) Scop. – fairly frequent; 7 loc.: 7b, 8e, 9a, 11e, 12a, 12e, 13e.

Cirsium oleraceum (L.) Scop. – frequent; 22 loc.: 1c, 3c, 4a, 4c, 5c, 6c, 7b, 7c, 8c, 8e, 9c, 9e, 10c, 10e, 11e, 12c-12f, 13a, 13c, 13e.

Cirsium palustre (L.) Scop. – very rare; 1 loc.: 11e.

Convallaria majalis L. – rare; 6 loc.: 1b, 2c, 3b, 6b, 8a, 12a.

Corydalis cava Schweigg. et Körte – fairly frequent; 11 loc.: 1b, 2b, 3a, 4a, 5a, 7a, 7c, 9c, 10c, 11c.

Corydalis intermedia (L.) Mcrat – very rare; 2 loc.: 8c, 9c.

Corydalis solida (L.) Clairv. – rare; 6 loc.: 8c, 11c, 12c, 13a, 13c, 13e.

Corylus avellana L. – frequent; 16 loc.: 1b, 2b, 3b, 4b, 5a, 5c, 6b, 7b, 7c, 8a, 9a, 9c, 10c, 11c, 13a, 13c.

**Cotoneaster horizontalis* Decne. – very rare; 1 loc.: 13e (planted on the embankment of a forest road).

**Cotoneaster dammeri* C. K. Schneid. – very rare; 1 loc.: 13a (planted on the embankment of a forest road).

Crataegus laevigata (Poir.) DC. – very rare; 2 loc.: 1b, 5c.

Crataegus monogyna Jacq. – very rare; 2 loc.: 1b, 2b.

Crataegus rhipidophylla Gand. – rare; 4 loc.: 6c, 8a, 9a, 10a.

Crepis biennis L. – very rare; 2 loc.: 10e, 12e.

Crepis paludosa L. – fairly frequent; 11 loc.: 1b, 1c, 2b, 2c, 3c, 4c, 5c, 6c, 8c, 11c, 12c.

Dactylis glomerata L. – frequent; 13 loc.: 2c, 7b, 9a, 9e, 10c, 10e, 10f, 11e, 12a, 12c, 12e, 13c, 13e.

Dactylis polygama L. – rare; 5 loc.: 6b, 9a, 10a, 10c, 13a.

Daphne mezereum L. – fairly frequent; 8 loc.: 3c, 4c, 7b, 8a, 9a, 10a, 11c, 13e.

Daucus carota L. – very rare; 1 loc.: 12e.

Dentaria bulbifera L. – rare; 6 loc.: 4b, 10a, 11a, 11d, 12a, 13a.

Deschampsia caespitosa (L.) P. Beauv. – rare, 6 loc.: 1c, 9a, 10a, 10e, 10f, 12a.

Deschampsia flexuosa (L.) Trin. – fairly frequent; 11 loc.: 3b, 4b, 5a, 6b, 7b, 8a, 10a, 10f, 11a, 12a, 13a.

Digitalis grandiflora Mill. – very rare; 2 loc.: 12a, 12e.

Dryopteris carthusiana (Vill.) H.P. Fusch – frequent; 21 loc.: 2b, 3a, 3b, 4a, 4c, 5a, 5c, 6b, 6c, 7c, 8a, 8c, 9a, 9c, 10a, 10c, 11a, 11c, 12a, 12c, 13a.

Dryopteris dilatata (Hoffm.) A. Gray – rare; 3 loc.: 2b, 8a, 11a.

Drypteris filix-mas (L.) Schott – common; 30 loc.: 1b, 2b, 2c, 3b, 3c, 4a-4c, 5c, 6c, 7a-7c, 8a, 8c, 8e, 9a, 9c, 10a, 10c, 10d-10f, 11c, 12a, 12c, 12e, 13a, 13c, 13e.

Elymus repens (L). Gould – very rare; 1 loc.: 12e.

Epilobium hirsutum L. – rare; 5 loc.: 9c, 11c, 12e, 13c, 13e.

Epilobium lamyi F. W. Schultz – very rare; 1 loc.: 10f;

Epilobium montanum L. – frequent; 27 loc.: 1b, 2b, 3b, 4b, 4c, 5a, 6b, 7b, 7c, 8a, 8c, 8e, 9a, 9c, 9e, 10a, 10c, 10e, 11c, 11e, 11f, 12a, 12c, 12e, 13a, 13c, 13e.

Epipactis purpurata Sm. – rare; 4 loc.: 7b, 8a, 11a, 13a.

Equisetum arvense L. – fairly frequent; 8 loc.: 1c, 3b, 7b, 8e, 10e, 11e, 12c, 13e.

Equisetum fluviatile L. – very rare; 1 loc.: 6c.

Equisetum hyemale L. – very rare; 1 loc.: 11c.

Equisetum palustre L. – very rare; 1 loc.: 7c.

Equisetum pratense Ehrh. – frequent; 26 loc.: 1b, 2b, 3a, 4b, 4c, 5a, 5c, 6b, 6c, 8c-8e, 9a, 9c, 9e, 10a, 10c, 10e, 10f, 11c, 11e, 12c, 12e, 13a, 13c, 13e.

Equisetum sylvaticum L. – frequent; 18 loc.: 1b, 2b, 3b, 4a-4c, 5a, 5c, 6b, 7b, 8a, 9c, 9e, 10a, 10e, 10f, 11a, 13a.

Equisetum telmateia Ehrh. – fairly frequent; 12 loc.: 4c, 7c, 8a, 9c, 9e, 10a, 10c, 10e, 11c, 11d, 12a, 12e.

Erigeron acris L. – very rare, 1 loc.: 10e.

Erodium cicutarium (L.) L'Her – very rare; 1 loc.: 10e.

Euonymus europaea L. – fairly frequent; 11 loc.: 1b, 2b, 4a, 4c, 5a, 6b, 6c, 7b, 7c, 10c, 12e.

Eupatorium cannabinum L. – fairly frequent; 7 loc.: 7b, 9c, 11e, 12a, 12c, 12e, 13e.

Fagus sylvatica L. – common; 34 loc.: 1b, 1c, 2b, 3b, 4b, 4c, 5a, 5c, 6b, 6c, 7b, 7c, 8a, 8c, 8e, 9a, 9c, 9e, 10a, 10c, 10d, 10e, 10f, 11a, 11c, 11d-11f , 12a, 12c, 12e, 13a, 13c, 13e.

Festuca altissima All. – fairly frequent; 9 loc.: 4a, 10a, 10d, 10e, 11a, 12a, 12c, 12e, 13a.

Festuca gigantea (L.) Vill. – frequent; 26 loc.: 1b, 3b, 3c, 4b, 5a, 5c, 6c, 7b, 7c, 8a, 8e, 9a, 9c, 9e, 10c-10f, 11c, 11e, 12a, 12c, 12e, 13a, 13c, 13e.

Festuca pratensis Huds. – very rare; 2 loc.: 12e, 13a.

Ficaria verna Huds. – frequent; 20 loc.: 1b, 1c, 2b, 2c, 3a, 4a, 5c, 6c, 7c, 8c, 9c, 10c, 10d, 11a, 11c, 12a, 12d, 12c, 13a, 13c.

Filipendula ulmaria (L.) Maxim. – fairly frequent; 12 loc.: 1c, 2b, 4c, 5c, 6c, 7c, 8c, 8d, 9c, 9e, 10c, 11e.

Fragaria vesca L. – frequent; 16 loc.: 2b, 5a, 6c, 7b, 7c, 8a, 8c, 8e, 9a, 9e, 10e, 11c, 11e, 12a, 12e, 13e.

Fraxinus excelsior L. – frequent; 16 loc.: 1b, 2b, 4b, 4c, 6b, 6c, 7b, 8a, 8c, 8e, 9a, 9e, 10a, 11c, 12c, 13c.

Gagea lutea L. – frequent; 25 loc.: 1b, 1c, 2b, 3a, 4a, 5a, 5c, 6c, 7a, 7c, 8a, 8c, 9a, 9c, 10a, 10c, 11b, 11c, 12a, 12c-12e, 13a, 13c, 13e.

Gagea minima (L.) Ker Gawl. – very rare; 1 loc.: 1b.

Gagea spathacea L. – rare; 6 loc.: 1b, 1c, 5c, 10a, 10b, 12c.

**Galanthus nivalis* L. – very rare; 1 loc.: 1b (antropogenic locality; in an illegal refuse heap in forest)

Galeobdolon luteum Huds. – frequent; 23 loc.: 1b, 1c, 2b, 3b, 4b, 4c, 5a, 6b, 7b, 7c, 8a, 8c, 9a, 9c, 10a, 10c, 10e, 11a, 11c, 12a, 12c, 13a, 13c.

Galeopsis speciosa Moll. – fairly frequent; 9 loc.: 1b, 2b, 3b, 4a, 5a, 5c, 6c, 8c, 12d.

Galeopsis tetrahit L. – very rare; 1 loc.: 13a.

Galium aparine L. – frequent; 13 loc.: 1b, 1c, 2b, 4c, 5c, 6c, 8c, 10c, 10e, 11c, 11e, 12c, 12d.

Galium elongatum C. Presl. – very rare; 1 loc.: 6c.

Galium mollugo L. s.str. – very rare; 1 loc.: 11e.

Galium odoratum (L.) Scop. – common; 30 loc.: 1b, 1c, 2b, 3b, 4c, 5a, 5c, 6b, 6c, 7a-7c, 8a, 8c, 8e, 9a, 9c, 9e 10a, 10c, 10e, 11a, 11c, 11e, 12a, 12c, 12e, 13a, 13c, 13e.

Galium schultesii Vest – fairly frequent; 11 loc.: 1b, 3b, 4b, 7b, 8a, 8c, 9a, 9c, 12a, 13c, 13e.

Galium uliginosum L. – rare; 3 loc.: 1c, 8d, 12d.

Geranium robertianum L. – frequent; 28 loc.: 1b, 2b, 3b, 4a, 4c, 5c, 6b, 6c, 7a-7c, 8c-8e, 9c, 9e, 10a, 10c, 10e, 10f, 11a, 11c, 11e, 12c, 12e, 13a, 13c, 13e.

Geranium sylvaticum L. – very rare; 2 loc.: 2b, 5a.

Geum rivale L. – very rare; 2 loc.: 1b, 6c.

Geum urbanum L. – frequent; 26 loc.: 1b, 1c, 2b, 3b, 4c, 5a, 5c, 6b, 6c, 7b, 7c, 8a, 8c, 8e, 9a, 9c, 10c, 10e, 10f, 11c, 11e, 12c-12e, 13a, 13e.

Glechoma hederacea L. – frequent; 21 loc.: 3b, 4b, 4c, 5c, 6c, 7c, 8d, 8e, 9c, 10a, 10c, 10e, 10f, 11c, 11e, 12c-12e, 13a, 13c, 13e.

Glyceria fluitans (L.) R. Br. – fairly frequent; 11 loc.: 1c, 2c, 4c, 5c, 9c, 9e, 10c, 11c, 12d, 13a, 13c.

Glyceria nemoralis (R. Uechtr.) R. Uechtr et Korn – very rare; 2 loc.: 7c, 8c.

Gymnocarpium dryopteris (L.) Newman – frequent; 17 loc.: 2b, 3b, 5a, 6c, 7b, 7c, 8a, 8c, 9a, 9c, 10a, 10c, 11a, 11c, 13a, 13c, 13e.

Hedera helix L. – very rare; 1 loc.: 13c.

Hepatica nobilis Scherb. – frequent; 14 loc.: 1b, 2c, 3b, 4a, 4b, 6b, 6c, 9a, 11a-11c, 12a, 12c, 13c.

Hieracium murorum L. – very rare; 2 loc.: 9a, 12a.

Hieracium pilosella L. – very rare, 1 loc.: 11f.

Holcus lanatus L. – rare; 5 loc.: 8e, 10e, 11e, 12e, 13e.

Holcus mollis L. – rare; 4 loc.: 10e, 11e, 12a, 13e.

Hordelymus europaeus L. – very rare; 1 loc.: 8c.

Huperzia selago (L.) Bernh. ex Schrank et Mart. – rare; 3 loc.: 8a, 12a, 13a.

Hypericum maculatum Crantz. – rare, 6 loc.: 2c, 5s, 7b, 8e, 10e, 10f.

Hypericum perforatum L. – rare; 4 loc.: 6b, 8e, 10a, 13e.

Hypochoeris radicata L. – fairly frequent; 7 loc.: 2c, 9e, 10a, 10e, 10f, 12a, 12e.

Impatiens noli-tangere L. – frequent; 25 loc.: 1b, 2b, 2c, 3b, 3c, 4c, 5a, 5c, 6b, 8c-8e, 9a, 9c, 9e, 10a, 10c, 10f, 11a, 11c, 12a, 12c, 12d, 13a, 13c.

**Impatiens parviflora* DC. – common; 29 loc.: 1b, 1c, 2b, 2c, 3b, 4a-4c, 5a, 5c, 6b, 6c, 7b, 7c, 8a, 8c-8e, 9a, 9c, 10c-10f, 11a, 11e, 12c, 12d, 13a.

Iris pseudacorus L. – very rare; 1 loc.: 1c.

Juncus conglomeratus L. – very rare; 1 loc.: 2c.

Juncus effusus L. – frequent; 28 loc.: 2b, 3b, 4b, 4c, 5a, 5c, 6b, 6c, 7b, 7c, 8c-8e, 9a, 9c, 10a, 10c, 10f, 11a, 11c-11f, 12c, 12d, 13a, 13c, 13e.

**Juncus tenuis* Willd. – rare; 5 loc.: 8e, 10e, 11e, 12e, 13e.

**Lamium album* L. – very rare; 2 loc.: 7c, 12e.

Lamium maculatum L. – fairly frequent; 11 loc.: 2b, 4c, 8c, 9c, 10a, 10c, 10e, 11c, 12c, 12e, 13c.

Lapsana communis L. – frequent; 19 loc.: 1b, 2c, 4a, 5a, 6b, 6c, 7b, 7c, 8e, 9a, 9c, 9e, 10a, 10e, 11e, 12c, 12e, 13c, 13e.

Larix decidua Mill. – fairly frequent; 9 loc.: 4a, 4b, 7b, 9a, 9e, 10a, 10e, 11a, 12a.

Lathraea squamaria L. – frequent; 13 loc.: 1b, 2b, 3a, 4a, 8a, 9c, 10a-10c, 11b, 11c, 12a, 13c.

Lathyrus pratensis L. – very rare; 1 loc.: 2b.

Lathyrus sylvestris L. – very rare; 1 loc.: 2c.

Lathyrus vernus (L.) Bernh. – fairly frequent; 8 loc.: 2c, 4a, 7a, 7b, 8a, 9a, 10a, 13a.

Leontodon autumnalis L. – very rare; 2 loc.: 10e, 11e.

Linaria vulgaris Mill. – very rare; 1 loc.: 7b.

Lolium perenne L. – very rare; 1 loc.: 7b.

Lonicera xylosteum L. – fairly frequent; 8 loc.: 1b, 2b, 3b, 4b, 4c, 7b, 7c, 13c.

Lotus uliginosus Schkuhr – very rare; 1 loc.: 10a.

**Lupinus polyphyllus* Lindl. – very rare; 1 loc.: 12e.

Luzula campestris (L.) DC. – fairly frequent; 8 loc.: 6b, 7b, 9a, 9e, 10f, 11e, 11f, 12e.

Luzula luzuloides (L.) DC. – rare; 4 loc.: 2b, 3b, 12a, 13a.

Luzula pilosa (L.) Willd. – frequent; 19 loc.: 1b, 2b, 4b, 5a, 6b, 6c, 7a, 7b, 8a, 8c, 9a, 10a, 10c, 10e, 11a, 11e, 11f, 12a, 13a.

Lycopodium annotinum L. – rare; 4 loc.: 3b, 4a, 8a, 13a.

Lycopus europaeus L. – rare; 3 loc.: 6c, 11c, 13c.

Lysimachia nummularia L. – frequent; 18 loc.: 3c, 4c, 5c, 6c, 7c, 8a, 8c-8e, 9c, 10c, 11c, 11e, 12c-12e, 13c, 13e.

Lysimachia vulgaris L. – fairly frequent; 11 loc.: 1b, 1c, 2c, 5a, 5c, 7c, 8c, 9c, 10c-10e.

Maianthemum bifolium (L.) F. W. Schmidt – frequent; 19 loc.: 1b, 2b, 3b, 4b, 5a, 6b, 7b, 8a, 9a, 9e, 10a, 10c, 10f, 11a, 11c, 11f, 12a, 13a, 13c.

Medicago lupulina L. – very rare; 1 loc.: 12e.

Melandrium rubrum (Weigel) Garcke – fairly frequent; 10 loc.: 1b, 1c, 2c, 3a, 4a, 5a, 5c, 6c, 10c, 13e.

Melica nutans L. – fairly frequent; 9 loc.: 4b, 7b, 9a, 9e, 10a, 11a, 12a, 12e, 13e.

Melica uniflora Retz. – rare; 3 loc.: 9a, 10a, 12a.

Melilotus alba Medik. – very rare; 1 loc.: 10e.

Mentha aquatica L. – very rare; 1 loc.: 8c.

Mercurialis perennis L. – frequent; 27 loc.: 1b, 2b, 3a, 3b, 4a, 4b, 6b, 6c, 7a, 7c, 8a, 8c, 8d, 9a, 9c, 10a, 10c, 10e, 11a, 11c, 11e, 12a, 12c, 12d, 13a, 13c, 13e.

Milium effusum L. – frequent; 18 loc.: 1b, 2b, 3b, 4c, 5a, 5c, 6b, 6c, 8a, 9a, 10a, 10c, 10e, 11c, 12a, 12c, 13a, 13c.

Moehringia trinervia (L.) Clairv. – fairly frequent; 10 loc.: 2c, 8a, 8e, 9a, 10a, 10e, 10f, 11f, 12a, 13e.

Monotropa hypopitys L. – very rare; 1 loc.: 13a.

Mycelis muralis (L.) Dumort – frequent; 15 loc.: 1b, 3b, 5a, 6b, 7b, 8a, 8c, 8e, 10a, 10c, 11c, 11e, 12a, 13a, 13e.

Myosotis arvensis (L.) Mill. – very rare; 1 loc.: 7c.

Myosotis palustris (L.) L. em. Rchb. – frequent; 13 loc.: 1b, 2c, 3c, 4c, 6c, 8c, 8d, 9c, 9e, 10c, 11c, 12d, 13a.

Myosotis sylvatica Ehrh. ex Hoffm. – rare; 6 loc.: 8a, 9e, 10e, 11e, 12e, 13e.

Myosoton aquaticum (L.) Moench – very rare; 1 loc.: 2b.

Neottia nidus-avis (L.) Rich. – very rare; 1 loc.: 12a.

Oenothera biennis L. s.str. – very rare; 1 loc.: 12e.

Oxalis acetosella L. – common; 29 loc.: 1c, 1b, 2b, 2c, 3b, 4b, 5a, 5c, 6b, 6c, 7a, 7b, 8a, 8c, 9a, 9c, 10a, 10c, 10e, 11a, 11c, 11e, 11f, 12a, 12c-12e, 13a, 13c.

Padus avium Mill. – rare; 5 loc.: 9e, 10a, 11e, 12c, 13e.

Paris quadrifolia L. – rare; 3 loc.: 1b, 12a, 12c.

Petasites albus L. – frequent; 14 loc.: 4a, 4c, 7c, 8a, 8c-8e, 9a, 9c-9e, 10c, 11b, 11e.

Phalaris arundinacea L. – rare; 5 loc.: 1c, 2c, 10e, 12e, 13e.

Phegopteris connectilis (Michx.) Watt – frequent; 20 loc.: 2c, 3b, 4b, 6b, 7a-7c, 8d, 9a, 9c, 10a, 10c, 11a, 11c-11e, 12a, 12c, 13a, 13c.

Phleum pratense L. – very rare; 1 loc.: 10e.

Phragmites australis (Cav.) Trin. ex. Stend. – rare; 3 loc.: 7b, 9e, 11e.

Phyteuma spicatum L. – frequent; 16 loc.: 1b, 2b, 3b, 4b, 6b, 7b, 8a, 9a, 9c, 10a, 10e, 11a, 11c, 12a, 12c, 13a.

Picea abies (L.) H. Karst – frequent; 26 loc.: 2b, 3b, 4a-4c, 5a, 6b, 6c, 7b, 7c, 8a, 8c, 9a, 9e, 10a, 10c, 10e, 10f, 11a, 11c, 11e, 11f, 12a, 12c, 13a, 13c.

Pinus sylvestris L. – frequent; 13 loc.: 3b, 4a, 4b, 7b, 9a, 9e, 10a, 10e, 10f, 11a, 11f, 12a, 13a.

Plantago major L. – fairly frequent; 8 loc.: 7b, 8e, 9e, 10e, 11e, 12e, 13a, 13e.

Plantago lanceolata L. – very rare; 2 loc.: 11e, 12e.

Platanthera chlorantha Rchb. – very rare; 1 loc.: 10e.

Poa annua L. – rare; 6 loc.: 7b, 8e, 10e, 10f, 11e, 12e.

Poa nemoralis L. – frequent; 21 loc.: 1b, 3b, 4c, 5a, 7b, 7c, 8a, 8c, 8e, 9a, 9c, 9e, 10a, 10c, 10f, 11a, 11c, 12a, 12e, 13a, 13c.

Poa palustris L. – fairly frequent; 8 loc.: 1b, 2b, 2c, 3c, 10c, 10d, 11c, 12c.

Poa pratensis L. – rare; 6 loc.: 7b, 9e, 10e, 11e, 12e, 13e.

Poa trivialis L. – frequent; 17 loc.: 2b, 4a, 4c, 6c, 7c, 8c-8e, 9a, 11a, 11c, 11d, 12c, 12d, 13a, 13c, 13e.

Polygonum hydropiper L. – very rare; 1 loc.: 12a.

Polygonatum multiflorum (L.) Au. – rare; 5 loc.: 1b, 2b, 3b, 5a, 6b.

Polypodium vulgare L. – fairly frequent; 8 loc.: 4b, 8a, 8c, 11a, 11c, 12a, 12c, 13a.

Populus tremula L. – fairly frequent; 8 loc.: 2b, 2c, 3b, 4b, 9a, 9e, 10e, 11e.

Potentilla anserina L. – rare; 5 loc.: 8e, 9e, 11e, 12e, 13e.

Potentilla erecta (L.) Raeusch. – very rare; 1 loc.: 10e.

Prunella vulgaris L. – fairly frequent; 7 loc.: 7b, 10e, 11e, 12a, 12e, 13a, 13e.

**Prunus cerasifera* Ehrh. – very rare; 1 loc.: 1b.

Pteridium aquilinum (L.) Kuhn – fairly frequent; 8 loc.: 1b, 2b, 8a, 9a, 10a, 11e, 11f, 13a.

Pulmonaria obscura Dumort – fairly frequent; 8 loc.: 1c, 2b, 3b, 7a, 8a, 8c, 12a, 13a.

Pyrola minor L. – very rare; 1 loc.: 10e.

**Pyrus communis* L. – very rare; 1 loc.: 1c.

Quercus petraea (Matt.) Liebl. – fairly frequent; 9 loc.: 8a, 9a, 10a, 10c, 10e, 11a, 11f, 12a, 13a.

Quercus robur L. – frequent; 16 loc.: 1b, 2b, 3b, 4b, 4c, 5a, 5c, 6b, 7b, 8a, 9a, 10a, 10f, 11a, 12a, 13a.

**Quercus rubra* L. – rare; 3 loc.: 4b, 10e, 10f.

Ranunculus acris L. – rare; 4 loc.: 8e, 9a, 9e, 10e.

Ranunculus auricomus L. – very rare; 1 loc.: 8c.

Ranunculus cassubicus L. s.l. – very rare; 1 loc.: 11c.

Ranunculus lanuginosus L. – fairly frequent; 19 loc.: 1b, 2b, 2c, 3b, 6b, 7b, 7c, 8a, 8c, 9c, 9e, 10a, 10c, 10e, 11c, 11e, 12c, 13a, 13c.

Ranunculus repens L. – frequent; 20 loc.: 1c, 4c, 5a, 6c, 8a, 8c, 8e, 9a, 9c, 9e, 10a, 10c-10f, 11c, 12c, 12e, 13a, 13e.

Ribes alpinum L. – very rare; 1 loc.: 13a.

Ribes nigrum L. – fairly frequent; 7 loc.: 1c, 2b, 4a, 7c, 8c, 9c, 11c.

Ribes spicatum E. Robson – very rare; 1 loc.: 1c.

**Robinia pseudoacacia* L. – very rare; 1 loc.: 13e.

Rubus caesius L. – rare; 6 loc.: 3b, 4c, 9e, 11e, 12e, 13e.

Rubus idaeus L. – frequent; 28 loc.: 1c, 2b, 3b, 4b, 4c, 5a, 5c, 6b, 6c, 7b, 7c, 8a, 8c, 8e, 9a, 9c, 9e, 10a, 10c, 10e, 10f, 11a, 11c, 11e, 11f, 13a, 13c, 13e.

Rubus pedemontanus Pinkw. – frequent; 16 loc.: 2b, 4a, 4c, 5a, 5c, 6b, 6c, 7a-7c, 8c, 9a, 10a, 11a, 12a, 13a.

Rumex acetosa L. – rare; 4 loc.: 9e, 10e, 11e, 12e.

Rumex acetosella L. – rare; 4 loc.: 9e, 10e, 11f, 12a.

Rumex aquaticus L. – very rare; 2 loc.: 1c, 2c.

Rumex obtusifolius L. – frequent; 17 loc.: 4c, 5a, 5c, 6c, 7c, 8d, 8e, 9c, 10c, 10e, 10f, 11c, 11e, 12c, 12e, 13c, 13e.

Rumex sanguineus L. – fairly frequent; 9 loc.: 8d, 8e, 9e, 10a, 11c, 11d, 12d, 13a, 13e.

Salix alba L.- very rare; 1 loc.: 12e.

Salix caprea L. – frequent; 13 loc.: 1b, 8a, 8c, 9a, 9e, 10a, 10e, 11c, 11e, 12a, 12e, 13a, 13e.

Salix purpurea L. – very rare; 1 loc.: 9e.

Sambucus nigra L. – frequent; 14 loc.: 1b, 1c, 4b, 5a, 6b, 7b, 8a, 9a, 9c, 10a, 11c, 12c, 12e, 13c.

Sambucus racemosa L. – very rare; 2 loc.: 10a, 11c.

Scirpus sylvaticus L. – rare; 4 loc.: 8c, 9e, 10f, 12a.

Scrophularia nodosa L. – frequent; 26 loc.: 1b, 1c, 2b, 3b, 4c, 5a, 6b, 6c, 7b, 7c, 8c-8e, 9a, 9e, 10a, 10c, 10e, 11a, 11e, 11f, 12a, 12c, 12e, 13a, 13e.

Scutellaria galericulata L. – fairly frequent; 8 loc.: 7c, 9c, 10c, 10e, 11c, 12d, 13a, 13c.

Senecio jacobaea L. – very rare; 1 loc.: 10f.

Senecio vulgaris L. – very rare; 1 loc.: 11f.

**Sinapis alba* L. – rare; 4 loc.: 8c, 8e, 9c, 9e.

Solanum dulcamara L. – very rare; 2 loc.: 11c, 12a.

**Solidago canadensis* L. – fairly frequent; 7 loc.: 2b, 6b, 9e, 11d, 12d, 12e, 13e.

**Solidago gigantea* Aiton – fairly frequent; 3 loc.: 9a, 12e, 13a.

Solidago virgaurea L. s.str. – very rare; 1 loc.: 10e.

Sorbus aucuparia L. em. Hedl. – frequent; 19 loc.: 1b, 2b, 3b, 4b, 5a, 6b, 6c, 7b, 8a, 9a, 9c, 9e, 10a, 10c, 10f, 11a, 11e, 12a, 13a.

Stachys sylvatica L. – common; 30 loc.: 1b, 1c, 2b, 3b, 3c, 4b, 4c, 5a, 5c, 6b, 7b, 7c, 8c-8e, 9a, 9c, 9e, 10a, 10c, 10f, 11c-11e, 12c-12e, 13a, 13c, 13e.

Stellaria graminea L. – rare; 3 loc.: 10e, 11e, 12e.

Stellaria holostea L. – frequent; 28 loc.: 1b, 2b, 3b, 4b, 5a, 5c, 6b, 6c, 7b, 8a, 8d, 8e, 9a, 9c, 9e, 10a, 10c, 10e, 11a, 11c, 11d, 11f, 12a, 12c, 12e, 13a, 13c, 13e.

Stellaria media (L.) Vill. – fairly frequent; 9 loc.: 1b, 4b, 8e, 9e, 10e, 10f, 11c, 11e, 12e.

Stellaria nemorum L. – common; 31 loc.: 1b, 1c, 2b, 3b, 3c, 4a-4c, 5a, 5c, 6b, 6c, 7c, 8c-8e, 9a, 9c, 10a, 10c, 10e, 10f, 11c, 11e, 12a, 12c-12e, 13a, 13c, 13e.

Stellaria uliginosa Murray – rare; 4 loc.: 10a, 10d, 12d, 13a.

Tanacetum vulgare L. – rare; 4 loc.: 10e, 11e, 12e, 13e.

Taraxacum officinale F. H. Wigg – frequent; 17 loc.: 1b, 2b, 4a, 7c, 8c, 8e, 9a, 9c, 10e, 10f, 11e, 12a, 12c, 12e, 13a, 13c, 13e.

Thalictrum aquilegiifolium L. – frequent; 14 loc.: 1c, 2b, 4b, 4c, 6b, 7a, 7c, 8c, 9c, 10c, 11c, 12c, 13a, 13c.

Tilia cordata Mill. – fairly frequent; 10 loc.: 2b, 3b, 8a, 9e, 10e, 11a, 11c, 12a, 13a, 13e.

Trientalis europeaea L. – fairly frequent; 8 loc.: 4a, 9a, 10a, 10e, 11a, 11f, 12a, 13a.

Trifolium arvense L. – very rare; 1 loc.: 7b.

Trifolium campestre Schreb. – very rare; 2 loc.: 10e, 11e.

Trifolium medium L. – very rare; 1 loc.: 10e.

Trifolium pratense L. – rare; 3 loc.: 10e, 11e, 13a.

Trifolium repens L. – fairly frequent; 9 loc.: 7b, 8e, 9a, 9e, 10e, 11e, 12e, 13a, 13e.

Tussilago farfara L. – frequent; 13 loc.: 7b, 7c, 8e, 9a, 9e, 10a, 10e, 10f, 11e, 12e, 13a, 13c, 13e.

Ulmus glabra Huds. – frequent; 22 loc.: 1c, 2b, 4b, 4c, 5a, 5c, 6b, 7c, 8a, 8c, 8e, 9c, 10a, 10c, 10e, 11c, 11e, 12a, 12e, 13a, 13c, 13e.

Urtica dioica L. – common; 37 loc.: 1b, 1c, 2b, 2c, 3a, 3c, 4b, 4c, 5a, 5c, 6b, 6c, 7b, 7c, 8a, 8c-8e, 9a, 9c, 9e, 10a, 10c-10f, 11a, 11c, 11e, 11f, 12a, 12c-12e, 13a, 13c, 13e.

Vaccinum myrtillus L. – fairly frequent; 12 loc.: 4b, 5a, 6b, 7b, 8a, 9a, 10a, 10f, 11a, 11f, 12a, 13a.

Valeriana dioica L. – rare; 4 loc.: 2b, 6c, 8c, 9c.

Veronica beccabunga L. – frequent; 13 loc.: 1c, 2c, 3c, 4c, 5c, 6c, 7c, 8c, 8d, 9c, 11c, 12d, 13c.

Veronica chamaedrys L. – frequent; 13 loc.: 2c, 3b, 4b, 5a, 8c, 8e, 9c, 10c, 10e, 10f, 12a, 12e, 13e.

Veronica montana L. – frequent; 19 loc.: 1c, 2b, 2c, 4a, 4c, 5c, 6b, 6c, 7c, 8a, 8c, 8e, 9c, 10a, 10c, 11a, 11c, 13a, 13c.

Veronica officinalis L. – frequent; 14 loc.: 2c, 3b, 4b, 7b, 8a, 9a, 9c, 9e, 10a, 10e, 10f, 11e, 12a, 13a.

Veronica serpyllifolia L. – very rare; 2 loc.: 8a, 8e.

Viburnum opulus L. – very rare; 2 loc.: 1b, 2b.

**Vicia angustifolia* L. – very rare; 2 loc.: 10e, 12e.

**Vicia hirsuta* (L.) Gray – very rare; 2 loc.: 11e, 12e.

Vicia sepium L. – fairly frequent; 7 loc.: 7b, 8e, 9e, 10e, 11e, 12e, 13e.

Vicia sylvatica L. – frequent; 20 loc.: 3b, 4a, 4b, 6b, 7b, 8a, 8c-8e, 9a, 9e, 10a, 10c, 10e, 10f, 11e, 12a, 12e, 13a, 13c.

**Vicia tetrasperma* L. – rare; 3 loc.: 9c, 12c, 12e.

Viola mirabilis L. – very rare; 1 loc.: 2b.

Viola palustris L. – very rare; 2 loc.: 6c, 10f.

Viola reichenbachiana Jord. ex Boreau – fairly frequent; 10 loc.: 2b, 3b, 4a, 7b, 8a, 9c, 10a, 11a, 11c, 13a.

Most of species which were found are typical plants of shady deciduous forests of *Querco-Fagetea* class. The group of the most valuable taxa is large and includes 39 species of "special concern" (protected by law in Poland, endangered in Poland, or endangered in the region of Gdańsk Pomerania). Among them, 1 is strictly protected and 11 are partially protected (see Table 1) (Regulation of the Minister of the Environment of 2014 on the protection of plant species). In the investigated area 6 species endangered in Poland (acc. to ZARZYCKI and SZELĄG 2006) and 29 endangered within the Gdańsk Pomerania were recorded (see MARKOWSKI and BULIŃSKI 2004, OLSZEWSKI and MARKOWSKI 2005) (Table 2).

Table 1

The list of vascular plants protected by law occuring in the Grabianka river valley

Status of protection	Species	Number of species
P	*Epipactis purpurata*	1
pP	*Aconitum variegatum*, *Allium ursinum*, *Campanula latifolia*, *Daphne mezereum*, *Digitalis grandiflora*, *Galanthus nivalis*, *Huperzia selago*, *Lycopodium annotinum*, *Neottia nidus-avis*, *Platanthera chlorantha*, *Pyrola minor*	11

Explanations:
Species: P – strictly protected, pP – under partial protection

Table 2
The vascular plants of the Grabianka river valley included into the red list of plants in Poland and the red list of threatened plants in the region of Gdańsk Pomerania

Category of threat	Name of species	Number of species
Red list of plants in Poland[1]		
V	*Campanula latifolia*, *Gagea minima*	2
[V]	*Allium ursinum*, *Huperzia selago*	2
R	*Epipactis purpurata*, *Gagea spathacea*	2
Red list of threatened plants in the region of Gdańsk Pomerania[2]		
EN	*Platanthera chlorantha*	1
VU	*Aconitum variegatum*, *Allium ursinum*, *Dentaria bulbifera*, *Epipactis purpurata*, *Hordelymus europaeus*	5
NT	*Asarum europaeum*, *Campanula latifolia*, *Cardamine flexuosa*, *Corydalis cava*, *C. solida*, *Digitalis grandiflora*, *Gagea spathacea*, *Galium schultesii*, *Geranium sylvaticum*, *Glyceria nemoralis*, *Huperzia selago*, *Neottia nidus-avis*, *Petasites albus*, *Ranunculus cassubicus*, *Rumex aquaticus*, *Rumex sanguineus*, *Stellaria uliginosa*, *Veronica montana*, *Viola mirabilis*	19
LC	*Actaea spicata*, *Arctium nemorosum*	2
DD	*Circaea intermedia*, *Galanthus nivalis*	2

Explanations:
1 acc. to Zarzycki and Szeląg (2006): V – vulnerable; [V] – vulnerable, species that are endangered at isolated localities, situated beyond the main area of occurence; R – rare, potentially endangered;
2 acc. to Markowski and Buliński (2004) and Olszewski and Markowski (2005): EN – Endangered; VU – Vulnerable; NT – Near Threatened; LC – Least Concern; DD – Data Deficient

An interesting group of 9 mountain species (according to Zając 1996) were found in the analyzed area. The following species were observed almost exclusively on the valley bottom and along the stream: *Aconitum variegatum*, *Allium ursinum*, *Alnus incana*, *Equisetum telmateia* and *Veronica montana.* Numerous seepage spring areas were covered by large patches of *Petasites albus. Festuca altissima, Huperzia selago* and *Ribes alpinum* grew only in the beech forests. Most of the above mentioned species were common and abundant, besides *Alnus incana, Huperzia selago* and *Ribes alpinum* which were found at just a few (1–3) localities. Unfortunately, the study did not confirm any localities of *Pleurospermum austriacum* (L.) Hoffm. This mountain species, very rare in the northern part of Poland, was reported from the Grabianka river valley in the 1960s (Tokarz 1961). At present, only one locality of this species is known in the whole Elbląg Plateau, in the "Dolina Stradanki" nature reserve (Lewczuk et al. 2013).

The presence of mountain species, represented by both spermatophytes (Czubiński 1950, Zając 1996) and cryptogams (Szweykowski 1958), is a speci-

fic feature of the Elbląg Plateau flora. The occurence of such species is due to the existence of narrow valleys of small watercourses (SZAFER 1972), with cool and moist air lingering at their bottoms, similarly as in the mountain valleys (ZAJĄC 1996).

The group of alien vascular plants within the investigated part of the valley included 19 species, which accounted for around 6% of all the recorded plant species. Among these taxa, only highly invasive *Impatiens parviflora* (TOKARSKA-GUZIK et al. 2012) could threaten the natural species composition of the observed plant communities in the future. It was found at most of the analyzed localities, in all types of habitats, but it was usually not very abundant. Other species (e.g. *Centaurea nigra*, *Juncus tenuis*, *Lamium album*, *Quercus rubra*, *Robinia pseudoacacia*, *Solidago canadensis* and *Solidago gigantea*) were more rare and grew mainly along the road on the bottom of the valley. An interesting discovery was a locality of *Centaurea nigra,* a very rare ephemerophyte (RUTKOWSKI 2004). The roadside was also a habitat for the recorded native meadow and ruderal species. These plants did not penetrate into the forest communities, or only at very few localities.

The vascular flora of the upper part of the Grabianka river valley is characterized by only slightly changed natural composition and a presence of species that are rare and endangered on a national or regional scale, e.g. mountain species occurring in lowlands. These features were also confirmed for the flora of the erosion valleys of other small rivers within Gdańsk Pomerania, such as: Radunia (HERBICH and MARKOWSKI 1998), Stradanka (KRUK and TARATYKA 2000b, LEWCZUK et al. 2013) or Narusa (ŚLĘZAK 2012).

Conclusions

The results indicate that deep erosion valley of the Grabianka river is a refuge of vascular plants diversity, including many rare species. Domination of the natural forest species is related to the limited possibilities of economic exploitation of the steep slopes with numerous seepage spring areas.

Erosion valleys, cutting the landscape of the Gdańsk Pomerania, are distinguished by the presence of specific habitats and unique diversity of the flora and vegetation (BULIŃSKI 1995). Conservation of these exceptional qualities requires comprehensive protection of animate and inanimate nature, especially the sustainability of the natural hydrological features of watercourses (HERBICH 1997).

In the case of the analyzed area, the main threat to the flora is associated with the preparation and execution of the planned logging of the oldest beech trees, growing in easily accessible places. For example, rebuilding of the road

that runs along the Grabianka river at the valley section No. 7, carried out in 2010, involving reinforcement of the culverts and widening (e.g. for places of the temporary timber storage), resulted in the destruction of some localities of rare plants, such as *Equisetum telmateia* and *Petasites albus*. It is recommended to monitor the hazards and protect the reported localities of valuable species (particularly those protected by law) during the execution of planned economic activities.

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REMOVAL OF NITROGEN COMPOUNDS IN THE PROCESS OF AUTOTROPHIC DENITRIFICATION IN A SEQUENCING BATCH BIOFILM REACTOR (SBBR)

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K e y w o r d s: autotrophic denitrification, inorganic carbon, hydrogen gas, sequencing batch biofilm reactor (SBBR), synthetic wastewater.

A b s t r a c t

The paper discusses the impact of the C/N ratio of inorganic carbon ($KHCO_3$) and its interaction with carbon dioxide liberated by the oxygenation of a carbon electrode and with gaseous hydrogen produced by water electrolysis, on the concentration of oxygenated forms of nitrogen, during the process of autotrophic denitrification in a multi-catode reactor with immobilized biofilm. The experiment was set under anaerobic conditions, at the electric current density of 79 mA/m^2 and the C/N ratios of 0.5, 0.75 and 1.0. The results showed that a higher dose of inorganic carbon ($KHCO_3$) significantly decreased the concentration of nitrate. The concentration of this form of nitrogen was even lower in a reactor additionally loaded with CO_2 and H_2, in which the physicochemical parameters of sewage sludge (the redox potential and electrolytic conductivity) were therefore better for the denitrification process. Nitrate was not completely consumed by dissimilation reduction in either of the reactors. Some small amount of this compound was converted to the ammonium form on the assimilation pathway. In addition, the carbon electrode served as an acceptor of electrones, in the process of external oxygenation of organic compounds.

USUWANIE ZWIĄZKÓW AZOTU W PROCESIE DENITRYFIKACJI AUTOTROFICZNEJ W SEKWENCYJNYM REAKTORZE Z BŁONĄ BIOLOGICZNĄ (SBBR)

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S ł o w a k l u c z o w e: denitryfikacja autotroficzna, węgiel nieorganiczny, gazowy wodór, sekwencyjny reaktor z unieruchomioną błoną biologiczną (SBBR), ścieki syntetyczne.

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Abstrakt

W pracy przedstawiono wpływ stosunku C/N węgla nieorganicznego ($KHCO_3$) oraz jego współdziałania z dwutlenkiem węgla wydzielanym w wyniku utleniania elektrody węglowej i gazowym wodorem powstającym w procesie elektrolizy wody, na koncentrację utlenionych form azotu, w procesie autotroficznej denitryfikacji z wykorzystaniem reaktora wielo-katodowego z unieruchomioną błoną biologiczną (SBBR). Badania prowadzono w warunkach anaerobowych, przy gęstości prądu elektrycznego – 79 mA/m^2 i stosunku C/N = 0.5, 0.75, 1.0. Przeprowadzone badania pokazały, iż wzrost dawki węgla nieorganicznego ($KHCO_3$), istotnie wpłynął na zmniejszenie stężenia azotu azotanowego (V). W reaktorze wspomaganym dodatkowo CO_2 i H_2 ze względu na korzystniejsze, dla procesu denitryfikacji, parametry fizyko-chemiczne oczyszczanych ścieków (potencjał redox i przewodność elektrolityczna) odnotowano najniższą jego koncentrację. Azot azotanowy (III) w obu reaktorach, nie został całkowicie wykorzystany w redukcji dysymilacyjnej. Niewielka jego cześć przeszła w szlaku asymilacyjnym do formy amonowej. Elektroda węglowa pełniła dodatkowo rolę akceptora elektronów, w procesie zewnętrznego utleniania związków organicznych.

Introduction

Nitrates belong to compounds that dissolve very well in aquatic environment, which they enter from uncontrolled discharge of municipal and industrial wastewater, from landfill leachate and as a result of inadequate sewage and wastewater management solutions. A high concentration of this oxygenated form of nitrogen in surface waters has a negative impact on water, mostly by stimulating eutrophication. Moreover, nitrates can be transformed to nitrites in the digestive tract of animals and humans. In reaction with hemoglobin in blood, they cause methemoglobinemia, which inhibits the oxygen transport in cells. Bio-denitrification, both the heterotrophic and autotrophic one, has been known as the most frequently applied method in this respect (KARANASIOS et al. 2010, ZHAO et al. 2011). The heterotrophic denitrification, commonly applied in technological systems, consists in the consumption of organic compounds like methanol, ethanol or acetate by denitrifying bacteria as sources of carbon and electrons. Despite its high efficiency, this method has significant drawbacks that include the presence of carbon residues and process by-products in treated wastewaters and greater biomass growth from 0.6 to 0.9 g/gN-NO_3 (MATEJU et al. 1992, KULIKOWSKA et al. 2008).

Hence, it is essential to continue search for new technological solutions, which will ensure highly effective removal of these compounds. Recently, researchers have paid more attention to a possible combination of biological and physicochemical processes affected by an electric current. (KRZEMIENIEWSKI and RODZIEWICZ 2005, RODZIEWICZ et al. 2011, RODZIEWICZ et al. 2011a, RODZIEWICZ et al. 2011b, KŁODOWSKA et al. 2013). In order to reduce costs of providing a hydrogen donor (ex situ), researchers have postulated the use of an electro-biochemical reactor (in situ). The implementation of a bio-electrochemical reactor (SBBR), in which microorganisms are used to catalyze an

electrolytic process has been proven to be an effective solution. The biomass immobilized on the surface of a catode (discs) acts as a biocatalyst accepting electrons, which is a better solution than expensive chemical catalysts. The process of electrolytically supported denitrfication relies on the use of gaseous hydrogen biofilm, serving as substrate for microorganisms. This biofilm is produced on the surface of a catode during the hydrolysis of water, which functions as a donor of electrons in the reduction of nitrates to gaseous nitrogen. Less biomass is generated during autotrophic denitrification than in the course of heterotrohophic denitrification supplied with different carbon sources. The reason is that microorganisms expand more energy on digesting the compounds in which the degree of carbon oxygenation is higher than in biomass or which contain fewer C atoms (KULIKOWSKA et al. 2008). Low solubility of hydrogen (1.6 mg/L/bar at 20°C), no need for the removal of its excess and lack of process by-products make this method a good alternative to other electron donors for example: iron, sulfur, etc.(KULIKOWSKA et al. 2008, KARANASIOS et al. 2010, ZHAO et al. 2011). Autotrophic denitrification with a hydrogen donor can be additionally stimulated by inorganic substrate in the form of carbon dioxide, carbonates or bicarbonates. Carbon dioxide produced inside the system in the course of oxygenation of the carbon electrode (anode) and dosed bicarbonates may serve as an additional source of inorganic carbon for microorganisms. These compounds neutralize the reaction by capturing hydroxyl ions, while CO_2 ensures that appropriate anaerobic conditions are maintained for the growth of biofilm (ZHOU et al. 2007, SUKKASEM et al. 2008, ZHAO et al. 2011). Ions HCO_3^- and CO_3^{2-} produced by the dissociation of bicarbonates and carbon dioxide can improve the conditions for the course of hydrogenotrophic denitrification by stimulating a higher electrolytical conductivity of sewage and wastewater.

The available literature does not mention the possibility of supporting hydrogenotrophic denitrification with inorganic carbon in the form of potassium bicarbonate, or its interaction with carbon dioxide released by oxygenation of a carbon electrode and gaseous hydrogen generated during the electrolysis of water. This has encouraged the authors to examine the effect of the C/N ratio of inorganic carbon ($KHCO_3$) on changes in the concentration of oxygenated nitrogen forms, in the process of autotrophic denitrification, using a multi-catode reactor with immobilized biofilm (SBBR).

Material and Methods

The research was conducted on synthetic wastewater characterized by a low concentration of organic compounds and a high concentration of

oxygenated forms of nitrogen. Synthetic wastewater was produced as follows: 2.0 dm^3 of tap water was mixed with enriched broth (0.08 g/dm^3), $NaNO_3$ (30.36 g/dm^3), KCl (21 g/dm^3), $MgSO_4 \cdot 7H_2O$ (307.5 g/dm^3) and $CaCl_2$ (21 g/dm^3).

The average values of physicochemical parameters are given below:

– concentration of nitrates – 52.09 [mgN_{NO_3}/dm^3],

– concentration of organic compounds ($ChZT_{Cr}$) – 70.57 [mgO_2/dm^3],

– concentration of total organic carbon (TOC) for C/N ratio of 0.5, 0.75 and 1.0 – 31.89, 33.70, 35.29 [mgC/dm^3],

– concentration of inorganic carbon (IC) for C/N ratio of 0.5, 0.75 and 1.0 – 49.70, 69.50, 83.20 [mgC/dm^3],

– pH value – 7.5 [–],

– redox potential – 131.14 [mV],

– electrolytic conductivity – 1.58 [mS/cm].

The experiment was run in parallel, in 2 sequential batch biofilm reactors (SBBR) with vertical cyliners of the capacity of 3.0 dm^3, working under anaeorbic conditions (Figure 1). Each reactor contained a set of 12 stainless

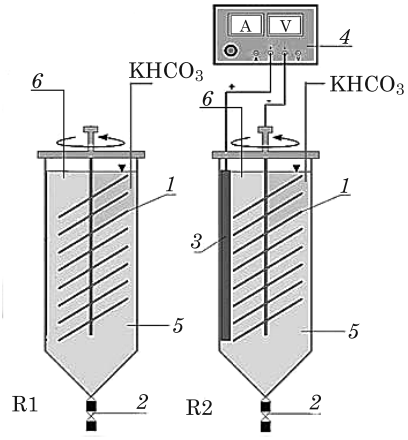

Fig. 1. The scheme of an experimental installation with anaerobic rotating multi-disc reactor: *1* – stainless steel cathode – discs with biomass, *2* – outlet, *3* – carbon anode, *4* – electric current source, *5* – reactor: R1 – control reactor 1 ($KHCO_3$), R2 – reactor 2 (CO_2 + $KHCO_3$ + H_2), *6* – inlet

steel catodes (discs) with immobilized biofilm, each of the diameter of 0.10 m and total surface of 0.19 m^2. The discs fitted axially on a vertical shaft rotated at a speed of 10 rev./min, and were 100% submerged. Reactor 1 (R1) was a control system, unaffected by an electric current. In contrast, reactor 2 (R2) worked under an electric current flow. It contained a carbon steel anode, whose total surface area was 0.003 m^2. Both reactors (R1 and R2) were fed

potassium bicarbonate ($KHCO_3$) at the C/N ratio of 0.5, 0.75 and 1.0. The catode and anode were conected to a laboratory power supply to ensure the required electric current, i.e. 15 mA, 3.0 V (electric current density of 79 mA/m^2). Prior to the proper tests, the reactors had been working until an adquate structure of the biofilm and a stable concentration of nitrates in the outflow were achieved, which took three months. Samples for analyses were taken once a day (1.0 dm^3). Afterwards, the reactors were emptied (1.0 dm^3) and refilled with sewage (2.0 dm^3).

The following physicochemical analyses were made in raw and treated wastewater:

– nitrates – with the colorimetric method [ISO 7890-3:1988],

– nitrites – with the colorimetric method [ISO 6777:1984],

– ammonia – with the colorimetric method [PN – 73/C-04576/01],

– organic compounds (COD) – with the dichromate method [ISO 6060:1989],

– total organic carbon and inorganic carbon – with an IL 550 TOC-TN analyzer by Hach,

– pH value – with an HI 123 pH meter by Hanna Instruments,

– electrolytic conductivity – with an HI 99301 conductivity meter by Hanna Instruments,

– redox potential – with an pH 211 meter by Hanna Instruments.

Results and Discussion

The experiments have demonstrated that an increased dose of $KHCO_3$ resulted in a decreased concentration of nitrate in treated wastewater, but did not guarantee a complete run of the autotrophic denitrification process. The said process proceeded through two steps: to ammonia nitrogen on the assmilation path and to molecular nitrogen by the dissimilation reduction. According to KLIMIUK and ŁEBKOWSKA (2008), the assmilation path is catalyzed by nitrate reductase type B (consititutive enzyme), while nitrate reductase type A (inductive enzyme) is the catalyst of the dissimilation.

In the R2 system, where – apart from potassium bicarbonate – the reactor was supplied with carbon dioxide produced by the anodic oxygenation of a carbon electrode and with gaseous hydrogen generated by water electrolysis, at the initial value of 52.09 mg NNO_3/dm^3, the final concentration of nitrate decreased to 12.38 (±0.64) mg NNO_3/dm^3 at C/N equal 0.5, 8.44 (±0.88) mg NNO_3/dm^3 at C/N = 0.75, and 4.46 (±0.26) mg NNO_3/dm^3 at C/N= 1.0 (Figure 3). At the same time, the consumption of $KHCO_3$ decreased as its dose increased from 31.51% (±5.86) (C/N = 0.5) to 15.67% (±3.37) (C/N = 1.0), while

the percentage of used of organic carbon rose from 73.77% (±2.98) (C/N = 0.5) to 76.19% (±2.50) (C/N = 1.0) (Figure 2).

In the R1 system fed with $KHCO_3$ alone, a much higher concentration of the examined nitrogen form was recorded in the discharge: 32.66 (±3.47) mg NNO_3/dm^3 (C/N = 0.5), 24.63 (±2.38) mg NNO_3/dm^3 (C/N = 0.75) and 18.34 (±0.58) mg NNO_3/dm^3 (C/N = 1.0) (Figure 3). Same as in reactor 2, the percentage of used potassium bicarbonate fell from 21.23% (±2.32) (C/N = 0.5) to 10.73% (±2.49) (C/N = 1.0) to the advantage of organic carbon, rising from 67.67% (±2.94) (C/N = 0.5) to 67.74% (±3.69) (C/N = 1.0) (Figure 2). This substantial decrease of the total carbon in the outflow of treated raw sewage is attributed to the fact that some of the organic form of carbon was converted to inorganic form; another reason is the participation of autotrophic and heterotrophic bacteria in the wastewater treatment process.

The nitrite in the outflow from both reactors, shown in figure 3, was not completely used up on the dissimulation pathway and a small amount of this compound changed into the ammonium form by the assimilation reduction. According to SZEWCZYK (2005), ammonium ions are used by microorganisms to build their cells. Thus, denitrification was participated by "*true denitrifying*" bacteria, which used nitrite as an acceptor of electrons in the conversion of nitrate to gaseous nitrogen, and by "*nitrate respiring*" bacteria, which did not take part in the transformation of atmospheric nitrogen (GLASS and SILVERSTEIN 1998). These microorganism are characterized by a much faster growth than "*true denitrifying*" bacteria (WILDERER et al. 1987). The lowest concentration of nitrite was recorded in reactor 1. On average, it was 2.26 (±1.18) mg NNO_2/dm^3 at the C/N ratio of 0.5; a large decline, down to 0.17 (±0.34) mg NNO_2/dm^3, was observed at C/N = 0.75, and a rise to 0.40 (±0.27) mg NNO_2/dm^3 appeared at C/N = 1.0.

The ammonium nitrogen concentration was low. An increase in the dose of inorganic carbon ($KHCO_3$) caused an increase in the outflow of this nitrogen form. On average, it reached 0.52 (±0.46) mg NNH_4/dm^3 at C/N = 0.5, 2.42 (±0.36) mg NNH_4/dm^3 at C/N = 0.75 and 2.85 (±1.00) mg NNH_4/dm^3 at C/N = 1.0. A much higher nitrite concentration was obtained in reactor 2. At the lowest carbon to nitrogen ratio (C/N=0.5), it was 5.88 (±0.68) mg NNO_2/dm^3 on average. A small decrease was noticed at the C/N ratio of 0.75 (5.60 (±0.31) mg NNO_2/dm^3), but then again the level of this nitrogen form increased at a higher C/N proportion (6.43 (±0.35) mg NNO_2/dm^3 at C/N = 1.0). Compared to reactor 1, reactor 2 had slightly higher concentrations of ammonia nitrogen at the C/N ratios of 0.5 and 0.75 (1.08 (±0.61) mg NNH_4/dm^3, 3.06 (±0.54) mg NNH_4/dm^3) and a lower concentration of this compound at C/N = 1.0 (1.16 (±0.19) mg NNH_4/dm^3).

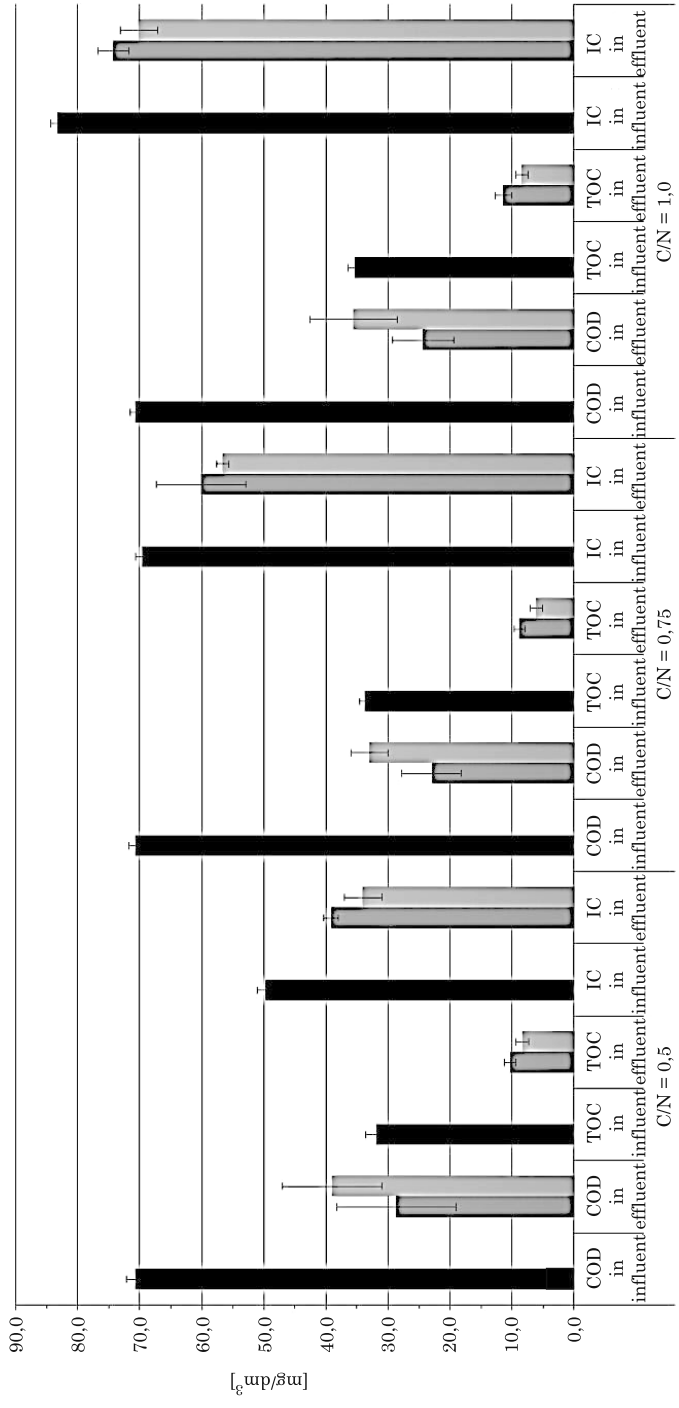

Fig. 2. Effect of C / N ratio on the concentration of organic compounds (COD), total organic carbon (TOC) and inorganic carbon (IC)

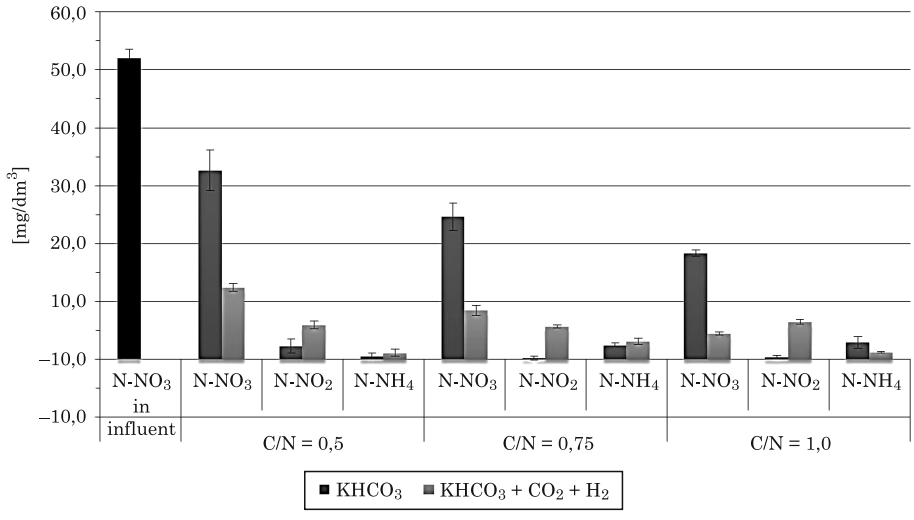

Fig. 3. Effect of C/N ratio on the concentration of mineral forms of nitrogen

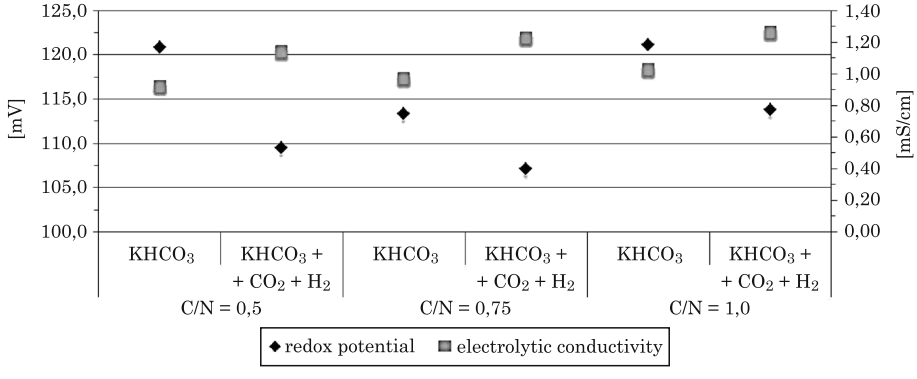

Fig. 4. Effect of C / N ratio to change the electrolytic conductivity and redox potential

Analogously to other studies (KURODA et al. 1996, KURODA et al. 1997, ZHOU et al. 2009), the COD value decreased (Figure 2). In reactor 2, organic substances were oxygenated by both microorganisms and hydroxyl radicals, generated by the oxygenation of water on the surface of the anode. During the flow of an electric current, the carbon electrode played the role of an electron acceptor in the process of external oxygenation of organic compounds, and was a source of CO_2 in the process of hydrogenotrophic denitrification. In raw sewage, organic compounds expressed by the COD index value, made up 70.71 mgO_2/dm^3. In the discharge from the control system (R1), the average concentration of COD at C/N = 0.5 was 28.68 (±9.61) mgO_2/dm^3; at the higher value of C/N = 0.75,

it fell to 22.93 (±4.81) mgO_2/dm^3, and for the highest C/N ratio of 1.0, it equalled 24.30 (±4.94) mgO_2/dm^3. A higher concentration of the discussed parameter was obserevd in the R2 system. At the C/N ratio of 0.5, it was 39.04 (±8.19) mgO_2/dm^3, it equalled 32.97 (±3.64) mgO_2/dm^3 at C/N = 0.75 and reached 35.53 (±7.80) mgO_2/dm^3 at the highest C/N ratio of 1.0.

Under the increased dose of potassium bicarbonate and availability of an additional source of carbon in reactor 2, such as carbon dioxide released from the oxygenation of a carbon electrode, an increase in the conductivity of wastewater and a large decrease in the redox potential appeared, which was in contrast to the process in reactor 1 (Figure 4).

A much larger decrease in the redox potential in reactor 2 was mainly caused by a much more intensive course of the denitrification process in that reactor. The higher conductivity was a result of the dissociation of potassium bicarbonate and hydrolysis of HCO_3^-:

$$KHCO_3 \rightarrow K^+ + HCO_3^- \quad (1)$$

$$HCO_3^- + H_2O \rightarrow H_2CO_3 + OH^- \quad (2)$$

$$H_2CO_3 + OH^- \rightarrow H_2O + HCO_3^- \quad (3)$$

$$HCO_3^- + OH^- \rightarrow H_2O + CO_3^{2-} \quad (4)$$

as well as the oxygenation of the carbon electrode. The carbon dixoide generated during the oxygenation of the anode underwent further dissociation according to the following reaction:

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad (1)$$

$$H_2CO_3 + OH^- \rightarrow H_2O + HCO_3^- \quad (2)$$

$$HCO_3^- + OH^- \rightarrow H_2O + CO_3^{2-} \quad (3)$$

As suggested by the course of the above reactions, amounts of soluble ions HCO_3^- and CO_3^{2-} in treated wastewater in R2 were higher than in R1.

Effect of inorganic carbon of C/N ratio and electric current density on the number of denitrifying bacteria (MPN), will be subject for further research.

Conclusions

1. An increase in the dose of inorganic carbon ($KHCO_3$) had a significant influence on the decrease in nitrate. In reactor 2, additionaly loaded with CO_2 and H_2, the concentration of nitrate was the lowest.

2. In neither of the reactors, nitrite was completely used up during the dissimilation reduction. A small amount of this compound was converted to the ammonium form on the assimilation pathway.

3. The substantial decrease in the discharge of total organic carbon available in raw sewage proves that some of the organic form of carbon has been converted to the inorganic form; it also indicates the participation of autotrophic and heterotrophic bacteria in the process of wastewater purification.

4. Increasing the C/N ratio of potassium bicarbonate ($KHCO_3$) as well as the presence of larger amounts of CO_3^{2-} and HCO_3^- ions originating from the dissociation of $KHCO_3$ and CO_2, prevented any larger decrease in the electrolytic conductivity in reactor 2.

5. The carbon electrode served as an acceptor of electrons during the process of oxygenation of organic compounds (COD).

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INFLUENCE OF FARMING TECHNOLOGY ON DRY MATTER CONTENT IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) MUSCLE TISSUE

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K e y w o r d s: aquaculture recirculation system, flow-through system, dry matter content, muscle tissue.

A b s t r a c t

Environmental and economic factors related to water exploitation have resulted in the development of recirculation techniques by trout fish farms. This study assesses the influence of farming technology on the content of dry matter in rainbow trout (*Oncorhynchus mykiss* Walbaum) muscles. Farming technology did not influence the dry matter content: mean content amounted to 25.7% in muscle tissue of trout caught in farms producing fish in recirculated water and 25.9% for fish from farms using flow-through water. Irrespective of the farming system, the catching season significantly influenced the dry matter content; in spring, dry content of muscle tissue of trout amounted to 25.3% and in autumn it was 26.3%. The place of catching (farm) influenced the dry matter content stronger in autumn than in spring. The kind of feed also influenced the dry matter content in rainbow trout muscle tissue depending on the season of catching.

WPŁYW TECHNOLOGII CHOWU NA ZAWARTOŚĆ SUCHEJ MASY W TKANCE MIĘŚNIOWEJ PSTRĄGA TĘCZOWEGO (*ONCORHYNCHUS MYKISS* WALBAUM)

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S ł o w a k l u c z o w e: system recyrkulacji akwakultury, układ przepływowy, zawartość suchej masy, tkanki mięśniowej.

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Abstrakt

Środowiskowe i ekonomiczne uwarunkowania wykorzystywania wody spowodowały rozwój technik recyrkulacyjnych na farmach pstrągowych. Oceniono wpływ technologii chowu na zawartość suchej masy w mięśniach pstrąga tęczowego (*Oncorhynchus mykiss* Walbaum). Technologia chowu nie miała wpływu na zawartość suchej masy: średnia zawartość suchej masy w mięśniach pstrągów odłowionych w gospodarstwach produkujących na wodzie recyrkulowanej wynosiła 25.7%, zaś 25.9% – w mięśniach ryb z farm stosujących jednokrotny przepływ wody. Niezależnie od systemu chowu, sezon odłowu znacząco wpływał na zawartość suchej masy; wiosną tkanka mięśniowa pstrąga zawierała 25.3% a jesienią – 26.3% suchej masy. Miejsce odłowu (farma) silniej wpływało na zawartość suchej masy jesienią, niż wiosną. Również rodzaj paszy miał wpływ na zawartość suchej masy w mięśniach pstrąga tęczowego w zależności od sezonu odłowu.

Introduction

Diminishing marine resources have resulted in an appreciable increase in fresh water organisms and aquaculture is nowadays becoming the most rapidly developing sector of the agriculture and food industry. In 2011, 64 million tons of fish were produced by aquaculture world-wide and this was 244% of the world;s production of beef meat, 141% of pork meat and 171% of poultry meat (FAO 2012, FAOSTAT). In Poland, in 2010, the production of fresh water fish amounted to 30,757 tons, together with 12,940 tons of trout (FAO 2010).

Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) was introduced to Poland from Canada at the end of the 19th century. Trout-breeding has been a subject of interest to fish-farmers and this is why trout, together with carp, became the most common fish in Polish aquaculture (TEODOROWICZ 2013, TKACZEWSKA and MIGDAŁ 2012). Trout is a cold water fish, living in a river environment. Clear and well oxidized water is a condition necessary for trout breeding. Rainbow trout production technology is based on breeding under near-natural conditions, using flow-through water technology (TURCHINI et al. 2004). Opened breeding facilities (FTS), based on single-flow-through water, consume on average 200–400 l of water per second for a production of 40 do 200 tons of trout per year (TURCHINI et al. 2004, LEVER et al. 2004, SINDILARIU et al. 2009). The kind of farming technology and environmental conditions are among the most important parameters determining the quality and nutritional properties of trout (ROQUE D'ORBCASTEL et al. 2008, SZCZEPANIK et al. 2011, TKACZEWSKA and MIGDAŁ 2012).

In countries with limited water resources, conserving water is necessary. In Poland, the EPRA project (Environmental Protection in Rural Areas) aims to reduce the nitrogen contribution to surface and ground waters from agricultural sources (MANTEUFFEL SZOEGE and SOBOLEWSKA 2004). Although minimizing of environmental contamination due to fish production is also desirable (ROQUE D'ORBCASTEL et al. 2008), a balance needs to be struck between

protecting the environment and employing profitable production technology. Increasing production yields is not possible when applying traditional methods of fish farming and meeting the strict demands of environmental protection. To lower production costs, producers are increasingly introducing water recirculation systems (RAS – Recirculation Aquaculture Systems) to re-use the water several times using filtration or chemical purification (DALSGAARD et al. 2013). However, Commission Regulation (WE) Nr 710/2009 (2009) lays down detailed rules on organic aquaculture production and does not allow the use of water recirculation in organic production until further studies are completed.

The aim of this study was to assess the effect of farming technology on dry matter content in rainbow trout muscle tissue.

Material and Methods

Material

Rainbow trout (*Oncorhynchus mykiss* Walbaum) was collected from six Polish farms: three of them produce fish by applying a flow-through water system and the other three farms use water recirculation (Fig. 1). Fish were fed

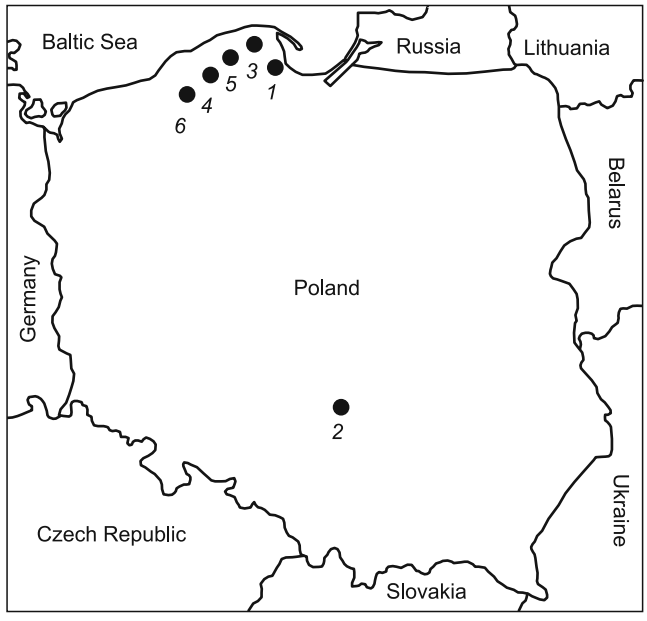

Fig. 1. Location of fish farms: *1, 2, 3* – farms with flow-through systems; *4, 5, 6* – farms with recirculation aquaculture systems

using feeds of similar composition (Tab. 1). The material consisted of 960 fish, i.e. 40 trout netted in each farm 4 times (terms of material collection is presented in table 2). At the collection point, the fish were killed after anaesthetization, washed, gutted, re-washed, packed into plastic bags and then transported into laboratory in ice. For analysis purposes, samples of muscle tissue (without skin and bones) of 5 cm width were cut out from the middle part of the fillet from the dorsal side to the abdominal side. Each sample was homogenized separately, frozen at -18°C and stored in plastic bags.

Table 1

Feed composition

Producer	Protein	FAT	Carbohydrates	Fibre	Ash
	%				
Agro-Fish	42	24	13.8	2	9
Aller	42	24	17	2	7
BioMar	40	20	18.2	3.8	6.0
Skretting	42	28	–	2.1	6.5

Determination of dry matter content

Dry matter content in rainbow trout muscle tissue was determined according to norm PN-ISO 1442:2000 in triplicates. The reference method on determination of water content in meat and meat products is grounded on drying the mixture of sample and sand at 103 ±2°C up to constant mass.

Statistical analysis

A statistical analysis was conducted using the STAT statistical software package (Statistica, Version 10.0). The average content of dry matter was determined at $p < 0.05$. The homogeneity of variances was examined using Levene's test. Non-parametric analysis (Mann-Whitney U test) was used to determine if there were differences in dry matter content, depending on the farming technology and season of catching. A test of the significance between the number of averages was performed using non-parametric analysis of multiple comparisons of means (Kruskal-Wallis). Spearman correlation coefficients were calculated to determine the strength and significance of the correlation.

Results

The dry matter (DM) content in the muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) from different farming technologies is presented in Table 2. Fish originating from RAS farms contained from 21.88% to 30.20% of dry matter and trout from FTS technology contained from 21.08% to 31.13%. No correlation between farming technology and dry matter content was found. The season of catching influenced DM, both on farms applying water recirculation and on farms with a flow-through system. The mean dry matter content in the muscles of trout netted in 2011 on RAS farms amounted to 25.95% and was significantly different from results obtained in the other years. Moreover, trout netted in the spring of 2012 in FTS farms contained a significantly higher content of dry matter compared to the fish netted on these farms in the other years.

Table 2

Terms of fish catching

Farm	I	II	III	IV
1	15.12.2010	05.06.2011	09.10.2011	13.05.2012
2	18.12.2010	04.06.2011	16.10.2011	05.05.2012
3	06.12.2010	22.05.2011	08.10.2011	02.06.2012
4	14.11.2010	11.07.2011	13.10.2011	20.05.2012
5	15.11.2010	18.06.2011	28.10.2011	03.06.2012
6	13.11.2010	12.06.2011	23.10.2011	20.05.2012

Table 3

Dry matter content according to the trout farming technology

Technology		RAS		FTS	
		Mean* + SD [%]	Min – Max [%]	Mean* + SD [%]	Min – Max [%]
		25.68 ± 1.335	21.88 – 30.20	25.88 ± 1.566	21.08 – 31.13
Year	2010	25.42 ± 0.978^{a}	23.16 – 29.20	26.08 ± 0.978^{a}	23.30 – 28.28
	2011	25.95 ± 1.422^{b}	21.97 – 30.20	26.11 ± 1.842^{a}	21.08 – 31.13
	2012	25.43 ± 1.369^{a}	21.88 – 29.30	25.21 ± 1.201^{b}	22.65 – 28.46
Season	Autumn	25.26 ± 1.322^{A}	21.88 – 30.20	25.38 ± 1.599^{A}	21.08 – 31.13
	Spring	26.11 ± 1.206^{B}	23.16 – 29.20	26.39 ± 1.402^{B}	23.07 – 30.33

* Values, in columns within the same groups (Technology, Season), marked with different characters are significantly different ($p < 0.05$).
RAS – recirculation aquaculture systems, FTS -technology with flow-through systems.

The mean content of DM of all the fish netted in autumn was 26.25% (Tab. 4) and was higher than in spring (25.32%). The collection site (farm) influenced DM more strongly in spring than in autumn; significant differences in the dry matter content of fish bred in different farms was found more often in spring than in autumn. The type of fish feed influenced DM content depending on the season of trout netting. There was no difference in DM content in trout netted in spring and fed with Biomar and Aller feed. The lowest (and significantly different) DM content in muscle tissue was determined in autumn in trout fed with Biomar.

In the FTS farms, the year of netting influenced muscle dry matter (-0.23, $p < 0.05$) the most and in the RAS farms it was the season (-0.32, $p < 0.05$) and type of feed (-0.24, $p < 0.05$). In spring, the DM content was slightly, but

Table 4

Dry matter content of trout, depending on the harvest season

Season	Spring		Autumn	
	Mean* + SD [%]	Min – Max [%]	Mean* + SD [%]	Min – Max [%]
	25.32 ± 1.447	21.08 – 31.13	26.25 ± 1.314	23.07 – 30.33
Farm				
1	25.44 ± 1.495^{a}	22.65 – 28.70	26.22 ± 1.424^{abc}	23.07 – 28.90
2	24.56 ±1.655^{bd}	21.08 – 31.13	26.56 ± 1.815^{abc}	23.33 – 30.33
3	26.18 ± 0.995^{ce}	24.05 – 29.35	26.38 ± 0.717a	24.74 – 28.23
4	25.19 ± 1.673^{a}	21.88 – 30.20	26.34 ± 1.188^{a}	23.90 – 29.20
5	24.71 ± 0.965^{d}	21.97 – 28.60	25.98 ± 1.346^{b}	23.16 – 28.77
6	25.88 ± 0.905^{e}	22.13 – 28.56	26.00 ± 1.036^{bc}	23.88 – 28.99
Feed				
Agro – Fish	26.53 ± 1.091^{b}	24.11 – 29.35	26.38 ± 0.717^{a}	24.74 – 28.23
Aller	25.18 ± 1.250^{a}	21.97 – 28.60	26.32 ± 1.290^{a}	23.07 – 29.20
BioMar	25.32 ± 1.672^{a}	21.08 – 31.13	25.02 ± 0.773^{b}	23.16 – 27.32
Skreting	25.14 ± 1.422^{c}	21.88 – 30.20	26.43 ± 1.576^{a}	23.20 – 30.33

* Values, in columns within the same groups (Technology, Year, Season), marked with different characters are significantly different ($p < 0.05$).
Agro-Fish, Aller, BioMas, Skreting – trade names of feed.

Correlation analysis confirmed the abovecorrelations between dry matter content and rainbow trout farming technology (Tab. 5). Regardless of farming technology, the most significant influence on DM content was the netting season (-0.31, $p < 0.05$). Farming technology did not influence DM content in trout muscle tissue. The year of sampling and feed type showed a small, yet statistically significant, correlation with dry matter (respectively, 0.10, -0.12, $p < 0.05$). No correlation was calculated between place (farm) of netting and DM content.

Table 5
Correlation of the dry matter content in trout depending on the technology and culture conditions

	Technology	Year	Season	Farm	Feed
Technology	0.07*	-0.10*	-0.31*	-0.01	-0.12*
RAS	–	0.03	-0.32*	0.06*	-0.24*
FTS	–	-0.23*	-0.30*	0.16*	-0.13*
Season					
Spring	0.07*	0.00	–	0.05*	0.00
Autumn	0.08*	0.38*	–	-0.08*	-0.17*
Farm					
1	RAS	-0.33*	-0.23*	–	0.24*
2	RAS	-0.11*	-0.53*	–	-0.14*
3	RAS	-0.32*	-0.12*	–	-0.31*
4	FTS	-0.04	-0.38*	–	-0.38*
5	FTS	-0.03	-0.48*	–	-0.10*
6	FTS	0.20*	-0.06	–	–

* Statistically significant correlations ($p < 0.05$).
RAS – recirculation aquaculture systems, FTS – technology with flow-through systems.
Aqua – Fish, Aller, Biomas, Prima-Skreting – trade names feed.

significantly, correlated with farming technology (0.07, $p < 0.05$) and also with the place of netting (0.05, $p < 0.05$). In autumn, the DM content was clearly significantly correlated with the year of trout sampling (0.38, $p < 0.05$), but only was only slightly significantly correlated with the other parameters.

Discussion

It is well-known that the content of dry matter depends on fish species. For example, POLAK-JUSZCZAK (2007) determined the DM content in various fish species: 39.68% – butter fish, Nile perch – 20.70%, African wels – 24.47%, panga – 17.18%. GRELA et al. (2010) found 21.6% of DM in carp meat, 20.39% in pike and 20.43% in zander. The authors stated that the content of nutrients depended on fish species, although they observed a tendency to increase DM content in the meat of carp and bream with netting prolongation. HARTMAN and MARGRAF (2008) found general relationships between the contents of dry matter and fat and protein, and suggested using the water content determination as a simple and economical predictor of body percent composition of rare species of fish.

The presented results of DM content in rainbow trout are similar to those reported by other authors. SKAŁECKI et al. (2013) determined DM content in rainbow trout from farms located in Lubelskie; the content was significantly different: 22.7% in fish of assortment S (fish of 350 – 500 g) and 25.4%

– D (501 – 800 g). The value of this parameter increased with fish size. WEATHERUP and MCCRACKEN (1999) also found a significant increase in DM content with trout age. TKACZEWSKA and MIGDAŁ (2012) analyzed samples of trout netted in fish farms situated in 4 regions of Poland (Malopolskie, Śląskie, Świetokrzyskie and Warmińsko-Mazurskie) and determined DM in a wide range (from 23.41% to 28.93%), depending on the farming system. SZCZEPANIK et al. (2011) analyzed changes in trout meat occurring during refrigeration storage; the dry matter content of trout bought in the Zachodniopomorskie provincial market (fresh fish stored in ice) amounted to 21.19%.

ÖZDEN (2005) determined 23.77% of DM in trout meat (*Salmo gairdneri*) bought at a market in Istanbul. UNUSAN (2007) analyzed trout originating from the Konya region, Turkey, located in the middle of Anatolia, netted in September, 1997. The mean dry matter content amounted to 28.71%. FALLAH et al. (2011) found from 24.92 to 26.08% of dry matter in meat of farmed trout and from 21.05 to 22.15% in wild ones. The authors concluded that the differences between farmed and wild fish DM contents resulted from fish diet composition and the environmental conditions of fish life.

Several works have confirmed the influence of trout feeding on dry matter content in fish meat. A feeding experiment was conducted by KENNETH et al. [2004] using four experimental feeds containing fats of different origin. At the beginning of the experiment, trout fillets contained 26% of dry matter and after 16 weeks this increased to 37-39% due to a considerable increase in both the protein and fat contents (the latter component doubled). GÜMÜŞ and YKIZ (2009) fed trout for 13 weeks with 4 diets equal in proteins and energy and different in carbohydrates and fats. The content of DM decreased with a decreased fat content (from 25.8 to 23.9%).

ZOCCARATO et al. [1994] conducted a twelve-week experiment to assess trout fillet composition depending on nutrition and fish density in pond. The dry matter content amounted to: 23.64 ±0.39% at low density and high feeding level (the highest results), 23.44 ±0.75% at high density and high feeding level, 22.74 ±0.67% at low density and low feeding level and 22.59 ±0.10% at high density and low feeding level. TURCHINI et al. (2004) proved that farming trout in an extensive system (rich fish community, not granulated feed) increased the general quality of the fish by improving the content and composition of fish fatty acids. HUNG and STOREBAKKEN (1994) proved that DM content in trout fillet fed continuously was lower than in fish fed four meals daily.

OO et al. (2007) showed that replacement of fish oil in trout fed by palm oil did not disturb fish growth but lowered dioxin contamination in trout fillet. SHAFAEIPOUR et al. (2008) also confirmed that applying plant oil (canola oil) instead of fish meal in rainbow trout feed did not affect the fish growth performance and could be successfully used in trout feed production. DEFRAN-

CESCO et al. (2004) did not find significant differences in DM contents in fillets of trout fed with fish meal addition (28%) and a mixture of plant protein sources (27%).

Conclusions

1. Farming technology did not influence the content of dry matter in the rainbow trout muscle tissue.

2. The season of fish netting significantly influenced the dry matter content; the content of dry matter in muscles of rainbow trout netted in autumn was higher than those collected in spring.

3. The place of netting (farm) more strongly influenced dry matter content in spring than in autumn.

4. The dry content of matter in muscles of fish caught in autumn significantly depended on kind of feed used for fish feeding.

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PRODUCTS OF BIOTRANSFORMATION OF TEA INFUSION – PROPERTIES AND APPLICATION

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K e y w o r d s: Kombucha, tea fungus, biotransformation, fermentation, chemical composition.

A b s t r a c t

Fermented tea broth (known as Kombucha) has been used for ages in many countries, especially in Japan, Russia, China and Eastern Europe. Nowadays, this beverage is generally regarded a universal natural medicament having a strengthening effect on the human body. Kombucha beverage is popular because of its favourable effect on human health. Its composition includes: B vitamins, C vitamin, mineral components and organic acids. It is believed that Kombucha decreases the risk of cancer, prevents circulation disorders, improves the function of the digestive system, mitigates inflammatory conditions and has a favourable effect on the skin, hair and nails.

The composition and properties of tea are well documented. Regrettably, the scientific information on the composition, effect on human body and properties of Kombucha is sparse. The goal of this paper is to present the properties and composition of Kombucha beverage as well as its biological activity and potential favourable effect on the human body.

PRODUKTY BIOTRANSFORMACJI NAPARU HERBATY – WŁAŚCIWOŚCI I ZASTOSOWANIE

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Abstrakt

Napój sfermentowanej herbaty (znany jako Kombucha) stosowany był od wieków w wielu krajach, szczególnie w Japonii, Rosji, Chinach i Europie Wschodniej. W dzisiejszych czasach uznawany jest jako środek naturalny wzmacniający organizm.

Kombucha zyskała popularność ze względu na potencjalny korzystny wpływ na organizm. Do głównych składników sfermentowanej herbaty należą: witaminy z grupy B, witamina C, związki mineralne i kwasy organiczne. Przypuszcza się, że Kombucha może obniżać ryzyko występowania nowotworów, zapobiegać zaburzeniom układu krążenia, poprawiać funkcjonowanie układu pokarmowego, łagodzić stany zapalne oraz korzystnie wpływać na skórę, włosy i paznokcie.

Skład i właściwości herbaty są dobrze udokumentowane. Doniesienia naukowe dotyczące składu, działania i właściwości Kombuchy są nieliczne. Celem pracy jest prezentacja właściwości i składu Kombuchy, jak również aktywności biologicznej oraz potencjalnego korzystnego wpływu na organizm.

Introduction

Tea has been known and appreciated in many countries and cultures for millennia. Presently, it is one of the most popular drinks worldwide. In China, it has been used already five thousand years ago, mainly as stimulating and detoxicating elixir (DUFRENSE and FARNWORTH 2000, BALENTINE et al. 1997). Recently many scientific reports have shown that tea has beneficial influence on human health and well-being (YEN et al. 1997). Tea brew regulates proper function of digestive system, strengthens walls of blood vessels, improves physical abilities and concentration (KUNTZE 2003).

Kombucha is acquired by fermentation process utilizing tea and sucrose. Despite common names, such as: Manchurian mushroom, tea fungus, tea beer, tea cider or Chinese mushroom, Kargasok tea (JARRELL et al. 2000, JAYABALAN et al. 2008, KURTZMAN et al. 2001) it is not fungus, but a symbiotic colony of multiple nonpathogenic bacteria and yeast (TEOH et al. 2004). Acetic acid bacteria synthesize bacterial cellulose (Fig. 1) in form of cream-colored or light-beige layers, also named microbial mats and biofilms (JARRELL et al. 2000, STAHL and CAUMETTE 1994).

The latter product of biotransformation occurs in form of slightly carbonated consumable broth and has specific sweet and sour taste (JAYABALAN et al. 2008). Its potential therapeutic properties have become the field of interest and research (MURUGESAN et al. 2009).

Development of knowledge about tea is a starting point for better understanding of Kombucha beneficial action. In popular medicine fermented tea drink was used by many cultures. It was claimed to be a universal wonderful drug, helping in many illnesses and strengthening organism. It was known as a potion which improves awareness and concentration, slimming, also purifying, regenerating and life extending (JARRELL et al. 2000, DUFRENSE and FARNWORTH 2000).

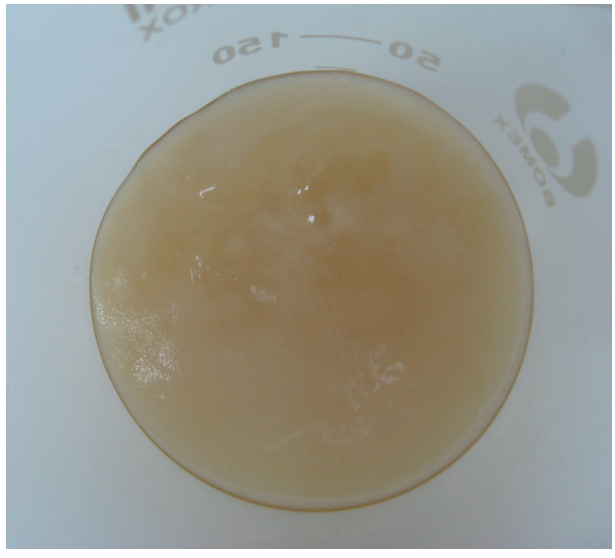

Fig. 1. Bacterial cellulose layer

There are theories in literature describing the origin of the tea mushroom. Kombucha drink is supposed to originate from the Far East (China) and the information on it dates back to 220 BC. According to some reports, Kombucha was brought from Korea to Japan in year 414 AD on the request of the Emperor by a Korean doctor named Kombu in order to treat the digestive problems of the Emperor (DUFRENSE and FARNWORTH 2000). It was brought to Russia by merchants from the Far East, later it was brought to Europe (HARTMANN et al. 2000).

It is possible that the name "Kombucha" originates from Japanese words "kombu", which means seaweed and "cha" that stands for tea. Different theory claims that the it may have connection with the name of previously mentioned Korean physician Kombu (JARRELL et al. 2000).

Kombucha mushroom and its beneficial therapeutic effect was again appreciated as late as at the beginning of the 20th century (HARTMANN et al. 2000). Presently, the tea mushroom is cultured in domestic conditions and passed from family to family.

The composition and properties of the tea are well documented. Regretfully, scientific reports on the composition, effect on the human body and properties of Kombucha are sparse. The aim of this paper is to review the properties and composition of Kombucha beverage as well as its biological activity and possibly favourable effect on the body.

The process of brewing Kombucha

The most common substrates used for Kombucha brewing are black tea and sucrose. The preparation is carried out in the following manner: base tea infusion usually made from black tea is enriched by sucrose. Next, the tea is cooled to room temperature and acidized by adding vinegar or readymade Kombucha beverage. Kombucha "mushroom" is inoculated into the surface area. The solution is left to ferment in room temperature for at least a week. The product contains carbohydrates, organic acids, vitamins and mineral components. Acetic acid bacteria is the most popular microorganism found in Kombucha. Through the process of yeast fermentation, sucrose is transformed into glucose and fructose (SIEVERS et al. 1995). Acetic acid stimulates production of ethanol which, in turn, may facilitate the growth of acetic acid bacteria and production of acetic acid (LIU et al. 1996). Acetic acid bacteria initially oxidises ethanol to acetic aldehyde and then to acetic acid. This bacteria also activate glucose oxidation to gluconic acid (DUFRENSE and FARNWORTH 2000).

Both ethanol and acetic acid (TEOH et al. 2004) have an antimicrobial and anti-pathogenic bacteria effect, providing protection against contamination of the tea mushroom. Besides, the fungi and bacteria present in Kombucha beverage as strong symbiotes are capable of inhibiting the growth of many potentially contaminating bacteria (LIU et al. 1996).

Despite the fact, that Kombucha drink is manufactured mainly in domestic conditions, its potential beneficial action brought much attention in scientific societies. More professional brewing methods with many variants have been incorporated.

The most common scenario can be found in (DUFRENSE and FARNWORTH 2000). As carbon source 50 g dm^{-1} of sucrose is used. Initial acidification can be achieved by either use of vinegar or previous Kombucha sample. Environmental conditions are observed, temperature is maintained between 20°C and 30°C for one to eight weeks of fermentation time.

Most researchers prove that fermentation cycle lasts about 7–10 days; after that time most of carbon sources are used up and all processes slow down (MALBAŠA et al. 2008, GOH et al. 2012(I), GOH et al. 2012(II)). On the other hand there are meaningful arguments towards sustaining prolonged fermentation even up to 60 days (CHEN and LIU 2000), (JAYASUNDARA et al. 2008).

Chemical composition and properties

Detailed knowledge of the composition and properties of tea is crucial for better understanding of Kombucha effect. Tea as a plant belongs to *Camel-*

liaceae family. The leaves are picked up from evergreen shrubs and may be processed using different methods.

Black tea is made from undeveloped leaf buds (*Theae nigre folium*) and the first (two or three-day) leaves which, after picking, are dried in the air and next, rolled or crushed in order to release enzymes, next left for fermentation and then dried. Green tea (*Theae viridis folium*) is obtained through the process of quick drying of the leaves after picking them, in order to inactivate enzymes (KUNTZE 2003).

The main tea components include: purine alkaloids (caffeine, theine, theobromine, theophylline); proantocianidinas and their esters; flavan derivatives (catechins, epicatechins); galotannins (esters of gallic acid with glucose); phenol acids; triterpene saponins (tea saponins); tea flavin yellow in colour, teaflagalin i tearubigenin red in colour (compounds formed during fermentation, absent in green tea); flavonoids (quercetin, kemperol, myricetin and their glycosides); mineral compounds (fluorine, potassium, magnesium and aluminium); aminoacids (theanine); volatile aromatic compounds (mainly teaspiran and other monoterpene, aldehydes, alcohols, ketones); carbohydrates; vitamins (ascorbic acid, B vitamins) (DUFRENSE and FARNWORTH 2000, action KUNTZE 2003).

The presence of alkaloids in black and green tea has influence on their stimulating effect, tannins however, cause constipating effect. Polyphenol compounds express an antioxidant, antivirus and antibacterial (KUNTZE 2003).

Kombucha beverage is consumed because of its favourable effect on health. The most important components, identified in the fermented beverage include: organic acids, mainly acetic acid, L-lactic acid, gluconic acid, glucuronic acid. The rich acid content is one of the most potent and desirable properties of Kombucha. Table 1 presents several measurement results acquired by different authors. Moreover, Kombucha contains B vitamins (B_1, B_2, B_3, B_6, B_{12}, folic acid), vitamin C, mineral components (ions of zinc, copper, iron and manganese), glucose, fructose, ethanol – summarized in Table 1 – (0,5–1,5%), glycerol, carbon dioxide, enzymes, gluconolactone, as well as tea catechines and caffeine (JARRELL et al. 2000, FRANK 1994, REISS 1987). The comparison of main mineral components between teas and Kombucha drink is presented in Table 2.

The main detoxicating compound present in Kombucha drink is gluconic acid. Its beneficial terapeutic action was described by many researchers (FRANK 1994, LONČAR et al. 2000). The antimicrobial effect of the drink is accredited to lactic acid (GREENWALT et al. 1998). Taste of Kombucha and its sensory properties derive from presence of alcohols, aldehydes, ketones, esters and amino acids (TEOH et al. 2004, JAYASUNDARA et al. 2008). Despite many studies and experiments conducted over the drink, there are many unidenti-

Table 1
Most common acids and ethanol contents for Kombucha tea in different days of fermentation cycle

Compound	[g/l]	Day	Growth conditions		Author
			sucrose [g/l]	black tea content [g/l]	
Acetic Acid	4.74	7	70	2.0	Velićanski A. (2013)
	2.44	9	100	1.2	Jayabalan R. et al. (2007)
	5.80	20	70	1.5	Sievers M. et al. (1995)
	5.00	14	100	4.0	Chen C. and Liu B. Y. (2000)
Ethanol	4.07	7	70	2.0	Velićanski A. (2013)
	5.50	14	100	4.0	Chen C. and Liu B. Y. (2000)
Glucuronic acid	1.69	9	100	1.2	Jayabalan R. et al. (2007)
	0.57	14	50	17.0	Talawat S. (2006)
	6.00	20	70	1.5	Sievers M. et al. (1995)

Table 2
Anion content comparison for Kombucha tea, Black tea and Green tea

Anion [mg g^{-1}]	Kombucha Tea	Black Tea	Green Tea	Authors
F^-	–	0.08	–	Alcazar et al. (2003)
	3.2	1.2	–	Kumar et al. (2008)
	–	0.06	–	Spiro et al. (1995)
Cl^-	–	0.6	–	Alcazar et al. (2003)
	–	–	1.78	Ding et al. (1997)
	0.96	3.12	–	Kumar et al. (2008)
	–	0.9	0.53	Spiro et al. (1995)
Br^-	0.04	0.04	–	Kumar et al. (2008)
NO_3^-	0.18	0.34	–	Kumar et al. (2008)
HPO_4^{-2}	–	2.93	–	Alcazar et al. (2003)
	–	–	7.88	Ding et al. (1997)
	0.04	0.08	–	Kumar et al. (2008)
	–	1.18	0.9	Spiro et al. (1995)
SO_4^{-2}	–	–	4.58	Ding et al. (1997)
	1.02	4.2	–	Kumar et al. (2008)
	–	1.45	2.13	Spiro et al. (1995)
I^-	1.04	0.44	–	Kumar et al. (2008)

fied compounds that have antibiotic properties (Jarrell et al. 2000). Full identification of ingredients of Kombucha is a difficult task because of multiple differences in substrate selection, way of preparation and routine of fermentation process (Blanc 1996).

The studies on the effect of Kombucha were mainly conducted by the scientists who performed chemical analysis of this beverage using high performance liquid chromatography (HPLC) (Chen and Liu 2000) and mass spectrophotometry. The results indicated that fructose, acetate and gluconic

acid were the basic components of the fermented tea. Researchers also found that the amount of vitamins present in Kombucha beverage was insufficient for dietary suppementation of the human body. Glucuronic acid was not detected in the studied samples. Steinkraus et al. however, stress that the results cannot be directly compared to other study results due to a significantly lower level of tea in the sample (STEINKRAUS et al. 1996). Total acid content was detected using titration with standard solution of sodium hydroxide with phenolphthalein as indicator (MALBAŠA et al. 2006).

The composition and concentration of the substances present in Kombucha beverage depend on the origin of tea mushroom, the amount of sucrose and the duration of fermentation process. Optimal concentration of ethanol and lactic acid in Kombucha is obtained after adding 50 g of sugar per one litre of the beverage (REISS 1994). It was also proved that the process of fermentation improves the synthesis of B vitamins and folic acid (DUFRENSE and FARNWORTH 2000).

Sucrose is metabolized into organic acids by bacteria and yeast which increases acidity of the beverage. The pH level decreases accordingly to increase of total organic acids content during fermentation (JAYABALAN et al. 2007, MALBAŠA et al. 2008, JAYASUNDARA et al. 2008).

One of the most promising and potent product of tea broth biotransformation is bacterial cellulose synthesized by *Gluconacetobacter xylinus* (former *Acetobacter xylinum*) strain (JARRELL1 et al. 2000). The cellulose has recently been used in production of medical dressing, known as "artificial skin" or "water mantle". Such dressing are utilized in case of hard healing wounds, for instance burnings (KUBIAK et al. 2009, FONTANA et al. 1991).

The US Food and Drug Administration had tested several samples of commercially available Kombucha. In result, neither pathogenic microorganisms nor other contamination were found (KURTZMAN et al. 2001, JAYABALAN et al. 2007, CDC Editorial Note 1996).

Biological activity and effect on the body

Kombucha is consumed not only because of its sensory values but also for multiple favourable effects on health (JAYABALAN et al. 2008). Kombucha is believed to decrease the risk of various types of cancer, prevent circulation disorders, improve digestive system function (in case of metabolic diseases), strengthen the immune system, mitigate inflammatory conditions and to have a favourable effect on skin, hair and nails (JAYABALAN et al. 2007). On the other hand however, only few among the described properties have been proved by the scientists in experimental studies (DUFRENSE and FARNWORTH 2000).

Starting from 1852 scientists, mainly from Europe, began more significant trials. The first meaningful reports come from Russia, from the beginning of the 20th century and the World War I when it was announced that the "Russian secret homemade remedy" also called a "Wonder drink" helped in headaches and digestive tract diseases and regulated intestinal function, often impaired due to exposure to stress in the army. In the years 1925–1950, several medical studies conducted by recognised physicians confirmed the traditional opinions about Kombucha beverage and its favourable effect was presented including: antibiotic properties, regulating digestive system and intestinal function and gland activity, bringing relief in rheumatism, arthritis and haemorrhoids, decreasing cholesterol level, preventing atherosclerosis, facilitating release of toxins and purifying blood, helping in neurosis and ageing-related problems (DUFRENSE and FARNWORTH 2000). Regretfully, methods used for these studies still remain unexplained. In 1951, the diagnostic poll conducted in the former Soviet Union by the Central Oncological Research Unit and the Russian Academy of Sciences in Moscow showed that there was a positive correlation between consumption of Kombucha and exceptionally high resistivity to cancer (JAYABALAN et al. 2007). Moreover, the study performed in 1960 confirmed the properties of Kombucha connected with cancer prevention, or its detoxicating effect; additionally, it was confirmed that regular consumption of Kombucha within a long period of time strengthens the immune system and increases interferon production. The properties of Kombucha presented by the Russians were next confirmed by the scientists from Switzerland, Germany and Holland (DUFRENSE and FARNWORTH 2000).

It has been suggested that consumption of Kombucha may regulate blood pressure, bring relief in rheumatoid arthritis, strengthen the immune system (popular with HIV-positives and sick with AIDS (TIMMONS 1994)), prevent cancer, mitigate hair greying, smooth wrinkles, reduce haemorrhoids, (JARRELL et al. 2000, FRANK 1994, STAMETS 1994–95, PETRO 1996). There are reports in literature on antibacterial properties of Kombucha beverage and its favourable effect on bacterial microflora, present in the human digestive tract. The antibacterial activity of Kombucha against *Helicobacter pylori* (being a frequent reason of digestive system disorders and gastric ulcers), *Escherichia coli, Staphylococus aureus* and *Agrobacterium tumefaciens* is supposed to be connected with acetic acid produced during fermentation process (STEINKRAUS et al. 1996), (TEOH et al. 2004). Black tea extracts, used in the same amount, did not show antibacterial effect.

Acetic acid is capable of inhibiting and destroying microorganisms if applied in proper concentration. It is assumed that acetic acid in the amount of only 1 g dm^{-1} inhibits the growth of pathogenic bacteria (ADAMS 1985). Many properties of Kombucha are connected with the acidic nature of the beverage.

The detoxicating effect probably results from the potential of binding toxin particles by glucuronic acid and increased excretion of these toxins by kidneys or intestines. Thanks to this, Kombucha may contribute to the elimination of redundant metabolic products and bring relief in conditions associated with accumulation of toxins in the organism, such as: rheumatism, arthritis or renal calculus (kidney stones) (DUFRENSE and FARNWORTH 2000). However, the presence of glucuronic acid in Kombucha beverage and formation of glucuronide complexes (glucuronic acid glycoside) is still open to discussion.

Recent studies indicate that the substance identified in Kombucha beverage as glucuronic acid is probably 2-keto-gluconic acid. A high level of glucuronides is formed in the urine of individuals drinking Kombucha, thus two explanations are possible. The first one suggests that the increase in glucuronide level is dependent on the consumption of glucuronic acid itself. The second suggestion indicates that the presence of glucuronides may be connected with the inhibition of strong β-glucuronidase by saccharic acid 1.4-lactone which is also present in Kombucha beverage (ROUSSIN 1999, WANG et al. 2010). This indicates that it is not glucuronic acid, but uridine diphosphate (UDP) glucuronic acid, the active form produced in the liver, that plays a role in detoxication processes (DUFRENSE and FARNWORTH 2000).

Positive influence of Kombucha on nervous system is probably connected with the presence of B vitamins (ROCHE 1998).

Laxative action can be caused by lactic acid content (REISS 1987). There are also assumptions that lactic acid bacteria may act in immunostimulatory way. However, according to our knowledge, there is no evidence of successful colonization of human digestive system by organisms derived from Kombucha (MARTEAU and RAMBAUD 1993).

Tea and Kombucha are presented in literature as two different beverages, having different properties. However, some effects of tea and Kombucha may be similar. The studies conducted after year 1945 by Russians among the population drinking Kombucha allowed them to observe a decreased prevalence of cancerous diseases (ROCHE 1998). These findings are probably not due to the presence of anticancer substances, not only in tea, but also in the fermented Kombucha beverage. However, the issue of transformation of tea components during the process of fermentation remains unexplained. Catechins, present in tea extract, are increasingly often known as the substances with a strong antioxidant, anti-atherosclerotic, anti-inflammatory, anti-cancerous and anti-diabetic effect (JAYABALAN et al. 2008, JAYABALAN et al. 2007). Therefore, we may assume that the above mentioned benefits of both tea and Kombucha consumption may be due to the presence of catechins in tea alone. However, catechin activity may be also modified by the chemical environment of the fermented beverage. Specific properties of the Kombucha beverage may

also result from the synergistic effect of its compounds. The described properties of Kombucha beverage, connected with strengthening of the immune and gastrointestinal systems, and metabolic function improvement, may be thus connected both with tea properties and the transformations occurring during the process of fermentation (DUFRENSE and FARNWORTH 2000).

Consumption of Kombucha drink usually does not cause harmful side-effects, but several intolerance cases were reported, such as digestion problems, allergic reactions (most likely connected with individual sensitivity to acids) and even kidney failure (DUFRENSE and FARNWORTH 2000). There are two reported cases of severe metabolic acidosis after admission of Kombucha (SRINIVASAN et al. 1997) one case of hepatotoxicity (PERRON et al. 1995), one case of skin reaction (SADJADI 1998), several cases of toxic gastrointestinal reactions and one probable fatal case (PERRON et al. 1995). However these reactions mechanisms have not been met and explained. There is an assumption that all above can be caused by domestic grow contamination (SRINIVASAN et al. 1997, KURTZMAN et al. 2001).

Conclusions

Despite the dynamic development of contemporary medicine, people still search new methods to improve their health and physical condition as well as to strengthen their bodies. The trend of healthy lifestyle and nutrition contributes to the growing interest in natural medicine. Kombucha beverage is consumed due to its favourable effect on health. Its composition includes B vitamins, C vitamin, mineral components (ions of zinc, copper, iron and manganese), acetic acid, lactate, gluconic acid and glucuronic acid.

Kombucha is described by its enthusiasts as a "miraculous medicament that cures every condition". It is supposed to eliminate grey hair, improve vision, purify the body and the strengthen immune system. It also provides protection against cancerous diseases. Consumption of Kombucha usually does not produce adverse side effects, however, some cases of intolerance were reported. These included digestive problems or allergic reactions (partly due to the predisposal to sensitivity to acids) and renal insufficiency. However, the mechanisms of these adverse reactions have not been explained so far. It should be emphasised that more studies are needed on the biological activity and safety of Kombucha consumption. All the components of the beverage should be identified, both tea components and the components of the fermented Kombucha beverage. Further studies are required as more information is also necessary for the explanation of the effect of Kombucha components within the human body. Although we need more studies on Kombucha

beverage effects, there are reasons to believe that its effect on the human organism is beneficial. Probably, the process of Kombucha beverage fermentation involves more complex processes, yet they have not been known and explained so far.

Translated by AUTHORS

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EFFECT OF STORAGE OF DILUTED CARBOXYMETHYLCELLULOSE ON RHEOLOGICAL PROPERTIES

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K e y w o r d s: carboxymethylcellulose, CMC, rheological properties.

A b s t r a c t

The study was aimed at determining changes in properties of CMC solutions over different storage times. Rheometric analyses were made for solutions with CMC concentrations 1%, 2%, 3%, 4% and stored between 24 and 120 hours. Courses of the flow curves were described by Ostwald-de Waele (O-dW), Herschel-Bulkley (H-B), Casson and Ellis models. The study showed that the best fitting of the curve to measuring points was provided by the power model. Changes in the rheological properties of diluted CMC were, therefore, evaluated based on the analysis of differences in shearing stress in the samples described with the O-dW model. Analyses demonstrated that the value of the estimated differences was changing from 0.51 Pa in the case of the 1% solution to 5.1 Pa in the case of the 4% solution, sheared at a shear rate of 72.9 s^{-1}. It seems that such minute changes in the properties of the solutions enable their application for research analyses for a period of at least five days.

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S ł o w a k l u c z o w e: karboksymetyloceluloza, CMC, właściwości reologiczne.

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Abstrakt

Wodny roztwór soli sodowej karboksymetylocelulozy (CMC) jest tanim i łatwo dostępnym płynem modelowym, zdolnym zastąpić podczas badań wiele rzeczywistych wyrobów spożywczych, symulować ich przepływ, badać pracę maszyn, projektować technologie. Celem pracy było określenie zmian właściwości roztworów CMC zachodzących w czasie jej przechowywania. Badaniom reometrycznym poddano roztwory wodne CMC o stężeniu 1, 2, 3 i 4%, magazynowane w czasie od 24 do 120 godzin. Przebiegi krzywych płynięcia oparte o średnie wartości naprężenia stycznego wyznaczonego przy rosnącej i malejącej szybkości ścinania opisano modelami Ostwalda-de Waele (O-dW), Herschela-Bulkleya (H-B), Cassona i Ellisa. Stwierdzono, że wyznaczone stałe równań H-B i Ellisa nie spełniają warunków stosowalności modeli, a spośród dwóch pozostałych, dokładniejsze dopasowanie krzywej do wyników pomiarów zapewnia model potęgowy. Zmiany właściwości reologicznych roztworzonego CMC oceniano więc w oparciu o analizę różnic wartości naprężenia stycznego w próbkach opisanych modelem O-dW, przechowywanych w czasie 24h i 120h, przy szybkości ścinania 72,9 s^{-1}. Stwierdzono, że wartość oszacowanych różnic zmieniała się od 0,51 Pa w przypadku roztworu o stężeniu 1% do 5,1 Pa w przypadku roztworu o stężeniu 4%. Wydaje się, że tak małe zmiany właściwości magazynowanych roztworów CMC umożliwiają ich wykorzystanie do badań przez okres co najmniej pięciu dni od chwili roztworzenia CMC.

Introduction

The difficulties faced when investigating real-life manufacturing processes in the food industry frequently arise from limited access to the product, its complex physical or chemical properties, changes occurring during the process and its price. The properties of non-Newtonian liquid products, which are the most commonly used in food industry, depend on the duration and type of processing, type and component contents in a mixture, temperature, pressure, etc. Hence, if it is possible, a real product is replaced with a model agent with similar properties at the study stage. One of the most commonly used agents is an aqueous solution of carboxymethylcellulose sodium, known under its technical name as CMC (JAWORSKI and KILJAŃSKI 2005). CMC is an easy and commonly available product, which can be used in experiments as a replacement for such foodstuffs as yoghurt, cottage cheese (LIMANOWSKI 2001), buttermilk (BUTLER and MCNULTY 1995), orange juice (TELIS-ROMERO et al. 1999) and many others. It can be used to simulate liquid flow in aseptic processes (FAIRHURST and PAIN 1999) or to investigate the operation of such machines and devices as extruders (POULESQUEN and VERGNES 2003a, 2003b), heat exchangers (ALCAIRO and ZURITZ 1999), separators (ALKHADDAR 2001). A CMC suspension is prepared by dissolving powder in water and waiting some time for it to swell. Despite the common use of CMC in experiments, the literature does not provide many reports on a safe time of use for the solution after dissolving the powder, so that it does not falsify the measurement results as a consequence of changes caused by ageing. This is important because in prolonged experiments, and when an agent with unchanged properties has to

be used, it is common practice to prepare larger amounts of a solution to be used in consecutive measurements.

The aim of the study was to determine the course and range of change of properties of carboxymethylcellulose dissolved in water, which take place during the period of its storage, and to find out whether it is possible to use stored CMC solution for experiments.

Materials and experimental methods

Solutions were prepared from powdered CMC of the AS-90/2 type, with the trade name of Jelocel-S, produced at Jeleniogórskie Zakłady Chemiczne Jelchem (PL). According to the data provided by the manufacturer, the chloride content in the product was less than 19%, and the viscosity of a 2% solution of CMC should not be lower than 70 mPa · s. Such information is not complete because it does not mention the measurement temperature or the range of the changes of shear rate at which the viscosity was determined. It was also found that the data provided in the control certificates for different CMC batches differed significantly. Therefore, in order to avoid errors, all the basic measurements of rheological properties of solutions were conducted on material from the same production batch.

CMC, which is poorly soluble in cold water, was dissolved in water heated up to about 60°C (ABDELRAHIM et al. 1994) with a rotary agitator. Subsequently, the solution was transferred to a larger vessel and made up with water. The solution was additionally stirred for about 20 minutes. The tank with the prepared solution was covered tightly from the top in order to prevent water evaporation and drying of the film formed on the walls. The solution was left for 24 hours for CMC to swell. Before the rheometric measurements were conducted, the solution was stirred briefly again and brought to a constant temperature of about 15°C. Two hundred liters of solution were prepared at a time.

In order to demonstrate the effect of time of storage of CMC solution on its properties, flow curves were plotted for solutions at 1%, 2%, 3%, and 4% stored for 24, 48, 72, 96 and 120 hours after the powder was dissolved. Shear stress was measured with a Rheotest-2 rotary rheometer with an S/S1 cylinder system, at an increasing and decreasing shear rate, ranging from 1.5 to 656 s^{-1}. The measurements were conducted at 15°C (FAIRHURST and PAIN 1999). The flow curves were analyzed by three of the most highly valued mathematical models used to estimate the properties of pseudoplastic liquids (BAILEY and WEIR 1998):

Ostwald-de Waele: $$\tau = K \cdot \dot{\upsilon}^n \quad (1)$$

Herschell-Bulkley: $$\tau = \tau_0 + K \cdot \dot{\upsilon}^n \quad (2)$$

Casson: $$\tau^{1/2} = \tau_0^{1/2} + (\eta \cdot \dot{\upsilon})^{1/2} \quad (3)$$

where:
τ – shear stress, Pa
τ_0 – yield stress, Pa
$\dot{\upsilon}$ – shear rate, s^{-1}
η – viscosity, $Pa \cdot s$
K – consistence coefficient, $Pa \cdot s^n$
n – flow behaviour index,

and the less-commonly used Ellis model, regarded as one of the most precise models which describes the flow of foodstuffs – pseudoplastic liquids (HOLDSWORTH 1971):

$$-\dot{\upsilon}^n = (\varphi_0 + \varphi_1 \cdot |\tau|^{\alpha-1}) \cdot \tau \quad (4)$$

gdzie:
φ_0 – equation constant, $m^2 \cdot s^{-1} \cdot N^{-1}$
φ_1 – equation constant, $m^{2\alpha} \cdot s^{-1} \cdot N^{-\alpha}$
α – equation constant

There are always three positive constants in this model φ_0, φ_1 and α. When $\alpha>1$ and at low values of the shear stress p and at $\alpha<1$ and at high values of τ, the behavior of a real liquid is approximated by the behavior of a non-Newtonian liquid. In extreme cases, the model describes a Newtonian liquid ($\varphi_1 = 0$) or a power-law liquid ($\varphi_0 = 0$). The Ellis equation was used in measurements of the properties of aqueous solutions of CMC, for example by PAVEZ 2002 and LAREO and FRYER 1998 found the model to be capable of describing the flow curve of a CMC solution more precisely than the power-law model.

Changes in the properties of CMC solutions caused by their storage were evaluated by comparing the differences between the values of a shear stress in samples stored for 24 hours and for 120 hours, sheared at a shear rate of 72.9 s^{-1}, which is the closest to feeling the consistency and texture of food by humans.

Results and Discussion

An analysis of the flow curves of the solutions under study showed that the maximum differences in shear stress between part of the hysteresis determined at an increasing shear rate and the part of the hysteresis determined at a decreasing shear rate occurred at shear rate exceeding 121.5 s^{-1}. This means that both parts of the curve were nearly coincident in the area responsible for human organoleptic sensations and, hence, there is no need to consider it as a curve specific to thixotropic liquids. The maximum difference in shear stress of 11.02 Pa, i.e. 5.33% of the stress measured at an increasing shear rate, was found for the 24-hour solution at a concentration of 4%, and only a slightly smaller difference, of 5.16%, was found for the 4% solution stored for 120 hours. Similar stress differences were found for solutions at smaller concentrations. In a 1% solution, the differences ranged from 0.73% to 3.72%, in a 2% solution they ranged from 1.57% to 2.92%, 3% – from 0.94% to 1.96% and in a 4% solution they ranged from 2.40% to 5.33%. Fig. 1 shows the flow curves for the solutions with the widest hysteresis in the group of solutions with the same concentration. The differences were not found to depend significantly on the duration of the storage period. This was the grounds for the conclusion that the measured shear stress differences are sufficient to estimate the rheological properties of CMC solutions based on mean values of stress determined at growing and decreasing shear rate.

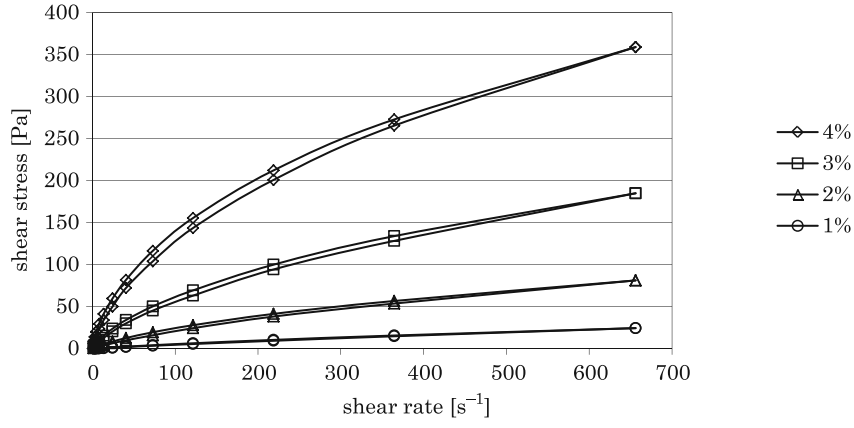

Fig. 1 Flow curves hysteresis for solutions with the greatest difference between the shear stress curves designated with increasing and decreasing shear rate

All of the analyzed flow curves have confirmed the non-Newtonian character of the solutions and the features typical of shear-thinned liquids (FERGUSON and KEMBŁOWSKI 1995, ILLICALI and ENGEZ 1996, PRUSOV et al. 2003,

STEFFE 1992). Attempts at describing the curves with model solutions 1–4 have shown that the highest values of correlation coefficients and the most exact fitting of model solutions to the measurement results was achieved by using Ellis and H-B models (Table 1–4). In both cases, the majority of the values of the yield stress τ_0^{HB} and constant a were smaller than zero, which is contrary to

Table 1

Results of the estimation of flow curves of a stored 1% aqueous solution of CMC

Model	Constants, correlation coefficient R	Storage time				
		24h	48h	72h	96h	120h
Ostwald-de Waele	K, Pa · s^n	0.07204	0.08192	0.08064	0.08617	0.08967
	n	0.88320	0.86544	0.87487	0.86306	0.86424
	R	0.99917	0.99925	0.99926	0.99928	0.99922
Herschel--Bulkley	τ_0^{HB}. Pa	-0.38998	-0.39625	-0.40191	-0.40674	-0.43931
	K, Pa · s^n	0.09858	0.11157	0.10917	0.11695	0.12279
	n	0.83670	0.81968	0.82998	0.81780	0.81767
	R	0.99974	0.99980	0.99979	0.99981	0.99979
Casson	τ_0^C, Pa	0.06189	0.08896	0.07872	0.09634	0.09803
	η_C, Pa · s	0.03073	0.03054	0.03227	0.03155	0.03315
	R	0.99805	0.99790	0.99805	0.99789	0.99782
Ellis	φ_0, m^2 · s^{-1} · N^{-1}	23.3980	21.5050	21.4930	20.559	19.814
	φ_1, m$^{2\alpha}$ · s^{-1} · N$^{-\alpha}$	0.10985	0.02829	0.14080	0.29065	0.21861
	α	2.32440	2.07870	-0.22946	-0.055	2.10584
	R	0.99985	0.99988	0.99989	0.99989	0.99988

Table 2

Results of the estimation of flow curves of a stored 2% aqueous solution of CMC

Model	Constants, correlation coefficient R	Storage time				
		24h	48h	72h	96h	120h
Ostwald-de Waele	K, Pa · s^n	0.90727	0.92004	0.91859	0.88395	0.89530
	n	0.69720	0.69331	0.69740	0.70268	0.69538
	R	0.99939	0.99910	0.99923	0.99935	0.99906
Herschel--Bulkley	τ_0^{HB}, Pa	-1.7035	-2.0244	-1.7553	-1.7546	-2.0339
	K, Pa · s^n	1.2017	1.2801	1.2206	1.17943	1.25415
	n	0.65592	0.64488	0.65567	0.66032	0.64596
	R	0.99986	0.99977	0.99972	0.99985	0.99975
Casson	τ_0^C, Pa	2.11427	2.12321	2.13868	2.04337	2.05737
	η_C, Pa · s	0.09373	0.09250	0.09505	0.09531	0.09151
	R	0.99398	0.99299	0.99363	0.99400	0.99297
Ellis	φ_0, m^2 · s^{-1} · N^{-1}	2.0006	2.7391	2.5186	2.4555	2.8244
	φ^1, m$^{2\alpha}$ · s^{-1} · N$^{-\alpha}$	0.25023	0.08693	0.12012	0.01531	0.08639
	α	0.28292	0.06439	0.14292	0.01935	0.05874
	R	0.99992	0.99998	0.99993	0.99996	0.99998

Table 3

Results of the estimation of flow curves of a stored 3% aqueous solution of CMC

Model	Constants, correlation coefficient R	Storage time				
		24h	48h	72h	96h	120h
Ostwald-de Waele	K, Pa · s^n	3.03494	2.96513	3.19242	3.23465	3.20138
	n	0.62986	0.63313	0.62673	0.62444	0.62633
	R	0.99926	0.99938	0.99918	0.99925	0.99916
Herschel--Bulkleya	τ_0^{HB}, Pa	-4.6786	-4.368	-5.1657	-4.9151	-5.1889
	K, Pa · s^n	4.14298	3.9816	4.43925	4.42778	4.45585
	n	0.58451	0.59014	0.57873	0.57872	0.57820
	R	0.99985	0.99990	0.99985	0.99985	0.99983
Casson	τ_0^C, Pa	7.24586	7.09933	7.59413	7.71615	7.61457
	η_C, Pa · s	0.18564	0.185993	0.19071	0.18959	0.19064
	R	0.99055	0.99109	0.99013	0.99023	0.99005
Ellis	φ_0, $m^2 \cdot s^{-1} \cdot N^{-1}$	0.83424	0.75964	0.80721	0.79951	0.81431
	φ_1, $m^{2\alpha} \cdot s^{-1} \cdot N^{-\alpha}$	0.01755	0.02598	0.015	0.01491	0.0143
	α	0.017192	0.087903	-0.00174	-0.0041	-0.01046
	R	0.99998	0.99998	0.99999	0.99998	0.99998

Table 4

Results of the estimation of flow curves of a stored 4% aqueous solution of CMC

Model	Constants, correlation coefficient R	Storage time				
		24h	48h	72h	96h	120h
Ostwald-de Waele	K, Pa · s^n	9.20391	9.8909	9.93985	9.51051	10.0187
	n	0.56257	0.553285	0.55528	0.56010	0.55465
	R	0.99866	0.99863	0.99853	0.99856	0.99879
Herschel--Bulkley	τ_0^{HB}, Pa	-14.205	-14.962	-15.535	-15.203	-14.196
	K, Pa · s^n	13.7803	14.9024	15.1116	14.4783	14.7187
	n	0.50456	0.49450	0.49520	0.49976	0.49943
	R	0.99968	0.99968	0.99964	0.99967	0.99972
Casson	τ_0^C, Pa	21.1573	22.5749	22.7003	21.782	22.9543
	η_C, Pa · s	0.32977	0.32861	0.33575	0.33421	0.33627
	R	0.98469	0.98393	0.98380	0.98420	0.98455
Ellis	φ_0, $m^2 \cdot s^{-1} \cdot N^{-1}$	0.34327	0.32913	0.36354	0.34996	0.30194
	φ_1, $m^{2\alpha} \cdot s^{-1} \cdot N^{-\alpha}$	0.86e-3	0.66e-3	0.41e-3	0.64e-3	0.87e-3
	α	-0.2828	-0.3234	-0.39585	-0.32444	-0.26918
	R	0.99996	0.99997	0.99993	0.99997	0.99997

the conditions of the model usability. The results of attempts at describing the curves with the other two equations indicated that a more exact fitting can be achieved with the power-law model. Correlation coefficients lower than 0.999 were achieved only for the 4% solution. The others were even higher. A less exact fitting was achieved with Casson's model. The description indicated the yield stress (21÷23 Pa) at the correlation coefficient ≅ of 0.984. In consequence,

the curves were described by the O-dW model. A comparison of the constants which described the solutions under study revealed that a higher content of CMC in a solution corresponds to a higher consistency coefficient and a lower value of the flow behavior index. This means that as the concentration of a solution grows, its consistency is stiffer and the solution is less susceptible to flowing.

Table 5
Standard deviations values for the constants in the CMC solution flow curve equations according to the Ostwald-de Waele involution model

CMC	K $Pa \cdot s^n$	σ_K $Pa \cdot s^n$	n	σ_n
1% 24h	0.072	0.009	0.883	0.019
1% 48h	0.082	0.009	0.865	0.018
1% 72h	0.081	0.009	0.875	0.018
1% 96h	0.086	0.009	0.863	0.017
1% 120h	0.090	0.010	0.864	0.018
2% 24h	0.907	0.065	0.697	0.012
2% 48h	0.920	0.081	0.693	0.015
2% 72h	0.919	0.075	0.697	0.013
2% 96h	0.884	0.067	0.703	0.012
2% 120h	0.895	0.081	0.695	0.015
3% 24h	3.035	0.209	0.630	0.012
3% 48h	2.965	0.189	0.633	0.011
3% 72h	3.192	0.230	0.627	0.012
3% 96h	3.235	0.222	0.624	0.011
3% 120h	3.201	0.234	0.626	0.012
4% 24h	9.204	0.729	0.563	0.013
4% 48h	9.891	0.772	0.553	0.013
4% 72h	9.940	0.807	0.555	0.014
4% 96h	9.511	0.775	0.560	0.014
4% 120h	10.027	0.737	0.555	0.012

The results of error analysis for the consistency coefficient and the flow behavior index for all the CMC solutions under analysis described by the model equation O-dW are shown in table 5.

Changes in the properties of the stored CMC solutions were evaluated by analyzing the shear stress differences in samples stored for the shortest and the longest periods (Fig. 2A). It was found that the difference in the stress for a 1% solution stored for 24 hours and 120 hours was only 0.51 Pa, but it accounted for up to 16.7% of the reference stress, i.e. the stress in a solution

stored for 24 hours. Such a large difference was a consequence of the low absolute value of the reference level (3.06 Pa). As the solution concentration grew, the shear stress and the differences between the shear of the 24h and 120h solutions increased. The reference stress for 2% solutions was 18.09 Pa and the difference was 0.57 Pa, i.e. 3.1% of the reference value. The shear stress for 3% solutions after 24 h storage was 45.87 Pa and the difference was 1.76 Pa, i.e. 3.8% of the reference value. The reference stress for a 4% solution was 104.90 Pa, and the stress in the solution after 120 h storage was 110 Pa. The difference accounted for 5.1% of the stress in the 24-hour solution. It is noteworthy that the shear stress in all the solutions stored for a long time was larger than the reference stress (Fig. 2B).

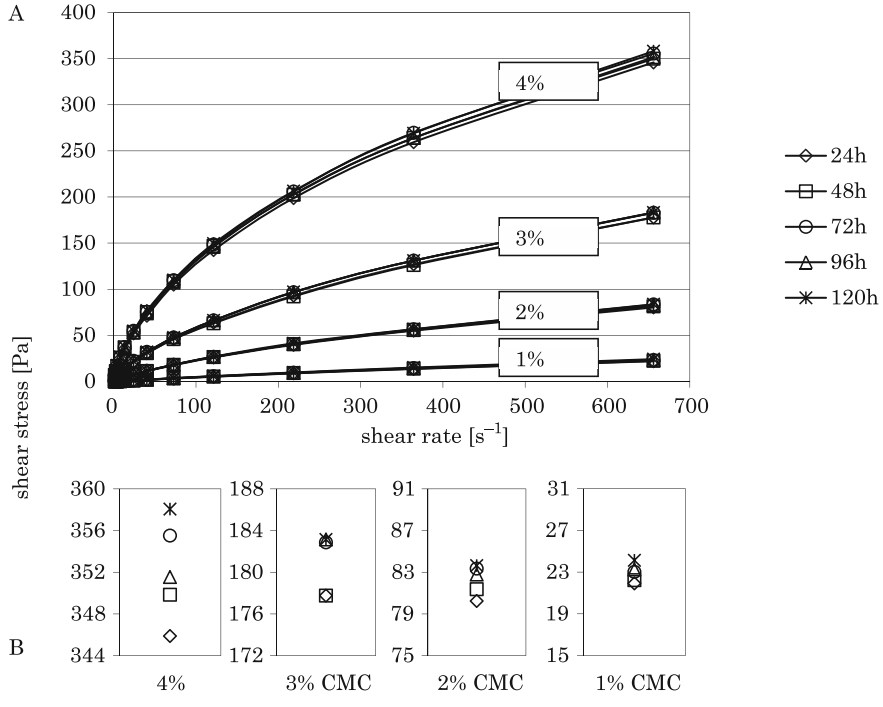

Fig. 2 *A* – Flow curves for stored CMC solutions, *B* – Distribution of measuring points at a shear rate 656 s^{-1}

This means that the storage time was a factor which strengthened the suspension structure and increased the shear stress. Only in the case of the 3% solution was the distribution of the measurement points not as distinctly proportional as for the other solutions, although this also shows the general tendency for changes in stress as a function of shear time. Therefore, it seems that aqueous solutions of CMC at concentrations below 4%, stored for

120 hours after dissolving powdered CMC can be used for experiments without fear of errors from changes in CMC properties caused by a considerable time of storage, provided that a measurement error of about 5% is accepted. This should be regarded as very convenient in planning and performing model studies of manufacturing processes in the food industry.

Conclusions

The hysteresis of the flow curve for aqueous solutions of carboxymethylcellulose at concentrations ranging from 1% to 4% can be replaced with a single curve based on mean values of shear stress determined at increasing and decreasing shear rate.

The calculated constants for Herschel-Bulkley and Ellis equations, used to describe the flow curves for the investigated solutions of CMC do not meet the conditions of the model usability. The best fitting of the curve to the measurement results can be achieved with the Ostwald-de Waele power-law model.

The difference between the shear stress in samples of CMC solutions stored for 24 hours and 120 hours, sheared at a shear rate of 72.9 s^{-1}, changed from 0.51 Pa in a 1% solution to 5.1 Pa in a 4% solution which is about a 5% change compared to the initial stress value. Such small changes in the properties of CMC solutions make it possible to use them for model studies of food products for a period of at least five days after powdered CMC is dissolved.

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DETERMINANTS OF COMPETITIVENESS LEVEL OF REFRIGERATED TRANSPORT SERVICES COMPANIES

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K e y w o r d s: competitiveness, services, refrigerated transport, foods.

A b s t r a c t

Transportation services can be evaluated from the perspective of a number of criteria. One aspect of the evaluation is the competitiveness of companies who provide the services. The most important factors determining the level of competitiveness of enterprises are discussed in reference books, while there are no tests determining their hierarchy of importance and their assessment for companies associated with the realization of transport function in the controlled temperature conditions.

The purpose of this study is defining the importance and assessing the main factors determining the level of competitiveness of transport companies.

The quantitative study was conducted using survey methods based on a questionnaire. Entities participating in the study were customers using refrigerated transport services. Among them were producers and distributors of food products, supermarkets and specialist grocery stores.

The study allowed for distinguishing two groups of importance of the competitiveness determinants, and determined the relationship between the level of selected assessed factors and the parameters characterizing the companies acquiring services.

DETERMINANTY POZIOMU KONKURENCYJNOŚCI PRZEDSIĘBIORSTW ŚWIADCZĄCYCH USŁUGI W ZAKRESIE TRANSPORTU CHŁODNICZEGO

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Abstrakt

Usługi transportowe mogą być oceniane z punktu widzenia wielu kryteriów. Jednym z aspektów ich oceny jest konkurencyjność przedsiębiorstw je świadczących. W literaturze przedmiotu omawiane są najważniejsze czynniki decydujące o poziomie konkurencyjności przedsiębiorstw, natomiast brak jest badań określających hierarchię ich ważności oraz ich oceny w zakresie przedsiębiorstw związanych z realizacją funkcji transportowej w warunkach kontrolowanej temperatury.

Celem niniejszej pracy jest określenie ważności i ocena głównych czynników decydujących o poziomie konkurencyjności przedsiębiorstw świadczących usługi transportowe.

Prowadzone badania miały charakter ilościowy z wykorzystaniem metod badań ankietowych w oparciu o kwestionariusz ankiety. Podmiotami biorącymi udział w badaniach byli klienci korzystający z usług w zakresie transportu chłodniczego. Należeli do nich producenci i dystrybutorzy produktów żywnościowych oraz hipermarkety i branżowe sklepy spożywcze.

Przeprowadzone badania pozwoliły na wyróżnienie dwóch grup ważności determinant konkurencyjności oraz określenie zależności poziomu wybranych ocen czynników od parametrów charakteryzujących przedsiębiorstwa nabywające usługi.

Introduction

Transportation services can be evaluated from the perspective of a number of criteria e.g. the level of competitiveness. The competitiveness of an enterprise is defined as *"a set of characteristics determining the attractiveness of given goods, services or the economy as a whole, and is mostly affected by price, performance characteristics or quality"* (Encyklopedia 2010, p. 60).

The most important factors determining the level of company competitiveness, associated with realizing transport functions, according to P. Romanov include: the credibility of the company (recommendations from other customers, the time of presence in the market), reliability of deliveries (promptness, accuracy, completeness), used means of transport (transport, reloading), transport route (distance), frequency of carriage, size of freight, delivery cost (price) and the level of alternative costs, (GOŁEBIOWSKI 1994, ROMANOW 2003).

According to KOŹLAK (2008), basic factors allowing the TSL sector companies to obtain an advantage over competitors can be: the level of company costs, its technological level (modern fleet, availability and quality of used infrastructure, IT systems), qualifications of staff, organizational efficiency and marketing strategies. BRDULAK (2009) in the research conducted on "The perfect TSL company profile" perceived by the customer takes into account such factors as: quality of service, terminal, fleet, customer service, promotional activities, prices and reliability of the company.

The aim of this study was to determine the importance and assess the main factors determining the competitiveness level of transport services. The scope of research included the evaluation of: the importance of competitiveness determinants, development of the level of service prices, quality of services provided, and the impact of co-operation with the service provider on the company satisfaction.

Material and Methods

Entities participating in the study were customers using the refrigerated transport services within the country. Among them were producers and distributors of food products, supermarkets and specialist grocery stores. For research purposes, a total of 206 questionnaires were obtained, by e-mail and the environmental method, from customers using these services.

Among the surveyed companies, manufacturers and distributors of food products constituted the largest group of more than 76%, while hypermarkets made up over 11%, and grocery stores over 12%.The largest number of companies, almost 52%, indicated that they operated in international markets; the smallest group of respondents included local-range companies (over 11%).

Research concerning competitiveness determinants in a transport company was of quantitative nature. A survey method based on a questionnaire prepared in accordance with official guidelines was employed in the quantitative survey (OPPENHEIM 2004, SAGAN 2004, COHEN et al. 2000).

Results obtained in the survey were subject to a statistical analysis. In order to test the significance of differences between the two dependent measurements the Wilcoxon signed-rank test was used, while the significance χ^2 test was used for comparison of groups containing the quantitative variables (ACZEL 2006, SOBCZYK 2002).

Results and Discussion

In the carried out research concerning the importance of competitiveness determinants of competitiveness, the factors proposed by ROMANOW (2003) were taken into account.

Table 1 shows the average assessment of the importance of factors determining the competitiveness level of a company providing transport services of products requiring controlled temperature.

The most important factors determining the level of service provider competitiveness, according to percentage rates of both high and very high importance factors, were: the cost of delivery, the company credibility and reliability of supply (81.6–83%). However, for the used means of transport the indicated share was about 74%, and for the remaining four factors the very high and high importance indicators were in the range between 62–67%.

The resulting ranking of the importance of leading features resembled the characteristics presented in the "Profile of ideal company providing logistics services" as seen by the customer. The results obtained by BRDULAK (2009) indicate that the "ideal company profile" TSL as perceived by the client in

Table 1

Assessment of the importance of factors determining a company competitiveness level

Factors determining the level of competitiveness of a company	% of indications						
	average evaluation of an importance (pt.)	not important (0 pts.)	very small importance of factor (1 pts.)	small importance of factor (2 pts.)	average importance of factor (3 pts.)	high importance of factor (4 pts.)	very high importance of factor (5pts.)
Company credibility	4.12	0	0	1.94	15.53	50.97	31.55
Reliability of supply	4.18	0	1.94	2.91	13.59	38.35	43.20
Means of transport used	3.93	0	0.49	2.43	23.30	51.46	22.33
Transport route	3.80	0	2.91	1.46	28.64	46.60	20.39
Frequency of carriage	3.77	0	0.49	3.88	33.98	40.78	20.87
Size of freight	3.76	0	0.49	6.80	29.61	42.23	20.87
Cost of delivery	4.26	0	0	1.94	15.05	38.35	44.66
Level of alternative costs	3.85	0	0	0	33.98	46.60	19.42

Source: own research.

Table 2

Hierarchy of factors determining the competitiveness level of the company

Average evaluation of an importance (pts.)	Factors determining the level of competitiveness of the company	Cost of delivery	Reliability of supply	Company credibility	Means of transport used	Level of alternative costs	Transport route	Frequency of carriage	Size of freight
4.26	cost of delivery	**1**	**0.27**	**0.05**	0	0	0	0	0
4.18	reliability of supply	**0.27**	**1**	**0.46**	0	0	0	0	0
4.12	company credibility	**0.05**	**0.46**	**1**	0	0	0	0	0
3.93	means of transport used	0	0	0	**1**	**0.22**	**0.17**	0.03	0.01
3.85	level of alternative costs	0	0	0	**0.22**	**1**	**0.72**	**0.26**	**0.16**
3.80	transport route	0	0	0	**0.17**	**0.72**	**1**	**0.99**	**0.65**
3.77	frequency of carriage	0	0	0	0.03	**0.26**	**0.99**	**1**	**0.72**
3.76	size of freight	0	0	0	0.01	**0.16**	**0.65**	**0.72**	**1**

Source: own research.

2009, shows that the most important were: quality of services, prices, then customer service, and reliability of the company.

The importance hierarchy for factors determining the competitiveness level of the company, in the opinion of the designated recipients according to average grade point along with the values of the variable p is given in Table 2. The *p*-values are the result of the Wilcoxon test verifying the null hypothesis of equal importance of different sets of criteria, i.e. the lack of significant differences between the analyzed groups. Test value below 0.05 indicates that the importance of the stated factors differs significantly from each other.

Two importance levels were appointed, on the basis of statistical inference, determining the company competitiveness to the greatest extent among the eight analysed factors. Groups of similarly important factors are as follows:

– cost of delivery, reliability of supply, credibility of company,

– means of transport used, the level of alternative costs, transport route, the frequency of carriage, the size of freight.

An important element of the competitiveness level for enterprises providing services in the field of refrigeration transport is the cost of delivery, so the price level of logistics services offered was evaluated. Figure 1 presents the obtained results.

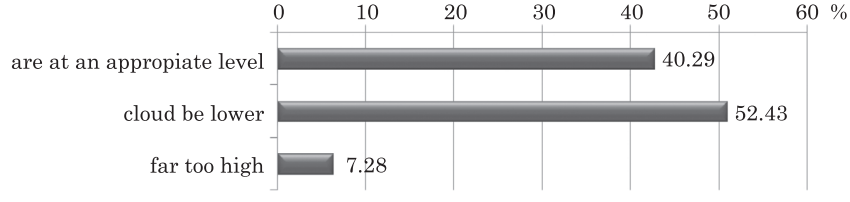

Fig. 1. Sheet of price evaluation for logistic services (% indications)
Source: own research.

More than half of the surveyed enterprises declared that the prices of the logistic services could be lower. However, for more than 40% of respondents prices of offered transport services are at an appropriate level, and for more than 7% the service prices are far too high.

The relationship between the assessment of the price level of the service and the area of the company activity were subject to the statistical analysis. The p coefficient value, resulting from the significance χ^2 test, lower than 0.05 indicates that there is a statistically significant relationship between the area of the company activity, and assessment of the level of service pricing. The results are given in Table 3.

Table 3

Relationship between the level of pricing and area of activity

Prices	Assessment of the level of pricing in relation to the area of company activity (% indications)			*P*-value
	production and distribution of food products	shopping malls	grocery stores	
Are at an appropriate level	**36.08**	**47.83**	**60.00**	0.16511
Could be lower	**56.96**	43.48	32.00	
Far too high	**6.96**	8.70	8.00	

Explanation to table 3: underlined values in table mean numbers of assessments by respondents between research variables >10.
Source: own research.

The relationship between the spatial range of the company and the assessment of the logistic service pricing was also examined. The obtained results of analyses are shown in Table 4.

Table 4

Relationship between the service price level and the spatial range of the company

Prices	Assessment of the transport service pricing in relation to the spatial range of the company (% indication)				*P*-value
	up to 9	10–49	50–249	more than 250	
Are at an appropriate level	34.78	33.33	**36.96**	**44.86**	**0.00073**
Could be lower	43.48	**66.67**	**54.35**	**49.53**	
Far too high	21.74	0	8.70	5.61	

Explanation to table 4: underlined values in the table mean numbers of assessments by respondents between research variables >10.
Source: own research.

On the basis of statistical analysis it was established that 60% of respondents functioning within specialist grocery stores found the prices of offered transport services to be on the appropriate level. On the other hand, 57% of food producers and distributors assessed that prices could be lower. Moreover, the statistic analysis indicated that the company activity area has no significant bearing on the assessment of pricing of the offered service ($p > 0.05$).

An important aspect of assessing the supply reliability, i.e. the quality factor of competitiveness of enterprises providing services for refrigerated transport, was also information about the opinion of recipient representatives (customers) concerning the level of service quality depending on the type of

cargo. Three main groups of products requiring controlled-temperature transport, such as: food products (fruits and vegetables), food products (meat, fish, eggs, milk and dairy products) and frozen or deep-frozen food products were analyzed. Table 5 shows the level of average ratings (in points) of transport services for the three food groups, where 0 is not satisfactory, 1 – very low, 2 – low, 3 – medium, 4 – high and 5 – very high level of quality of transport.

Table 5

Level of transport service quality depending on the type of cargo [pts.]

Groups of food products	Average evaluation
Food products (fruits and vegetables)	3.80
Food products (meat, fish, eggs, milk and milk products)	3.44
Food products frozen and deep-frozen	3.91

Source: own research.

Table 6

Dependence of the transport service quality level assessment for groups of food products on areas of the company activity

Level of quality for groups of food products		Assessment of quality level of transport service for groups of food transport on areas (% indications)			*P*-value
		production and distribution food products	shopping mall	grocery stores	
Food products (fruits, vegetables)	very low	0	17.39	8.00	**0.00007**
	low	2.53	0	8.00	
	medium	**17.09**	17.39	16.00	
	high	**39.87**	**56.52**	24.00	
	very high	**14.56**	8.70	20.00	
Food products (meat, fish, eggs, milk and milk products)	very low	6.41	0	0	**0.1330**
	low	3.85	19.05	0	
	medium	**14.74**	19.05	34.78	
	high	**18.59**	28.57	17.39	
	very high	**15.38**	19.05	17.39	
Food products frozen and deep-frozen	very low	1.27	0	0	0.32260
	low	2.53	8.70	16.00	
	medium	**17.09**	8.70	16.00	
	high	**39.87**	34.78	32.00	
	very high	**19.62**	26.09	16.00	

Explanation to table 6: underlined values in the table mean numbers of assessments by respondents between research variables >10.
Source: own research.

The research showed that recipient representatives (customers) similarly rated the quality level of logistic services for the three different groups of transported foods.

The dependence of assessment of the transport service quality level for three groups of food products on areas of activity of the company acquiring the services was also analysed. The coefficient p is the result of the significance test χ^2. The value of the coefficient p lower than 0.05 indicates that between the two variables there is a significant statistic correlation. The obtained results are presented in Table 6.

The carried out statistic analysis showed that there exists a relation between evaluating the quality level of transport services for two types of fresh food produce, and the areas of the company activity. However, no correlation was shown between the evaluation of transport service quality for frozen and deep-frozen food products and areas of the company activity.

The study was completed by assessing the impact of cooperation with the service provider on satisfaction of company. The subject of the statistical analysis was assessment of the relationship of positive impact of cooperation with the logistic services company for the refrigerated transport on the satisfaction of the company in relation to the spatial extent of its activity. The obtained results are presented in Table 7.

Table 7

Assessment of the relationship of positive impact of cooperation with the logistic services company for the refrigerated transport assessment on the satisfaction of the company in relation to the spatial extent of its activity

Impact of cooperation	Assessment of impact of cooperation with the transport company on the satisfaction of the company with the spatial extent of its actions (% indications)				*P*-value
	local	regional	domestic	international	
No significant impact	13.04	6.67	2.17	0.931	**0.00001**
Low impact	8.70	**40.00**	13.04	4.67	
Medium impact	17.39	0	19.57	**20.56**	
High impact	43.48	**40.00**	**30.43**	**42.06**	
Very high impact	17.39	13.33	**34.78**	**31.78**	

Explanation to table 7: underlined value in the table means numbers of assessments by respondents between researches variables >10.
Source: own research.

On the basis of the statistical analysis, a statistically significant relationship was noticed between the spatial range of the company activities, and the assessment of the positive impact of cooperation with the company providing logistic services for the refrigerated transport on its functioning (p-value <0.05). It has been shown, that the broader spatial extent of the company activities, the higher the assessment of the positive impact of cooperation with the company providing logistics services.

Conclusions

In accordance with the stated objective of the study, the order of priority for the factors determining the competitiveness level of the transport company was established, and then their assessment was made. The research showed that the most important factors determining the competitiveness level of the provider are: the cost of delivery, the reliability and credibility of the company. The means of transport used, the level of alternative costs, and frequency of transport, route and size of cargo belong to the second group of importance. It has also been shown that, in the opinion of more than half of recipients, the prices of offered transport services could be lower. Furthermore, the statistical analysis showed that the area of the company operations has no significant effect on the assessment of the transport service pricing. The research showed that recipient representatives (customers) similarly rated the quality level of services in three groups of transported foods. It was also found that there is a statistical relationship between the assessments of the quality of transport services for two groups of freight: food products (fruits, vegetables) and food products (meat, fish, eggs, milk and dairy products) and the areas of the company activity. There has also been shown a significant correlation between the positive impact of cooperation assessment on the company satisfaction, and the quality of the spatial extent of its activity. The broader the spatial range of activity (from local to international), the higher the assessment of the positive impact of cooperation with the service provider.

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