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SELECTED PROBLEMS OF SULFUR MANAGEMENT IN CROPS

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K e y w o r d s: sulfur sources, sulfur uptake by plants, plant metabolism.

A b s t r a c t

The study deals with the assessing the effect of sulfur on the metabolism occurring in crops. These changes largely reflect upon the size and quality of harvested crops. The plants are well fed with sulfur, because they are resistant to various biotic stresses, less prone to diseases and pests, and give better yields. Mechanism of plant resistance stimulated by the presence of sulfur results from a number of organic compounds present in a plant biomass and abundant in this component, such as H_2S, glutathione (GSH), sulfolipids, glucosinolates (GSL), phytoalexins, and alliins. These compounds significantly increase plant resistance to the impact of pathogens and stress caused by other environmental factors. In addition, sulfur is involved in the synthesis of, among others, proteins, lignin, fatty acids, nitrogen metabolism, sugar metabolism, affects the amount and quality of fat in rapeseed, as well as taste and aroma of certain plants, particularly onions and garlic. Therefore, the role of this nutrient in crop fertilization is increasingly emphasized.

WYBRANE PROBLEMY GOSPODARKI SIARKĄ ROŚLIN UPRAWNYCH

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S ł o w a k l u c z o w e: źródła siarki, pobieranie siarki przez rośliny, metabolizm roślin.

A b s t r a k t

W pracy przeanalizowano wpływ siarki na przemiany metaboliczne zachodzące w roślinach uprawnych. Przemiany te w dużej mierze rzutują na wielkość i jakość pozyskiwanych plonów roślin. Rośliny dobrze odżywione siarką są bowiem odporne na różnego rodzaju stresy biotyczne, mniej

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podatne na choroby i szkodniki i lepiej plonują. Mechanizm odporności roślin stymulowanej przez siarkę wynika z obecności w biomasie roślinnej wielu związków organicznych zasobnych w ten składnik, takich jak: H_2S, glutation (GSH), sulfolipidy, glukozynolany (GSL), fitoaleksyny oraz alliiny. Związki te wyraźnie zwiększają odporność roślin na wpływ patogenów oraz stres wywołany działaniem innych czynników środowiskowych. Dodatkowo siarka bierze udział min. w syntezie białek, lignin, kwasów tłuszczowych, przemianach związków azotu, metabolizmie cukrów, wpływa na ilość i jakość tłuszczu w nasionach rzepaku oraz walory smakowe i zapachowe niektórych roślin, zwłaszcza cebuli i czosnku. Coraz częściej podkreśla się więc rolę tego składnika pokarmowego w nawożeniu roślin uprawnych.

Introduction

Metabolic changes that occur in plants impinge heavily on the size and quality of the crop yields. Sulfur is clearly a nutrient, which plays an important role in plant metabolism. It is a component of many important compounds, the lack of which causes deterioration of harvested plant material quality and disorders in humans and animals. Plants well fed with sulfur are more resistant to biotic stresses, less prone to diseases and, in consequence, give better yields (BEDNAREK et al. 2008, MOTOWICKA-TERELAK and TERELAK 2000, PODLEŚNA 2005).

Unfortunately, there are more and more signs of a deepening deficit of this nutrient in crop production (Table 1).

Table 1

Sulfur pools and fluxes in agricultural land (KACZOR and ZUZAŃSKA 2009)

Income	Amount [kg S ha^{-1} rok^{-1}]	Outflows	Amount [kg S ha^{-1} rok^{-1}]
Atmosphere	12÷21	uptake by plants	13÷42
Fertilization	0÷26	leaching nutrients	30÷80
Earth water	0÷295	gas losses	0.2÷3
Mineralization	10÷30	–	–
Gases	1.5	–	–

Data published by the Sulfur Institute in Washington suggests that the global sulfur deficit in 2013 will reach nearly 11.9 million tons. Also, the results of research conducted by IUNG-PIB in Pulawy clearly show that more than half of the soil of our country (53%) is characterized by a low abundance of sulfur available to plants (less than 20 mg S-SO_4 kg^{-1}), 26% has an average, and 16% high abundance; the remaining 5% of Polish soils are contaminated due to

anthropogenic activities. Deficiency of this component in a plant growth environment affects primarily the cultivation of crops with high demands for sulfur (GONDEK 2010, MOTOWICKA-TERELAK and TERELAK 2000, WIELEBSKI 2011).

Therefore, the aim of this study was to evaluate the role of sulfur in metabolic transformations occurring in crops. This assessment based on the research of literature related to this subject.

Crop requirements for sulfur

The nutritional requirements of crops for sulfur depend largely on the crop species and the size of harvested crop (GAJ and KLIKOCKA 2011, KACZOR and ZUZAŃSKA 2009).

Primarily plants from *Brassicaceae* and *Liliaceae* families belong to the group with a particularly high demand for sulfur: rape, mustard, radish, turnip, onion, and garlic. They uptake 40 or even 80 kg of sulfur from 1 ha from the average yield (FILIPEK-MAZUR and GONDEK 2005, KOZŁOWSKA-STRAWSKA and KACZOR 2009, SZULC et al. 2000). Another group of plants with a high demand for sulfur mainly include: alfalfa and clover, which produce large amounts of protein, as well as corn and beets because of the quantity of the biomass produced. The last group of plants consists of those with a relatively small demand for sulfur, which take an average of 12 to 25 kg S ha^{-1} (grasses, potatoes). In the case of this group of plants, it is often underlined that although their requirements for sulfur are small, but at higher levels of fertilization using other nutrients, especially nitrogen and phosphorus, the demand of plants for sulfur may also increase due to the increased yield of crops (BARCZAK and NOWAK 2010, FILIPEK-MAZUR and GONDEK 2005, MCGRATH et al. 1996).

Mechanism of sulfur uptake by crops

Sulfur is taken mainly by roots. Sulfate uptake mechanism can be both passive and active. Passive process occurs mainly by means of diffusion consisting in equalizing the concentrations between the external solution and the so-called *apparently free space* in the root. Sulfates contained within this space can be exchanged into other ions or be removed by washing. The penetration of sulfates within apparently free space occurs relatively rapidly and generally the balance between the external solution and apparently free space is achieved after 30 minutes (MARSCHNER 1995).

Sulfur active uptake may occur against concentration gradient, wherein the acquired ions are not washed away, which requires some energy efforts expended by living cells (MARSCHNER 1995).

Sulfur can also be taken up by plants in the form of SO_2. The proportion of sulfur absorbed from the air in relation to its total content at plants amounts to 15–30% for well provided plants and more than 50% at plants suffering from sulfur deficiency (DECHNIK et al. 1990).

Sulfur sources for crops

Undoubtedly minerals containing sulfur in their crystal structures are the primary source of the element in the soil. Among minerals abundant in sulfur, the most important are: gypsum, iron sulfides found in the soil under reductive processes, and hydrotroilite making dark color of some clays and silts. Sphalerite, chalcopyrite, and cobaltite are also present, yet in smaller quantities. Readily soluble sulfates can be found in dry climate conditions, while sodium alum and tamarugite in a very dry climate. In agricultural soils containing pyrite in deeper profiles, iron, sodium, potassium, magnesium sulfate, and compounds at lower oxidation state such as sulfites, thiosulfates, pentathionates, and elemental sulfur are often present (MARSKA and WRÓBEL 2000, MOTOWICKA-TERELAK and TERELAK 1998).

Some organic compounds are another very valuable source of sulfur, among which the following dominate: amino acids, particularly methionine, and cysteine, the peptides (glutathione), protein, sulfolipids, and vitamins (thiamine and biotin) (FILIPEK-MAZUR and GONDEK 2005, MARSCHNER 1995).

The sulfur is introduced into the soil along with certain mineral fertilizers. These fertilizers are divided into two main groups: simple and complex. The simple fertilizers contain sulfur in the forms directly available to plants or as elemental sulfur, which must be oxidized in the soil prior to it is taken up by plants. Complex fertilizers can contain a variety of chemical forms of sulfur, hence their bioavailability is generally diverse (ERIKSEN and MORTENSEN 2002, MCGRATH et al. 1996, WIELEBSKI and WÓJTOWICZ 2000).

Natural fertilizers, especially farmyard manure, is a valuable source of sulfur, the content of the element varies from 0.3 to 0.6 kg S Mg^{-1} d.m. of fertilizer (MARSKA and WRÓBEL 2000, WIELEBSKI 2006).

Sulfur penetrating the soils can also origin from the atmosphere. Sulfur(IV) oxide, that is a major component of air contamination, can be absorbed onto the soil surface in the gaseous form and then dissolved and oxidized in the soil solution. The process is then referred to as *dry deposition*.

Sulfur oxides can be also oxidized in the atmosphere forming sulfuric acid and it is then called *wet deposition* (ERIKSEN and MORTENSEN 2002, MARSKA and WRÓBEL 2000).

Sulfur functions in crop metabolism

Sulfur is involved in numerous physiological processes taking place in all crops. Its deficiency inhibits primarily the protein synthesis. In the absence of sulfur, plants produce proteins of worse quality, which is associated with a lower content of essential amino acids such as methionine and cysteine. In addition, sulfur is a component necessary for the activity of enzymes responsible for nitrates reduction. Plants grown under the sulfur deficiency, accumulate non-protein nitrogen forms in their medium: nitrates, amides, and other compounds, such as ammonia (MARSCHNER 1995).

Moreover, in the absence of sulfur, photosynthetic intensity is lowered, as well as the decrease in protein synthesis and nucleic acids content, can reach up to about 50% or more. Sulfur stimulates the synthesis of lignin, fatty acids, it is a part of vitamin B_1 (thiamine) and vitamin H (biotin). It is also involved in enzymatic reactions responsible for biological nitrogen fixation from the air by nitrogen-fixing root bacteria, and is a component of some volatile compounds (aliphatic thioesters, polysulfides, sulfoxides) responsible for the taste and aroma of certain plants such as onions and garlic (ERIKSEN and MORTENSEN 2002, MARSCHNER 1995).

Sulfur deficiency in plant growth medium also causes disturbances in the metabolism of sugars. The starch content may be increased up to 2–3-fold, while reducing the amount of reducing sugars. This is undoubtedly related to the decrease in the photosynthetic activity of plants, at which chlorosis is the consequence of the sulfur absence (PARK 1988).

Similar correlations also showed in their study on the effect of sulfur on quality parameters of spring wheat PODLEŚNA and CACAK-PIETRZAK (2006) – Table 2.

Table 2
Chemical features of flour in dependence on sulfur fertilization (PODLEŚNA and CACAK-PIETRZAK 2006)

Income	Ash [% s.m.]	Total protein [% s.m.]	Gluten [%]
-S [0 S ha^{-1}]	0.65	14.0	29.1
+S [50 kg S ha^{-1}]	0.64	14.1	29.6
LSD [α = 0.05]	0.010	0.052	r.n.

r.n. – non-significant differences.

The use of sulfur is one of the most important factors influencing on the synthesis and accumulation of glucosinolates in rapeseed (Table 3).

Table 3

Main glucosinolates occurring in cruciferous plants (OLESZEK 1995)

Common name	Occurrence
Synigrine	*Brassica nigra*
Glukonapine	*B. napus*
Progoitrine	*B. napus*
Glukobrassicanapine	*B. napus*
Glukonapoleipheryne	*B. napus*
Glukonasturcine	*Nasturtium officinalis*
Glukotropaeoline	*Lepidium sativa*
Synalbine	*Sinapis alba*
Glukobrasicine	*B. oleracea*
4-OH Glukobrasicine	*B. napus*
4-OCH_3 Glukobrasicine	*B. napus*
Neoglukobrasicine	*B. napus*

The mature seeds of rape contain the largest quantities of the following four alkene glucosinolates: progoitrine, gluconapine, glucobrassicanapine, napoleipherine, and among indole glucosinolates: 4-hydroxyglucobrassicine and glucobrassicine. Excessive accumulation of glucosinolates in rapeseed is considered as a negative phenomenon, because too high content of these compounds eliminates the use of post-extraction meal as a feed for animals. It is assumed that about 25–30% of the total sulfur, on average, present in the seed can be found in glucosinolates. This reduces the nutritional value of the post-extraction meal, making the taste worse at the same time. Although it is assumed that glucosinolates are not harmful to animals, but the products of their enzymatic decomposition due to myrosinase enzyme become toxic. They mainly cause disturbances in the thyroid, liver, and kidneys functioning. As permitted in Poland and the EU, total glucosinolate levels in industrial rapeseed 00, the level of 25 μM per 1 g of dry de-fatted matter is accepted. However, from the viewpoint of metabolism occurring at plants, glucosinolates act as a storage of sulfur during its deficit within the plant growth medium (FIGAS 2009, GAJ and KLIKOCKA 2011, KACZOR and ZUZAŃSKA 2009, MALARZ et al. 2011).

The role of glucosinolates or their hydrolysis products is also associated with the defense functions of plants and interactions between *Brassicaceae* or *Cruciferae* plants vs. pests and pathogens. Glucosinolate catabolism products

exhibit a significant toxicity to a number of pathogenic fungi, bacteria, viruses, insects, and higher plants. They play functions of repellents, attractants, or compounds modifying the pest behavior, but also play a role in allelopathic interactions (GAJ and KLIKOCKA 2011, MAJCHRZAK et al. 2010, PODLEŚNA 2005).

Under conditions of good sulfur nutrition and at the same time in response to pathogens, a plant mobilizes its specific metabolic mechanism, which is referred to as sulfur-induced resistance – SIR (Sulfur Induced Resistance). The mechanism involves sulfur applied in accordance with a planned strategy of fertilization. Free cysteine resources appearing at plants well supplied with this nutrient become a factor of the plant resistance, while non-protein cysteine is a precursor of all relevant compounds containing sulfur and having a mechanism most probably related to the immunity stimulated by the component (GAJ and KLIKOCKA 2011).

Mechanism of plant resistance stimulated by the presence of sulfur in the biomass results from a number of organic compounds abundant in this component, such as H_2S, glutathione (GSH), sulfolipids, glucosinolates (GSL), phytoalexins, and alliins. These compounds significantly increase the plant resistance towards the impact of pathogens and stress caused by the action of other environmental factors. The protective effect of sulfur was found particularly with respect to the black cross, powdery mildew, leaf spot light, and to a lesser extent, in the case of dry rot in cabbage (GAJ and KLIKOCKA 2011, MAJCHRZAK et al. 2010, PODLEŚNA 2005).

Summary

The role of sulfur in plant nutrition has gained its importance especially in the last few years, when a clear deficit of this nutrient in crop production was found both in Poland and in most European countries.

Sulfur plays a specific role in the plant metabolism. It participates in the synthesis of proteins, lignin, fatty acids, nitrogen metabolism, sugar metabolism, affects the quantity and quality of fat in the seeds of oilseed plants, taste and flavor of certain plants and participates in plant defense mechanisms. Given the functions that this element plays in a plant life, it can be counted into the group of nutrients determining both the size and quality of harvested crops. Therefore, sulfur, besides nitrogen, phosphorus, and potassium, should be included in the group of nutrients regularly supplied to grown plants within the fertilization plan.

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EFFECT OF THE *ECKLONIA MAXIMA* EXTRACT ON SELECTED MICRO- AND MACROELEMENTS IN ABOVE-GROUND BIOMASS OF *FESTULOLIUM BAUNII* (K. RICHT.) A. CAMUS

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K e y w o r d s: seaweed extract, microelements, macroelements, biomass, *Festulolium baunii*.

A b s t r a c t

Studies with *Festulolium braunii* (variety Felopa), cultivation was carried out in the polyurethane rings with a diameter of 36 cm and hight of 40 cm, which were dug to a depth of 30 cm and filled with soil material. In this experiment Kelpak SL (extract from *Ecklonia maxima*) was used as bioregulator. It consists the natural plant hormones such as auxin (11 mg in dm^3) and cytokines (0.03 mg in dm^3). Experimental objects: A1 – control (no extract), A2 – extract. The preparation was applied to all regrowth in the form of spray at a dose of 3 cm^3 $ring^{-1}$ during the grass shooting stage (2 dm^3 preparation diluted in 350 dm^3 of water). The full period of this experiment was in the years 2010–2011. The study traits were: the content of macroelements – phosphorus, potassium, calcium, magnesium (g kg^{-1} DM) and trace elements – manganese, zinc and copper (mg kg^{-1} DM) in the aboveground biomass of *Festulolium baunii*. In the paper ratio of Ca : P and K : (Ca + Mg) was also calculated. The results were analyzed statistically using analysis of variance and the average were compared by according to Tukey's test. As a result of spraying with the extract from seaweed, in *Festulolium baunii* aboveground biomass increased content of phosphorus, potassium, zinc and manganese. The contents of magnesium, calcium and copper did not undergo differentiation under the influence of study factor. In the consequence of differentiation of macroelements content the decrease of the ratio value of calcium to phosphorus and the increase in the ratio of potassium to calcium and magnesium was appered.

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WPŁYW EKSTRAKTU Z *ECKLONIA MAXIMA* NA ZAWARTOŚĆ WYBRANYCH MIKRO- I MAKROELEMENTÓW W BIOMASIE NADZIEMNEJ *FESTULOLIUM BAUNII* (K. RICHT.) A. CAMUS

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Słowa kluczowe: ekstrakt z wodorostów, mikroelementy, makroelementy, biomasa, *Festulolium baunii.*

Abstrakt

Badania z uprawą *Festulolium braunii* (odmiana Felopa) przeprowadzono w pierścieniach poliuretanowych o średnicy 36 cm i wysokości 40 cm, które wkopano na głębokość 30 cm i wypełniono materiałem glebowym. Czynnik doświadczalny stanowił bioregulator o nazwie handlowej Kelpak SL (ekstrakt z *Ecklonia maxima*), w skład którego wchodzą naturalne hormony roślinne, tj. auksyny (11 mg dm^{-3}) i cytokininy (0,03 mg dm^{-3}). Obiekty doświadczalne: A1 – kontrola (bez ekstraktu), A2 – ekstrakt. Preparat aplikowano na wszystkie trzy odrosty w formie oprysku w dawce 3 $cm^3 \cdot$ pierścień$^{-1}$ w fazie strzelania w źdźbło traw (2 dm^3 preparatu rozpuszczonego w 350 dm^3 wody). Okres pełnego użytkowania obiektów doświadczalnych przypadał na lata 2010–2011. Badano następujące cechy: zawartość makroelementów – fosforu, potasu, wapnia, magnezu (g kg^{-1} s.m.) i mikroelementów – manganu, cynku i miedzi (mg kg^{-1} s.m.) w biomasie nadziemnej *Festulolium baunii*. W pracy wyliczono również stosunek Ca : P oraz K : (Ca + Mg). Wyniki opracowano statystycznie, stosując analizę wariancji oraz NIR0,05 według testu Tukey'a. W efekcie stosowania oprysku ekstraktem z wodorostów w biomasie nadziemnej *Festulolium baunii* nastąpił wzrost zawartości fosforu, potasu cynku i manganu. W konsekwencji zróżnicowania zawartości makroelementów nastąpiło zawężenie stosunku wapnia do fosforu i rozszerzenie stosunku potasu do wapnia i magnezu.

Introduction

According to ABOU EL-YAZIED et al. (2012), seaweed is one of the most important marine resources of the world. There are used as human food, animal feed and raw materials for many industries. They are also used as a fertilizer for agricultural and horticultural crops (TEMPLE et al. 1988, BECKETT et al. 1994, OUEDRAOGO et al. 2001, BAI et al. 2007, ZODAPEA et al. 2009, NOUR et al. 2010, ZODAPE et al. 2010, ABOU EL-YAZIED et al. 2012). After applying these extracts in agricultural crops, were found better production results than after the mineral fertilizers (AITKEN and SENN 1965, THIRUMARAN et al. 2009, NOUR et al. 2010). Due to the presence of minerals and hormonal substances (MOLLER and SMITH 1998, 1999), they cause an increase of plant resistance to stress and disease conditions (VERKLEIJ 1992). The chemistry of

seaweed is complex, it has a very high content of organic carbon (particularly carbohydrates such as alginic acid, laminaren and mannitol), seaweed is also high in polysaccharides but yet very low in N, P, K, seaweed is well known for its trace mineral content and the presence of a range of biologically active, growth promoting substances. Seaweed concentrates are known to cause many beneficial effects on plants as they contain growth promoting hormones (IAA and IBA, cytokinins), trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins and amino acids (KHAN et al. 2009, ZODAPE et al. 2010, ABOU EL-YAZIED et al. 2012). Today, these preparations are seen as a natural organic fertilizer of new generation (AITKEN and SENN 1965, SINGH and CHANDELA 2005, SRIDHAR and RENGASAMY 2010, ZODAPE et al. 2010, ABOU EL-YAZIED et al. 2012). The beneficial effect of biostimulators fitohormons on based primarily reflected on yield increase (TEMPLE and BOMKE 1989, LIU et al. 1991, VERKLEIJ 1992, MOSTAFA and ZHEEKH 1999, ZODAPE 2001, MATYSIAK and ADAMCZEWSKI 2006, MATYSIAK et al. 2012). The application of these products also affected the content of micro-and macronutrients in plant material (RATHORE et al. 2009, ZODAPE et al. 2009, ABOU EL-YAZIED et al. 2012). In literature already described the effect of extracts on cereals and vegetables, but there is no response studies on the application of such preparations *Festulolium baunii* (K. Richt.) A. Camus.

Study was undertaken to determine the effect of the extract from *Ecklonia maxima* on content of selected micro- and macroelements in above-ground biomass of *Festulolium baunii*. The experiment was to demonstrate that the annually use of extract over the next two years of *Festulolium baunii* growing, influenced the content of P, K, Mg, Ca, Mn, Zn, and Cu in the tested plant material.

Materials and Methods

Studies of with growing of *Festulolium braunii* cv. 'Felopa', was carried out in the polyurethane rings, with 4 replications at the experimental object of Grassland and Development of the Department of Landscape Architecture. Rings with a diameter of 36 cm and height of 40 cm were dug to a depth of 30 cm and filled with soil material belonging to the soil of hortisole type, formed from weakly loamy sand. On the basis of chemical analysis performed at the Regional Chemical Station in Wesoła it was stated, that the soil in the rings was characterized by a neutral reaction (pH in 1 mol KCl dm^{-3} = 7.2), high abundance in humus (3.78%), available phosphorus (P – 395 mg kg^{-1}) and magnesium (Mg – 84 mg kg^{-1}) and the average abundance of nitrogen (N total – 1.8 g kg^{-1}) and soluble potassium (K – 157 mg kg^{-1}). For each of the rings

(3 April 2010), six seeds of tested grass species were sown. After seeds germination when seedlings reached the 2–3 leaf stage, negative selection was made by removing the two weakest plants, and than it was introduced an experimental factor as a growth regulator – Kelpak SL. It is a preparation, consisting from natural plant hormones such as auxin (11 mg in dm^3) and cytokinins (0.03 mg in dm^3). It is prepared from brown algae *Ecklonia maxima* (TEMPLE et al. 1988). Experimental objects: A1 – control (noextract), A2 – extract. The preparation was applied to all regrowth in the form of spray at a dose of 3 cm3 $ring^{-1}$ during the grass shooting stage (2 dm^3 preparation was diluted in 350 dm^3 of water). In addition, mineral fertilizers were used in the annual dose: N – 0.6 g $ring^{-1}$ and K – 0.74 g $ring^{-1}$. The full period of three cut using of experimental objects was in the years: 2010–2011.

The content of micro- and macroelements in the biomass has determined for all the cuts over three years of this crop. The following methods for analizing was used: P – flow spectroscopy, K – emissions by flame spectroscopy, Ca and Mg – atomic absorption spectroscopy. Furthermore, based on macroelements the content of the following ratios were calculated Ca : P and K: (Ca + Mg). Chemical analyzes on the contents of Mn, Zn and Cu by atomic absorption spectrometry.

The obtained results were evaluated statistically by using analysis of variance for multivariante experiments. Differentiation of medium was verified by Tukey's test at significance level $p \leq 0.05$.

Weather conditions of research area were typical for IX – eastern district of agro-climatic of Poland (RADOMSKI 1977). Average annual air temperature ranged from 6.7–6.9 °C, and in summer the average daily temperature is 15°C. Annual precipitation are at the level 550–650 mm, while they are not frequent, but heavy. The vegetation period usually begins in the first decade of April and ends in the third one of October, and so takes from 200 to 220 days. Meteorological data from the research years were obtained from the Hydrological and Meteorological Station in Siedlce. However, in order to determine the temporal and spatial variability of meteorological elements and their effects on vegetation the hydrothermal index of Sielianinov (BAC et al. 1993) was calculated. This values for individual months and years of research are presented in Table 1.

The data in Table 1 indicate, that the most favorable distribution and the amount of rainfall, with optimum air temperatures within the vegetation period for plants growing characterized 2011. In that year there were no months with strong drought inverse to the year 2010.

Table 1

Value of hydrothermal index of Sielianinov (K) in individual months of vegetation

Year	Month						
	IV	V	VI	VII	VIII	IX	X
2010	0.40	2.21	1.19	1.18	1.79	2.81	0.53
2011	1.10	0.89	0.72	2.19	0.84	0.78	0.94

$K < 0.5$ – serve drougth; 0.51–0.69 – drougth; 0.70–0.99 – poor drougth; $K > 1$ – no drought

Results and discussion

The spray using the extract from *Ecklonia maxima* in the cultivation of *Festulolium baunii* resulted in a significant increase in phosphorus content over 13,5% and over 16,5% of potassium (Table 2). The statistical analysis also showed that the content of these elements were also dependent on the year. The smallest amount of phosphorus (3.73 g kg^{-1} D.M. – mean for cut), independently of the research factor was occurred in the second year of cultivation, but the greatest was in the first year (4.23 g kg^{-1} D.M. – mean for cut). However, the potassium content increased with aging plants. It is worth noting that the use of extract in each year of the experiment did not significantly affect the content of magnesium and calcium in the tested plant material. This is confirmed in a study conducted by ABOU EL-YAZIED et al. (2012), but does not correspond to the results obtained by ZODAPE et al. (2009), which reported a significant increase in the content of these macronutrients in cereal grains.

Numerous studies (FEATONBY-SMITH, STADEN 1983, VERKLEIJ 1992, GALBIATTIA et al. 2007) have shown that the beneficial effects with the use of seaweed extracts as natural regulators increased yield, improved plant vigor, and the ability to resisting unprofitable environmental conditions. The using of the extract as an organic biostimulator, was quickly accepted by practice in horticulture because of the beneficial production effects (VERKLEIJ 1992, CROUCH, STADEN 1993). According to SANDERSON and JAMESON (1986) or STIRK and VAN STADEN (1997) the main components of extracts affecting on the plants are cytokinins and auxins, which have been identified in most seaweed concentrates.

According to WIERZBOWSKA, BOWSZYS (2008), these hormones induce many processes connected with histological and cytological aspects of plants and influence on the content of some macronutrients. From the research of ABOU EL-YAZIED et al. (2012) on the effects of seaweed extracts on the beans quality resulted that the application during two growing seasons, resulted in an increase in the content of phosphorus and potassium in the leaves in compare

to control. The same trend also concerned magnesium content. Similar results are presented by PISE and SABALE (2010). The increase in the content of phosphorus and potassium, in plant material after extract spray application were also found by SHEHATA et al. (2011). Also NOUR et al. (2010) studying the effect of extracts spray from seaweed on the chemical composition of tomato showed that the most of K and P occurred in vegetables grown on the objects with this factor. Studies on soybean (RATHORE et al. 2009), relating to the response of this plant to the different concentrations of the extracts from seaweed, also showed a significant increase in P and K content after extract application regardless of the concentration. The ZODAPE et al. (2009), using 1% spray with extract on wheat stated in the grain more than 15% increase of K content 18% P, 45% Ca and 28% Mg. The increase in the content of macronutrients in plants after application of extracts was also reported by BECKETT et al. (1994) and ZAHID (1999).

Table 2

Effect of the extract from *Ecklonia maxima* on the content of selected macroelements [g kg^{-1} D.M.] in biomass of *Festulolium braunii* in each study years and cuts

Years	Cut 1			Cut 2			Cut 3			Extract		Mean
	extract			extract			extract					
	A1	A2	mean	A1	A2	mean	A1	A2	mean	A1 control	A2 factor	
P												
2010	4.11^{Aa}	4.01^{Ba}	4.06^{B}	2.92^{Bb}	3.93^{Ba}	3.43^{B}	3.23^{Aab}	4.11^{Aa}	3.67^{B}	3.44^{Bb}	4.03^{Ba}	3.74^{B}
2011	4.30^{Aa}	4.63^{Aa}	4.47^{A}	3.81^{Aa}	4.41^{Aa}	4.11^{A}	3.92^{Aa}	4.20^{Aa}	4.06^{A}	4.02^{Ab}	4.42^{Aa}	4.22^{A}
Mean	$4.20a$	4.32^{a}	4.26^{a}	3.41^{b}	4.22^{a}	3.77^{b}	3.63^{b}	4.20^{a}	3.87^{ab}	3.73^{b}	4.23^{a}	–
K												
2010	33.5^{Aa}	35.8^{Aa}	34.7^{A}	$32,9^{Ab}$	38.0^{Aa}	35.5A	32.9^{Ab}	38.7^{Aa}	35.6^{A}	33.1^{Ab}	37.5^{Aa}	35.3^{A}
2011	32.4^{Ab}	39.1^{Aa}	35.8^{A}	33.0^{Ab}	40.6^{Aa}	36.8^{A}	34.5^{Ab}	40.0^{Aa}	37.3^{A}	33.3^{Ab}	39.9^{Aa}	36.6^{A}
Mean	33.0^{a}	37.5^{a}	35.3^{a}	33.0^{b}	39.3^{a}	36.2^{a}	33.7^{b}	39.4^{a}	36.5^{a}	33.2^{b}	38.7^{a}	–
Mg												
2010	5.81^{Aa}	5.10^{Aa}	5.46^{A}	4.82^{Bb}	5.23^{Aa}	5.03^{A}	5.61^{Aa}	5.30^{Aa}	5.46^{A}	5.41^{Aa}	5.21^{Aa}	5.31^{A}
2011	5.32^{Aa}	5.61^{Aa}	5.47^{A}	5.20^{Aa}	5.31^{Aa}	5.26^{A}	5.41^{Aa}	5.34^{Aa}	5.38^{A}	5.31^{Aa}	5.42^{Aa}	5.41^{A}
Mean	5.57^{a}	5.36^{a}	5.47^{a}	5.01^{a}	5.27^{a}	5.14^{a}	5.51^{a}	5.32^{a}	5.42^{a}	5.41^{a}	5.31^{a}	5.31^{A}
Ca												
2010	7.20^{Aa}	7.10^{Aa}	7.15^{Aa}	7.23^{Aa}	6.65^{Aa}	6.94^{Aa}	6.90^{Aa}	7.01^{Aa}	6.96^{Aa}	7.11^{Aa}	6.92^{Aa}	7.02^{Aa}
2011	7.01^{Aa}	7.01^{Aa}	7.01^{Aa}	7.44^{Aa}	7.00^{Aa}	7.22^{Aa}	7.21^{Aa}	7.03^{Aa}	7.12^{Aa}	7.22^{Aa}	7.01^{Aa}	7.12^{Aa}
Mean	7.11^{a}	7.06^{a}	7.09^{a}	7.34^{a}	6.83^{a}	7.08^{a}	7.06^{a}	7.02^{a}	7.04^{a}	7.21^{a}	7.01^{a}	–

Mean values marked with the same small letters do not differ significantly, and mean values marked with the same capital letters do not differ significantly

In the literature relating to the nutritional value of the feed material emphasizes the importance of quantitative relationships of individual minerals as a parameter describing the nutritional value of plants (STANIAK 2004, JANKOWSKA-HUFLEJT, WRÓBEL 2008, NOWAK et al. 2008). According to STANIAK (2004), it is important to determine the ratio of Ca:P and K: (Ca+Mg). In the analyzed plant material, regardless of the study year, the using of an extract significantly reduced the ratio of calcium to phosphorus (Table 3), from 1.94 to 1.66 (mean for cut). The experiment biomass has a very high content of K in relation to animal nutrition standards (JANKOWSKA-HUFLEJT, WRÓBEL 2008). It influenced to a significant decrease in the ratio K: (Ca+Mg), which in plant material collected from control crops amounted 2.66. It should be noted, however, that spraying the *Festulolium braunii* with extract caused a statistically significant, over 31% increase in its value. In the subsequent study years occurred an increase of K content (Table 2), which also resulted in the increase of K: (Ca+Mg) ratio to a value greater than 3 (Table 3).

Table 3

Macronutrient ratios in biomass of *Festulolium braunii* depending on the extract of *Ecklonia maxima* and the growing years and cuts

Years	Cut 1			Cut 2			Cut 3			Extract		Mean
	extract		mean	extract		mean	extract		mean			
	A1	A2		A1	A2		A1	A2		A1 control	A2 factor	
K : (Ca+ Mg)												
2010	2.57^{Aa}	2.93^{Aa}	2.75^{A}	2.73^{Ab}	3.20^{Aa}	2.97^{A}	2.63^{Ab}	3.14^{Aa}	2.89^{A}	2.65^{Ab}	3.10^{Aa}	2.87^{A}
2011	2.63^{Ab}	3.10^{Aa}	2.87^{A}	2.61^{Ab}	3.30^{Aa}	2.96^{A}	2.73^{Ab}	3.23^{Aa}	2.98^{A}	2.66^{Ab}	3.22^{Aa}	2.94^{A}
Mean	2.60^{b}	3.02^{a}	2.81^{a}	2.67^{b}	3.25^{a}	2.97^{a}	2.68^{b}	3.19^{a}	2.94^{a}	2.66^{b}	3.16^{a}	–
Ca : P												
2010	1.75^{Aa}	1.77^{Aa}	1.76^{A}	2.48^{Aa}	1.69^{Ab}	2.09^{A}	2.14^{Aa}	1.71^{Aa}	1.93^{A}	2.08^{Aa}	1.73^{Ab}	1.91^{A}
2011	1.63^{Aa}	1.51^{Aa}	1.57^{A}	1.95^{Aa}	1.58^{Aa}	1.77^{A}	1.84^{Aa}	1.67^{A}	1.76^{A}	1.80^{Aa}	1.59^{Aa}	1.70^{A}
Mean	1.69^{b}	1.64^{a}	1.67^{a}	2.22^{a}	1.64^{a}	1.93^{a}	1.99^{a}	1.69^{a}	1.84^{a}	1.94^{a}	1.66^{b}	–

Mean values marked with the same small letters do not differ significantly, and mean values marked with the same capital letters do not differ significantly

Spraying of *Festulolium braunii* plants with extract from seaweed led to a significant increase (Table 4) the content of Mn and Zn in aboveground biomass. The manganese content increased from 127 on control object to 149 mg kg^{-1} DM – objects with preparation (mean for cut). This represented more than 17% increase in value. However, in the case of zinc has been noted the increase more than 9% compared to control object.

The statistical evaluation regardless of study years hadn't significant medium differentiation after application of factor in relation to the content of copper. In addition, analyzing the micronutrients content should be noted, that in the biomass of *Festulolium braunii* significant differentiated copper content only. The largest amount of this element was observed in the material collected in the first study year. The increase in the content of some micro elements was also stated in the study of ZODAPE et al. (2009).

Table 4

Effect of the extract from *Ecklonia maxima* on the content of selected microelements [mg kg^{-1} D.M.] in biomass of *Festulolium braunii* in each study years and cuts

Years	Cut 1			Cut 2			Cut 3			Extract		Mean
	extract			extract			extract					
	A1	A2	mean	A1	A2	mean	A1	A2	mean	A1 control	A2 factor	
Mn												
2010	130^{Aa}	150^{Aa}	140^{A}	130^{Ab}	151^{Aa}	141^{A}	127^{Ab}	149^{Aa}	138^{A}	129^{Ab}	150^{Aa}	140^{A}
2011	137^{Aa}	148^{Aa}	143^{A}	117^{Ab}	148^{Aa}	133^{A}	121^{Ab}	148^{Aa}	135^{A}	125^{Ab}	148^{Aa}	137^{A}
Mean	134^{a}	149^{a}	142^{a}	124^{b}	150^{a}	137^{a}	124^{b}	149^{a}	137^{a}	127^{b}	149^{a}	–
Zn												
2010	44.7^{Ab}	51.0^{Aa}	47.9^{Ab}	47.0^{Aa}	50.5^{Aa}	48.8^{Ab}	46.0^{Ab}	50.3^{Aa}	48.2^{Ab}	45.9^{Aa}	50.6^{Aa}	48.3^{Ab}
2011	45.8^{Aa}	50.1^{Aa}	47.9^{Ab}	47.1^{Aa}	49.4^{Aa}	48.3^{Ab}	46.0^{Aa}	49.9^{Aa}	48.0^{Ab}	46.3^{Ab}	49.8^{Aa}	48.1^{Ab}
Mean	45.3^{b}	50.6^{a}	47.9^{a}	47.1^{a}	50.0^{a}	48.6^{a}	46.0^{b}	50.1^{a}	48.1^{a}	46.1^{b}	50.2^{a}	–
Cu												
2010	9.50^{Aa}	8.88^{Aa}	9.19^{A}	9.76^{Aa}	9.11^{Aa}	9.44^{A}	9.60^{Aa}	9.01^{Aa}	9.31^{A}	9.62^{Aa}	9.00^{Aa}	9.31^{A}
2011	9.10^{Aa}	9.49^{Aa}	9.30^{A}	9.32^{Aa}	9.24^{Aa}	9.28^{A}	9.06^{Aa}	9.41^{Aa}	9.24^{A}	9.16^{Aa}	9.38^{Aa}	9.27^{A}
Mean	9.30^{a}	9.19^{a}	9.25^{a}	9.54^{a}	9.18^{a}	9.36^{a}	9.33^{a}	9.20^{a}	9.28^{a}	9.39^{a}	9.19^{a}	–

Mean values marked with the same small letters do not differ significantly, and mean values marked with the same capital letters do not differ significantly

According to the authors, the application of the extract from *K. alverezii* in cereals, regardless of the preparation concentration, resulted in an increase of zinc content (4.9%) and manganese (9.42%) in wheat grain. Only the copper content did not undergo differentiation under the influence of the extract.

Conclusions

1. The application of the extract from *Ecklonia maxima* in the cultivation of *Festulolium braunii* resulted in a statistically significant increase in the content of P and K in the above-ground parts of this plant.

2. The content of Mg and Ca in the biomass of *Festulolium braunii* did not undergo significant differentiation both under the influence of an extract of seaweed as well as research years.

3. Both the K: (Ca+Mg) ratio Ca:P and levels are changed significantly as a result of the extract application. The ratio of K to Ca and Mg increased by 31% and Ca to P was reduced by 17%.

4. Spraying with seaweed extract resulted in an increase in the content of Mn and Zn in the dry matter of *Festulolium braunii*. Copper did not undergo to significant variation under the preparation influence.

5. The study did not show clearly in which conditions there was the most favorable effect of the extract of *Ecklonia maxima* for the content of micro- and macronutrients in *Festulolium braunii* biomass.

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COMPOST, BENTONITE AND CALCIUM OXIDE USED FOR ALLEVIATION OF THE IMPACT OF PETROLEUM PRODUCTS ON SOME SOIL PROPERTIES*

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K e y w o r d s: petrol and diesel oil contamination, compost, bentonite, calcium oxide, soil, acidity, sorptive properties.

A b s t r a c t

The purpose of this study has been to test the effect of compost, bentonite and calcium oxide as substances alleviating light contamination with petrol and diesel oil (0, 2.5, 5 and 10 cm^3 kg^{-1}) on some of the soil properties. The effect of petroleum products on soil properties depended on their dose and the application of alleviating substances to soil. Petrol (unlike diesel oil) had a rather negative effect on sorption characteristics of soil, raising hydrolytic acidity and decreasing its pH, total exchangeable bases, cation exchange capacity and base saturation of soil. Bentonite and calcium oxide produced a much positive effect on the analyzed soil properties than compost, generally causing an increase in pH, total exchangeable bases, cation exchange capacity and base saturation while decreasing hydrolytic acidity.

WYKORZYSTANIE KOMPOSTU, BENTONITU I TLENKU WAPNIA W ŁAGODZENIU WPŁYWU SUBSTANCJI ROPOPOCHODNYCH NA WYBRANE WŁAŚCIWOŚCI GLEBY

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S ł o w a k l u c z o w e: zanieczyszczenie benzyną i olejem napędowym, kompost, bentonit, tlenek wapnia, gleba, kwasowość, właściwości sorpcyjne.

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Abstrakt

Celem badań było zastosowanie kompostu, bentonitu i wapna do złagodzenia wpływu niewielkiego zanieczyszczenia benzyną i olejem napędowym (0, 2,5, 5 i 10 cm^3 kg^{-1}) na niektóre właściwości gleby. Oddziaływanie substancji ropopochodnych na właściwości gleby zależało od ich dawki oraz dodatku substancji do gleby. Benzyna (w odróżnieniu od oleju napędowego) oddziaływała negatywnie na właściwości sorpcyjne gleby, przyczyniając się do zwiększenia kwasowości hydrolitycznej oraz zmniejszenia pH, sumy wymiennych kationów zasadowych, całkowitej pojemności wymiennej i stopnia wysycenia gleby kationami zasadowymi. Bentonit i tlenek wapnia korzystniej wpływały na badane właściwości gleby niż kompost, powodując na ogół wzrost pH, sumy wymiennych kationów zasadowych, pojemności wymiennej, stopnia wysycenia gleby zasadowymi kationami wymiennymi oraz przyczyniając się do zmniejszenia kwasowości hydrolitycznej.

Introduction

The dynamic growth of the global economy, strictly dependent on the development of transportation, stimulates fuel consumption. Because Poland has scarce fuel resources, most of them are imported (WYSZKOWSKI et al. 2004, ZIÓŁKOWSKA and WYSZKOWSKI 2010). The transport of fuels and crude oil, their processing, storage and distribution may pose a threat to the environment, such as possible leakage of pollutants (WYSZKOWSKI and ZIÓŁKOWSKA 2011b). Also, machines and mechanical devices used in agriculture can spill small amounts of these substances, which permeate into soil and are dangerous not only to soil and grown crops but also to the following links in the food chain, including animals and people (MCGRATH 1992, KRAHL et al. 2002, OGBOGHODO et al. 2004). Petroleum products have an extremely bad effect on the quality of soils, as they block void spaces, inhibit or completely stop transport of air and water, and consequently cause formation of soil lumps (XU et al. 1996). As a result, physical, chemical and biological properties of soil suffer, which means that their productivity and fertility are inferior (ŁEBKOWSKA et al. 1995, KUCHARSKI and WYSZKOWSKA 2001, CARAVACA and RODÁN 2003 and WYSZKOWSKI, WYSZKOWSKA 2005 and WYSZKOWSKA, WYSZKOWSKI 2006, 2010, WYSZKOWSKI and ZIÓŁKOWSKA 2009a, 2009b, WYSZKOWSKI and SIVITSKAYA 2012). It is therefore important to control the influence of petroleum products on soil properties through application of various substances, including waste products (WYSZKOWSKI and ZIÓŁKOWSKA 2009b, 2011a, WYSZKOWSKI and SIVITSKAYA 2012, 2013).

The purpose of this study has been to test the effect of compost, bentonite and calcium oxide as substances alleviating light contamination with petrol and diesel oil, and to analyze some of the soil properties.

Material and Methods

The experiment was conducted in a greenhouse at the University of Warmia and Mazury in Olsztyn (north-eastern Poland). The soil used for the trials was proper brown soil originating from light loamy sand, and had the following properties: pH_{KCl} 5.6, hydrolytic acidity (HAC) 21.0 mmol(+) kg^{-1}, total exchangeable bases (TEB) 112.0 mmol(+) kg^{-1}, cation exchange capacity (CEC) 133.0 mmol(+) kg^{-1}, base saturation (BS) 84.2%, content of C_{org} 6.2 g kg^{-1}, content of available phosphorus 27.4 mg kg^{-1}, potassium 50.6 mg kg^{-1}, and magnesium 66.0 mg kg^{-1}. The experiment was set up to determine the effect of petrol and diesel oil introduced to soil in the following quantities: 0, 2.5, 5 and 10 cm^3 kg^{-1} of soil on the acidity and sorption properties of soil. In order to assess potential alleviation of the effect of the petroleum products on soil, the soil was enriched with compost (3%), bentonite (2% versus the mass of soil) and calcium oxide (50% CaO) in a dose corresponding to one full hydrolytic acidity. The compost was made from waste material originating from an agricultural farm: leaves (44%), manure (33%) and peat (23%). It was composted for 6 months. The concentration of macroelements in these substances (in g kg^{-1}) was as follows: compost: P – 2.32, K – 1.33, Mg – 1.47, Ca – 15.86, Na – 0.12; bentonite: P – 0.47, K – 2.43, Mg – 5.03, Ca – 26.72, Na – 12.11; calcium oxide: P – 0.10, K – 0.77, Mg – 2.65, Ca – 347.99, Na – 0.07. The detail specification of petroleum products was given in our previous article (WYSZKOWSKI and ZIÓŁKOWSKA 2009b). Additionally, each pot received macro- and micronutrients (in mg kg^{-1} of soil): N – 25 $CO(NH_2)_2$, P – 30 (KH_2PO_4), K – 70 (KH_2PO_4 + KCl), Mg – 50 ($MgSO_4 \cdot 7H_2O$), Mn – 5 ($MnCl_2 \cdot 4H_2O$), Mo – 5 [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] and B – 0.33 ($H_3BO_3$). All the components, i.e. petrol, diesel oil, compost, bentonite and calcium oxide as well as macro- and micronutrients (as aqueous solutions) were thoroughly mixed with 9.5 kg of soil and placed in polyethylene pots. Yellow lupine (*Lupinus luteus* L.) cv. Parys was grown as the main (first) crop, and followed by maize (*Zea mays* L.) cv. Scandia as the subsequent (second) plant. Plants harvest was 62 (yellow lupine) and 60 days (maize) after sowing. During the experiment, the soil moisture was maintained at the level of 60% of capillary water holding capacity. The trials ran in four replicates. Soil samples for laboratory analyses were taken during the harvest of the second crop, that is maize.

The soil samples were analyzed to determine: soil reaction (pH) potentiometrically in KCl aqueous solution of the concentration of 1 mol dm^{-3}, hydrolytic acidity (HAC) and total exchangeable bases (TEB) with Kappen method (LITYŃSKI et al. 1976). The results of hydrolytic acidity (HAC) and total exchangeable bases (TEB) were used to calculate cation exchange capacity (CEC) and base saturation (BS) from the following formulas: CEC = TEB

+ HAC; BS = TEB · CEC^{-1} · 100 (LITYŃSKI et al. 1976). Additionally, before the trials were set up, the following soil concentrations were determined: organic carbon (C_{org}) with Tiurin method, available phosphorus and potassium with Egner-Riehm method and available magnesium with Schachtschabel method (LITYŃSKI et al. 1976). The results of these determinations underwent statistical processing using a Statistica software package (StatSoft, Inc. 2010) and applying a three-factor analysis of variance ANOVA.

Results and Discussion

The presence of the petroleum products and the applied neutralization substances had a significant effect on the soil properties, analyzed after the test crops had been harvested (Tables 1, 2). The soil in the pots contaminated with petrol and seeded with yellow lupine and maize was characterized by a soil reaction similar to that in the pots with diesel oil. The petrol-polluted treatments were 26% higher in hydrolytic acidity than the pots with diesel oil. The total exchangeable bases, cation exchange capacity and base saturation were higher in the treatments polluted with diesel oil than with petrol.

In the objects where no contamination alleviating substances were added, the increasing rates of petrol contributed to a gradual decrease in the soil reaction (Tables 1, 2). In turn, in the analogous objects with diesel oil, the pollutant was not found to produce a direct effect on the soil pH. It was rather an indirect influence, attributable to a much stronger adverse effect of petrol than diesel oil on the mass of plants (WYSZKOWSKI and ZIÓŁKOWSKA 2009b), whose cultivation under normal conditions contributes to a rise in soil pH. In the objects without alleviating substances, the rising doses of petrol caused an evident increase in hydrolytic acidity, whereas in the pots with diesel oil, the acidity of the tested soil was lower than in the control. The total exchangeable bases and cation exchange capacity of soil were positively correlated with the rate of petroleum products in most of the objects, except the objects without soil amendments and with the CaO application in petrol-pollutes treatments, where the correlation was negative. In the non-amended objects, the incremental doses of petrol caused a gradual decrease in the total exchangeable bases and cation exchange capacity of the analyzed soil. In that objects, the lowest total exchangeable bases was noticed when the substratum contained 10 cm^3 of petrol per 1 kg of soil, where it was 13% lower than in the control treatment (unpolluted). A reverse relationship occurred in the analogous objects polluted with diesel oil (+26%). The highest cation exchange capacity, 14% higher than in the control (no diesel oil) was recorded in the treatments polluted with 10 cm^3 diesel oil kg^{-1} of soil. In the objects without alleviating amendments,

Table 1

pH and hydrolytic acidity (HAC) of soil after plants harvest

Dose of Pet or DO [cm^3 kg^{-1} of soil]	Kind of contamination									
	petrol (Pet)					diesel oil (DO)				
	kind of substance neutralizing effect of Pet and DO									
	without substances	compost	bentonite	CaO	average	without substances	compost	bentonite	CaO	average
$pH_{1M\ KCl}$										
0	6.06	6.01	6.62	7.16	–	6.06	6.01	6.62	7.16	–
2.5	6.02	6.10	6.78	7.14	–	6.40	6.23	6.87	7.07	–
5	5.84	6.16	6.89	7.13	–	6.12	6.21	6.93	7.04	–
10	5.72	6.10	6.92	7.07	–	6.27	6.28	7.05	7.23	–
Hydrolytic acidity (HAC) in mmol(+) kg^{-1} of soil										
0	17.6	18.4	11.3	9.0	14.1	17.6	18.4	11.3	9.0	14.1
2.5	19.1	16.9	12.4	9.8	14.5	11.3	12.0	10.1	9.4	10.7
5	20.6	16.5	10.5	9.8	14.4	12.0	12.0	9.0	9.8	10.7
10	22.5	17.3	10.1	10.1	15.0	11.3	10.5	9.8	10.9	10.6
Average	20.0	17.3	11.1	9.7	14.5	13.0	13.2	10.0	9.8	11.5
LSD	a – 0.37**, b – 0.52**, c – 0.52**, ab – 0.73**, ac – 0.73**, bc – 1.04**, abc – 1.46**									

LSD for: a – kind of petroleum substances, b – petroleum dose, c – kind of neutralizing substance
** – significant for $p = 0.01$, * – significant for $p = 0.05$

Table 2

Total exchangeable bases (TEB), cation exchange capacity (CEC) and base saturation (BS) in soil after plants harvest

Dose of Pet or DO [cm^3 kg^{-1} of soil]	Kind of contamination									
	petrol (Pet)					diesel oil (DO)				
	kind of substance neutralizing effect of Pet and DO									
	without substances	compost	bentonite	CaO	average	without substances	compost	bentonite	CaO	average
Total exchangeable bases (TEB) in mmol(+) kg^{-1} of soil										
0	78.0	72.0	101.0	126.0	94.3	78.0	72.0	101.0	126.0	94.3
2.5	74.0	84.0	106.0	118.0	95.5	84.0	90.0	114.0	124.0	103.0
5	70.0	90.0	112.0	116.0	97.0	88.0	84.0	116.0	120.0	102.0
10	68.0	86.0	116.0	110.0	95.0	98.0	94.0	122.0	128.0	110.5
Average	72.5	83.0	108.8	117.5	95.4	87.0	85.0	113.3	124.5	102.4
LSD	a – 2.16**, b – 3.06**, c – 3.06**, ab – 4.33**, ac – 4.33**, bc – 6.45**, abc – 8.66**									
Cation exchange capacity (CEC) in mmol(+) kg^{-1} of soil										
0	95.6	90.4	112.3	135.0	108.3	95.6	90.4	112.3	135.0	108.3
2.5	93.1	100.9	118.4	127.8	110.1	94.9	101.6	124.1	133.4	113.5
5	90.6	105.6	122.5	125.8	111.1	100.0	96.0	125.0	129.8	112.7
10	90.5	103.3	126.1	120.1	110.0	109.3	104.5	131.8	138.9	121.1
Average	92.5	100.1	119.8	127.2	109.9	100.0	98.1	123.3	134.3	113.9
LSD	a – 2.05**, b – 2.91**, c – 2.91**, ab – 4.12**, ac – 4.12**, bc – 5.82**, abc – 8.23*									
Base saturation (BS) [%]										
0	81.6	79.6	89.9	93.3	86.1	81.6	79.6	89.9	93.3	86.1
2.5	79.5	83.3	89.5	92.3	86.2	88.5	88.6	91.9	93.0	90.5
5	77.3	85.2	91.4	92.2	86.5	88.0	87.5	92.8	92.4	90.2
10	75.1	83.3	92.0	91.6	85.5	89.7	90.0	92.6	92.2	91.1
Average	78.4	82.9	90.7	92.4	86.1	87.0	86.4	91.8	92.7	89.5
LSD	a – 0.52**, b – 0.74**, c – 1.05**, ab – 1.05**, ac – 1.49**, bc – 1.49**, abc – 2.10**									

LSD for: a – kind of petroleum substances, b – petroleum dose, c – kind of neutralizing substance
** – significant for $p = 0.01$, * – significant for $p = 0.05$

the application of petrol in a dose as low as 2.5 cm^3 kg^{-1} of soil depressed the base saturation, and higher rates of petrol caused its further decrease compared to the unpolluted treatment. In the diesel oil objects, a higher degree of soil saturation with base cations was observed than in the control.

Petroleum products have a strong influence on soils and modify their properties (MEGHARAJ 2000, OGBOGHODO et al. 2004, WYSZKOWSKA and WYSZKOWSKI 2006, 2010). In general, the present experiments does not confirm the results obtained in the previous years (WYSZKOWSKI and ZIÓŁKOWSKA 2011a), when petrol contamination of soil caused an increase in the soil pH and its base saturation but lowered the hydrolytic acidity, total exchangeable bases and cation exchange capacity. The effect of diesel oil on these properties was much weaker than that produced by petrol, although the hydrolytic acidity was observed to have risen whereas the values of the total exchangeable bases, cation exchange capacity and base saturation were lower. The correlation between increased contamination with petroleum products in soil versus its higher pH has also been verified by VINNIK and OVCHAROV (2004). In a study completed by AKUBUGWO et al. (2009), soil contamination with petroleum products raised exchangeable acidity, total exchangeable bases, cation exchange capacity and base saturation. Likewise, ONYEIKE et al. (2000) found an increase in the total of exchangeable cations, while CARAVACA and RODÁN (2003) noticed a higher pH in polluted soil. In an experiment reported by KUCHARSKI and JASTRZĘBSKA (2005), diesel oil contamination of soil led to a higher acidity and lower total exchangeable bases, cation exchange capacity and base saturation.

Obviously, pH and the other analyzed soil properties were also affected by the type of pollution alleviating substances (Table 1). Compost, bentonite and CaO had significant influence on the pH of soil in pots polluted with petrol or with diesel oil, causing its growth. The most successful was the application of bentonite or calcium oxide. In the pots with petrol or diesel oil, introduction of either of these substances contributed to a considerable and significant rise in the value of pH compared to the control variant (no amendments).

All the applied substances alleviated the effects of soil pollution with petrol products, and produced a significant effect on reducing hydrolytic acidity of soil in the amended compared to non-amended pots (Table 1). Among the tested substances, particularly desirable effects were obtained after the application of bentonite or CaO, when the average hydrolytic acidity in the soil on which all the test crops were grown was significantly lower than in the treatments without amendments. A weaker effect, although generally a positive one, on hydrolytic acidity was also produced by compost. It was only in the treatments polluted with diesel oil and amended with compost that the average value of hydrolytic acidity was slightly higher than in the control (without

pollution alleviating substances). The positive effect on the total exchangeable bases was observed in the objects with bentonite and CaO, whose application caused an increase by 50% and 62% in petrol-polluted soil and 30% and 43% in pots contaminated with diesel oil, respectively, in the average value of this parameter versus the average value in the control treatments (no amendments) – Table 2. In the pots polluted with petrol, all the alleviating substances added to soil raised the average value of cation exchange capacity compared to the control treatment (no amendments). When the soil was contaminated with either petrol or diesel oil, the cation exchange capacity was most strongly raised by bentonite and CaO, which contributed to a considerable and significant increase in the average value of this parameter compared to the non-amended objects. The substances used to neutralize soils contaminated with petrol or diesel oil (especially bentonite and CaO) had a positive effect on the base saturation of soil, contributing to its high increase versus the control treatments (without amendments),

In general, application of organic matter, bentonite or lime has positive influence on soil properties (ESTEFANOUS and SAWAN 2003, GARAU et al. 2007, HU CHENG and CAO ZHIPING 2007, OGBOGHODO et al. 2004, WYSZKOWSKA and WYSZKOWSKI 2006, WYSZKOWSKI and ZIÓŁKOWSKA 2009a, 2009b, 2011a). Bentonite added to soil can cause a high increase in soil pH. In a study carried out by QUEROL et al. (2006), the soil pH rose from 3.3 up to 7.6 under the influence of bentonite. Also, CROKER et al. (2004) demonstrate improved cation exchange capacity of soil after application of bentonite. In some earlier research by WYSZKOWSKI and ZIÓŁKOWSKA (2011a), bentonite and calcium oxide produced the strongest and most positive effect on the analyzed soil properties, especially hydrolytic acidity. The effect of compost, although generally positive, is weaker than that produced by bentonite or calcium oxide. In experiments conducted by CAIRES et al. (2004) as well as FAGERIA et al. (2010), liming had a significant effect on soil pH and saturation with base cations. JOVANOVIC et al. (2006) demonstrated a positive effect of liming on soil pH. The application of lime to soil in a study reported by KUCHARSKI and JASTRZĘBSKA (2005) caused increased pH, total exchangeable bases, cation exchange capacity and base saturation while decreasing hydrolytic acidity COX et al. (2001) showed that compost had the most positive influence on soil quality, for example by rising its pH. Also WALKER et al. (2004) conclude that soil pH can be raised when organic matter such as manure or compost is introduced to soil. According to LIU et al. (2009), this effect is achieved when compost is added to soil. Apart from improving soil pH, compost has favourable influence on other soil properties, e.g. it increases the soil sorption capacity (OUÉDRAOGO et al. 2001, WALKER and BERNAL 2008).

Conclusions

1. The effect of petroleum products on soil properties depended on their dose and the application of compost, bentonite and calcium oxide to soil.

2. Petrol (unlike diesel oil) had a negative effect on sorption characteristics of soil, raising hydrolytic acidity and decreasing its pH, total exchangeable bases, cation exchange capacity and base saturation of soil.

3. Bentonite and calcium oxide produced a much stronger effect on the analyzed soil properties than compost, generally causing an increase in pH, total exchangeable bases, cation exchange capacity and base saturation while decreasing hydrolytic acidity.

4. The application of bentonite and calcium oxide proved to be most successful. Compost had a weaker albeit generally positive effect on the analyzed soil properties.

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PERFORMANCE EVALUATION OF ALPINE AND SAANEN GOATS IN POLAND IN THE YEARS 2000–2011

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K e y w o r d s: goats, milk performance, reproductive performance.

A b s t r a c t

Our study was based on the following data provided by the Central Statistical Office and the Polish Goats Breeders Association for the years 2000–2011: the total of all goats in Poland, including the Alpine and Saanen breeds; the number of goats under performance evaluation; milk and reproductive performance results. To determine developmental directions of the traits in the analysed period, we used the trend method; and to calculate the trends, we used a first degree linear function. Among the Polish goats evaluated in 2011, the most numerous is the Boer breed (meat), which constitutes 45.87% of the total; whereas among milk breeds, the most popular are the White Improved (14.95%), Coloured Improved (9.79%), Saanen (6.19%) and Alpine (4.64%). Reproductive performance for the Alpine goats was 83.5% to 100%, and for the Saanen goats 73.3% to 100%; whereas prolificacy was respectively 139.6% to 193.3%, and 126.1% to 213.1%. Analysing trend lines of milk performance traits in the examined goats in the years 2000–2011, we observed an extension of the milking period by approx. 2.5 days per year, as well as an increase of milk yield per lactation by approx 1.7 kg (Alpine breed) and approx. 1.6 kg (Saanen breed), fat yield by approx 0.5 kg and approx 0.9 kg, and protein yield by approx 0.4 kg and 0.5 kg per year. All milk from evaluated goats had similar fat and protein content.

OCENA UŻYTKOWOŚCI KÓZ RAS ALPEJSKIEJ I SAANEŃSKIEJ W POLSCE W LATACH 2000–2011

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S ł o w a k l u c z o w e: kozy, użytkowość mleczna, użytkowość rozpłodowa.

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Abstrakt

W pracy uwzględniono: stan pogłowia kóz w Polsce ogółem, w tym rasy alpejskiej i saaneńskiej, liczbę kóz objętych kontrolą użytkowości oraz wyniki użytkowości mlecznej i rozpłodowej, korzystając z danych opublikowanych przez Główny Urząd Statystyczny oraz Polski Związek Owczarski w latach 2000–2011. Do nakreślenia linii rozwojowych badanych cech w omawianym okresie zastosowano metodę trendów, a do wyliczenia trendów wykorzystano funkcję liniową pierwszego stopnia. W krajowym pogłowiu kóz dominującą rolę odgrywa rasa burska (mięsna), która stanowi 45,87% pogłowia kóz objętych oceną użytkowości, z ras mlecznych – biała uszlachetniona (14,95%), barwna uszlachetniona (9,79%), saaneńska (6,19%) i alpejska (4,64%). Płodność kóz rasy alpejskiej wynosiła od 83,5% do 100%, saaneńskiej od 73,3% do 100%, natomiast plenność odpowiednio od 139,6% do 193,3% i od 126,1 do 213,1%. Analizując linie tendencji cech użytkowości mlecznej ocenianych kóz w latach 2000–2011, zaobserwowano wydłużanie się okresu dojenia o około 2,5 dnia na rok, wzrost wydajności mleka za laktację o około 1,7 kg (rasa alpejska) i ok. 1,6 kg (rasa saaneńska) oraz wzrost wydajności tłuszczu o ok. 0,5 kg i ok. 0,9 kg, a białka o ok. 0,4 i 0,5 kg na rok. Mleko ocenianych kóz charakteryzowało się zbliżoną zawartością tłuszczu i białka.

Introduction

Rearing and breeding of goats have a long history in Poland. After World War II, there were approx 800 thousand goats in the country, but over the following years, this number was constantly decreasing. In 1970, it was already down to 40 thousand, resulting in discontinuation of breeding and performance evaluation, and the animals were even no longer listed in the statistics prepared by the Central Statistical Office (RYNIEWICZ and KRZYŻEWSKI 1997, STRZELEC and NIŻNIKOWSKI 2009). The subject of goat breeding returned in the 1980s, which was caused by social and economical changes occurring in Poland and people's growing interest in goat milk (RYNIEWICZ and KRZYŻEWSKI 1997). In 1983, goat performance evaluation was resumed, mainly focussing on goat milk recording, selection of kids and young animals, choosing animals for further breeding, recording animals in their flock books, and keeping goat breeding documentation. In 1991, a standard was introduced for qualitative requirements of pasteurized goat milk (BAGNICKA and ŁUKASZEWICZ 2000, MROCZKOWSKI et al. 1997, SZYMANOWSKA and LIPECKA 2000).

Data gathered so far on our goats by milk recording indicate that milk from Polish goats is not the same in terms of yield and chemical content (RYNIEWICZ and KRZYŻEWSKI 1997, STRZELEC and NIŻNIKOWSKI 2009). Milk yield improvement in goats can be achieved through selection of existing goats and improvement of native breeds, which may be done by mixing them with bucks from countries with a strong record of highly productive bucks, such as France (RYNIEWICZ and KRZYŻEWSKI 1997, BAGNICKA and ŁUKASZEWICZ 2000).

The objective of the research was to analyse both reproductive and milk performance in Alpine and Saanen goats in Poland evaluated in 2000–2011.

Material and Methods

Our study was based on the following data provided by the Central Statistical Office and the Polish Goats Breeders Association for the years 2000–2011: the total of all goats in Poland, including the Alpine and Saanen breeds; the number of goats under performance evaluation; milk and reproductive performance results. To determine developmental directions of the traits in the analysed period, we used the trend method. we calculated the trends using a first degree linear function, as in the following formula (ZAJĄC 1988):

$$y_t = a_t + b$$

where:
a_t – slope (of regression line), indicating annual rate of a given trait's increase;
t – time indicated as subsequent years;
b – trait level in a given period.

Accuracy of trend lines was evaluated based on coefficients of determination (R^2), where:

$$R^2 = \frac{\sum_{t=1}^{n} \left(\hat{y}_t - \bar{y} \right)^2}{n}$$

y_t – actual value of the y variable at the t time;
$y \wedge t$ – theoretical value of the dependent variable (based on model);
$\bar{y}$ – arithmetic means of dependent variable empirical values.

Results and Discussion

According to the Central Statistical Office, the total number of goats in Poland in 2011 was 111,824, and 194 animals were under performance evaluation, which constitutes merely 0.18%. Currently, among all goats in Poland, the most numerous is the Boer meat breed, which constitutes 45.87% of the total; whereas among milk breeds, the most popular are the White Improved (14.95%), Coloured Improved (9.79%), Saanen (6.19%) and Alpine (4.64%) – Figure 1.

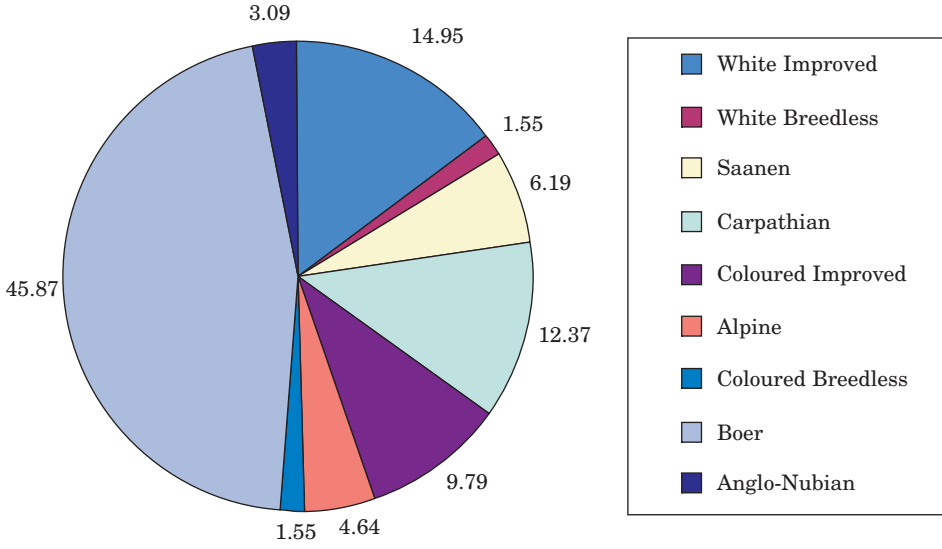

Fig. 1. Percentage of goats evaluated in 2011

As shown by data in Figure 2, the number of evaluated goats decreased from 278 animals for the Alpine breed, and 477 for the Saanen breed in 2009, down to 9 and 12 animals respectively in 2011. A dramatic drop in goat population was noted in 2007, which saw the number of Alpine goats falling from 144 (in 2006) to only 20, and Saanen from 726 to 23. This could have been caused, among other reasons, by introduction of payments for evaluation of milk performance of goats.

Temporal trends for the population of Alpine and Saanen goats evaluated in terms of their performance were negative. This means that the number of goats of both evaluated breeds has decreased over the 10 years, by the annual average of approx 33 Alpine and approx. 67 Saanen goats (Figure 2).

When milk production is the predominant use of goats, reproductive indexes play a very important role, not only because they define milk production but also economic effectiveness of goat rearing. Supplied details indicate that, over 12 years, fertility of evaluated goats was 83.5% to 100% in Alpine goats, and 73.3% to 100% in Saanen goats; whereas prolificacy 139.6% to 193.3% and 126.1% to 213.1% respectively. The highest fertility (in proximity of 100%) was found in goats in the years 2008–2010. In 2011, this result was lower by approx. 8% as compared to 2010. Analysing prolificacy in Alpine goats, we found an increase until 2008, and then a drop. In Saanen goats, prolificacy grew in the years 2002–2010, except for 2007, when it was the lowest at 108.5%. In 2011, a prolificacy increase was observed in Alpine goats

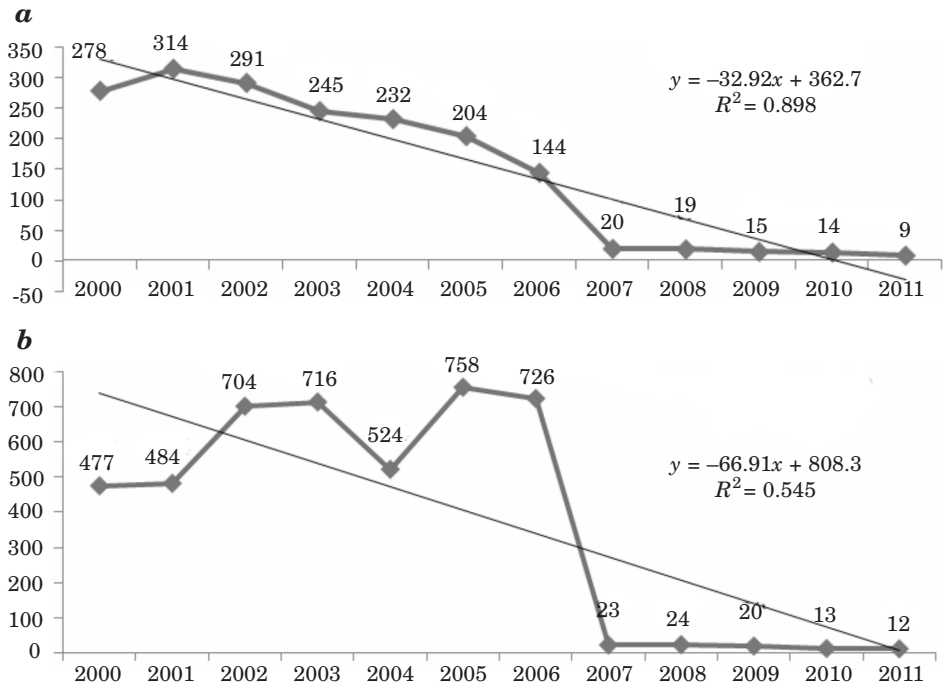

Fig. 2. Temporal trends for the population of Alpine (*a*) and Saanen (*b*) goats evaluated in terms of performance

by nearly 10 percent, whereas in Saanen goats a significant drop by as much as 50% as compared to 2010 (Figure 3).

Developmental trend lines for traits related to reproductive performance of Alpine and Saanen goats indicate constant, however small, increase in fertility in the years 2000–2011, on average by approx. 1 and 0.5%, and prolificacy respectively by 2.4 and 5.2% annually. Coefficient of determination (R^2) varied from 0.049 to 0.433 (Figure 3).

SZYMANOWSKA and LIPECKA (2000), analysing fertility and prolificacy of goats evaluated in terms of their performance in the years 1990–1999, observed that fertility in Polish goats was 85.3% to 97.4%, whereas prolificacy 164% to 187%. These values are similar to those found in our study (2000–2011). Higher prolificacy in Saanen goats in the years 2008–2010 (188.1–213.1%) may be connected with a small number of animals under performance evaluation.

Data shown in Figure 4 and Table 1 indicate that the average milk yield for Alpine goats in 2000 was 639 kg per a 243-day lactation, which translates to a daily yield of 2.6 kg. The lowest yields were found in 2002 (510.7 kg) and 2006

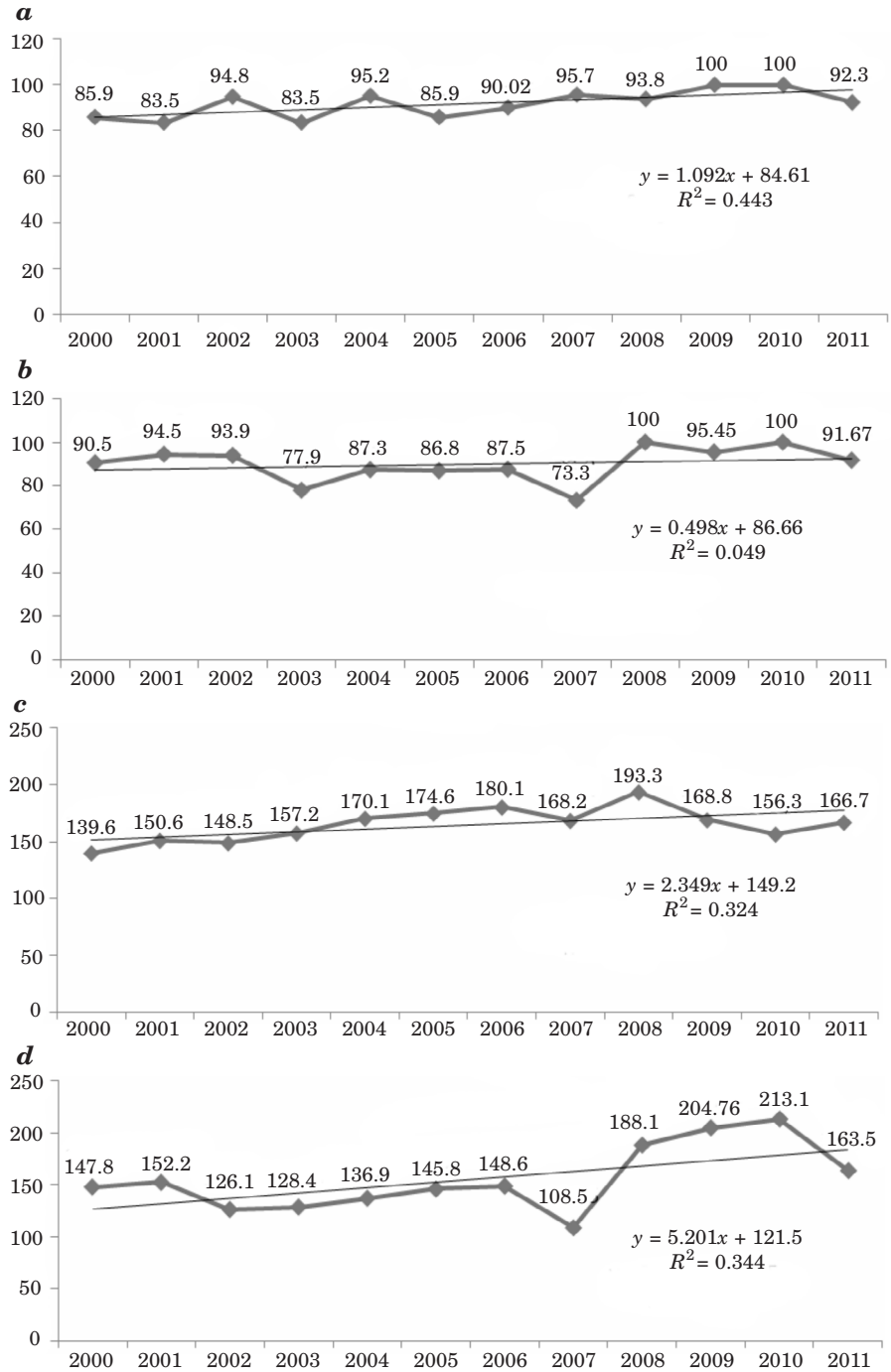

Fig. 3. Temporal trends for reproductive performance traits in Alpine and Saanen goats: *a* – Alpine gosts fertility, *b* – Saanen gosts fertility, *c* – Alpine goats prolificacy, *d* – Saanen goats prolificacy

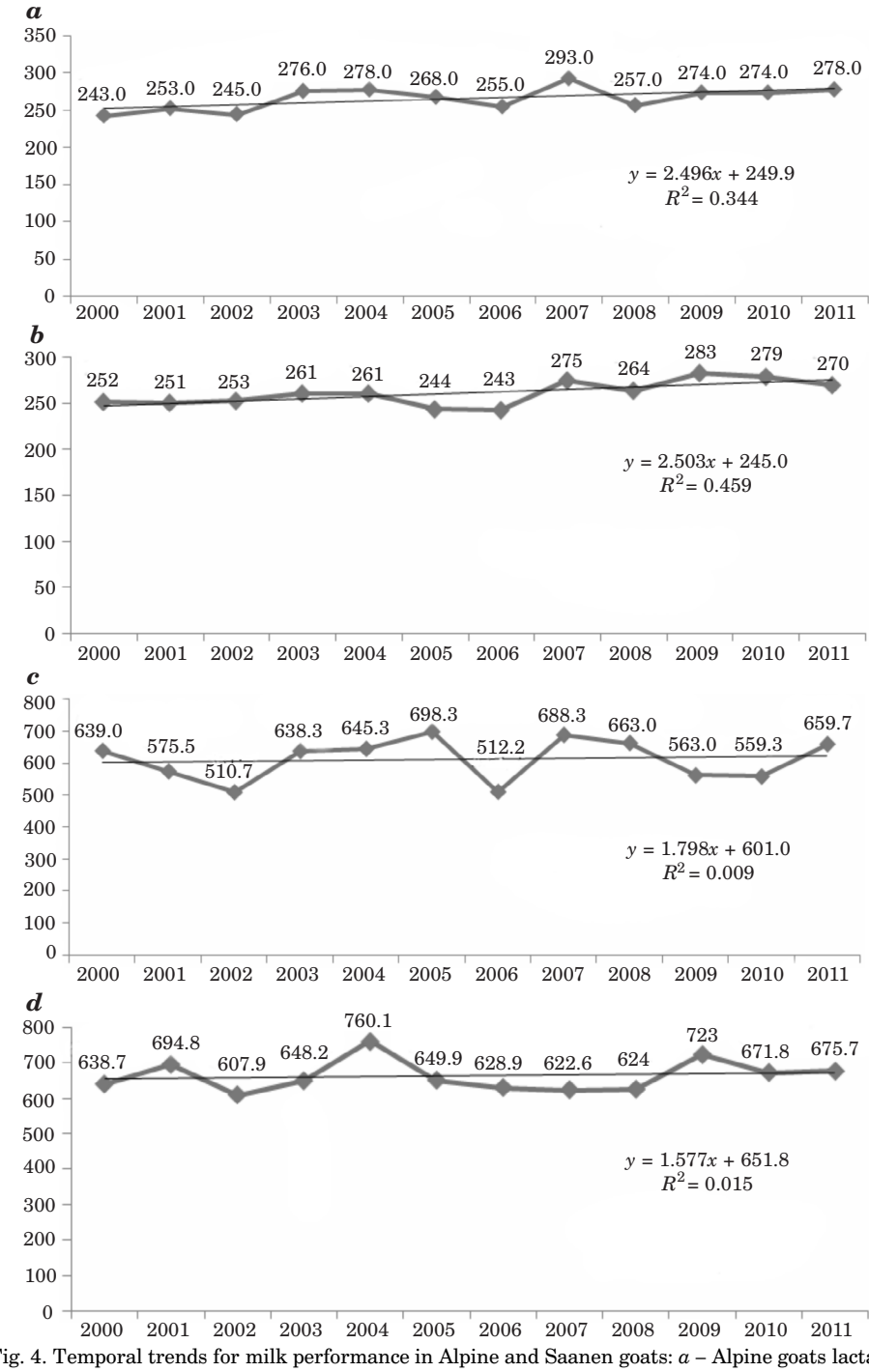

Fig. 4. Temporal trends for milk performance in Alpine and Saanen goats: *a* – Alpine goats lactation time, *b* – Saanen goats lactation time, *c* – Alpine goats milk yield, *d* – Saanen goats milk yield lactation

(512.2 kg). In 2011, lactation of evaluated goats lasted up to 278 days with the yield of 660 kg, and a daily yield of approx 2 kg (Figure 4 and Table 1). Milk performance in the year 2000 was similar in both analysed breeds. The longest lactation and the highest milk yield in these goats was recorded in 2009, equalling respectively 283 days and 723 kg of milk. In 2011, lactation was shortened by 13 days, and yield per lactation decreased by approx. 50 kg (Figure 4).

Table 1
Trait trends for daily milk yield as well as fat and protein yield and concentration in the milk of Alpine and Saanen goats in the years 2000–2011

Traits	Breed	Trend	Coefficient of determination (R^2)
Daily milk yield [kg]	Alpine	$y = 0.068x + 1.992$	0.3215
	Saanen	$y = 0.031x + 1.292$	0.3325
Fat yield [kg]	Alpine	$y = 0.503x + 17.223$	0.1613
	Saanen	$y = 0.889x + 20.765$	0.2314
Fat content [%]	Alpine	$y = -0.055x + 3.559$	0.4453
	Saanen	$y = 0.036x + 3.578$	0.1427
Protein yield [kg]	Alpine	$y = 0.371x + 14.653$	0.1433
	Saanen	$y = 0.493x + 16.902$	0.2674
Protein content [%]	Alpine	$y = 0.015x + 2.815$	0.1349
	Saanen	$y = 0.018x + 2.836$	0.5405

Similar dependencies were pointed out by BAGNICKA and ŁUKASZEWICZ (2000) in their analyses concerning assessment of the national goat milk performance database.

In milk production breeding of goats, not only the amount of milk is important but also its quality, which defines suitability of milk for processing. Average fat content in milk from Alpine goats was 3.0% to 3.8%, and protein content 3.0% to 3.4%; in milk from Saanen goats, these values equalled 3.0% to 3.8% and 2.9% to 3.2% respectively (Table 1).

Similar results of goat productivity assessment in the region of the town of Lublin was observed by SZYMANOWSKA et al. (2008). Whereas ŽAN et al. (2006), who evaluated milk quality in Saanen and Alpine pasture-fed goats, found that their milk contained the average of 3.77% (Saanen) and 3.36% (Alpine) of fat, and 3.40% and 2.95% of protein respectively. Alpine goat milk in a study conducted by SORYAL et al. (2005) contained the average of 2.76% fat and 2.53% of protein, which is less than what we established in our study. Whereas milk from Greek goats in a study by KONDYLI et al. (2007) was rich in these substances: concentration of fat was 3.93–4.46% and protein 3.18–3.70%.

Analysing trend lines for milk performance of evaluated goats in the years 2000–2011, we observed that the milking period lengthened by approx. 2.5 days a year, milk yield per lactation grew by approx 1.7 kg (Alpine goats) and approx. 1.6 kg (Saanen goats) – Figure 4. We also established an increase in fat yield in Alpine and Saanen goat milk by approx 0.5 kg and approx 0.9 kg, as well as protein by approx 0.4 and 0.5 kg per year respectively. We found a minor increase in fat concentration in Saanen goat milk (by approx 0.04%) and a decrease of fat content in Alpine goat milk by approx. 0.06% per year. Protein content in milk from both evaluated breeds increased by approx. 0.02% annually. The R^2 value equalled 0.009 to 0.5405 (Table 1).

Earlier research on goat milk performance conducted for the entire population of evaluated animals showed that the milking period becomes shorter and the yield decreases. Furthermore, fat content in milk was higher, whereas protein content lower (MISTRZAK and BERNACKA 2011, NIŻNIKOWSKI 1996, STRZELEC and NIŻNIKOWSKI 2009, SZYMANOWSKA and LIPECKA 2000).

The conclusion is that over the years 2000–2011 the number of Alpine and Saanen goats evaluated in terms of their performance decreased dramatically. The current goat breeding situation in Poland predominantly results from the lack of breeding programmes and small interest of breeders in animal evaluation. Consequently, possibilities of any efficient goat breeding in Poland are limited, mainly due to the small population size of animals under performance evaluation and the fact that they are scattered over a large area.

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EFFECT OF ENVIRONMENTAL FACTORS ON THE STRUCTURE OF POPULATIONS OF PULSATILLA PATENS (L.) MILL.

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K e y w o r d s: *Pulsatilla patens*, N-E Poland, gradient of variability, redundancy analysis.

A b s t r a c t

Pulsatilla patens is a rare and endangered component of the European flora. In Poland, the principal area occupied by the species is in north-eastern part of the country. The study of the *Pulsatilla patens* populations was conducted in 2011–2012, in the 15 sites in the two Natura 2000 wildlife refuges: the Myszynickie Bory Sasankowe and the Military Training Grounds in Orzysz. The purposes of the study have been to analyze the structure of populations of *Pulsatilla patens* growing in two types of habitats: forest and non-forest ones; to analyze the influence of selected habitat-specific characteristics on the structure of Easter pasque flower populations; to work out a model which will explain the dependence of the structure of a population of *Pulsatilla patens* on environmental conditions. Most of the populations (10) grew at the forest sites. They were less numerous, with an average of 9 rosettes at mean in site, in total, comprised 89 rosettes of *P. patens*.

On the non-forest populations, in total 206 rosettes of *P. patens* were counted, the average number of rosettes in a population was about 41; the average number of flowering rosettes reached *ca* 16. On forest sites, flowering rosettes had on average 3 flowers, whereas on non-forest sites the analogous number was 5.5. In forest sites statistical analysis has shown the positive correlation between the number of fruiting rosettes and the shading in the shrub layer as well as the negative correlation between the number of fruiting rosettes and the share of space potentially suitable for germination. In non-forest habitats, the relationship between the number of fruiting rosettes and the shading in the herbaceous vegetation layer is worth noticing. The comparative analysis accomplished with U Mann-Whitney test on both population and habitat characteristics relative to the habitat as a variable (forest or non-forest) showed statically significant differences between the number of rosettes and shading in layer of trees. Ordinance RDA explains about 62.7% of the total population variability.

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WPŁYW CZYNNIKÓW SIEDLISKOWYCH NA STRUKTURĘ POPULACJI *PULSATILLA PATENS* (L.) MILL.

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S ł o w a k l u c z o w e: sasanka otwarta, północno-wschodnia Polska, gradient zmienności, analiza redundancji.

A b s t r a k t

Pulsatilla patens to rzadki i zagrożony element flory europejskiej. W Polsce sasanka otwarta występuje najczęściej w północno-wschodniej części kraju. Badania prowadzono w latach 2011–2012 na 15 stanowiskach, na terenie dwóch obszarów Natura 2000 – w Myszynieckich Borach Sasankowych i na Poligonie Orzysz. Badania miały na celu: analizę struktury populacji *Pulsatilla patens* występujących na dwóch typach siedlisk – leśnych oraz nieleśnych, analizę wpływu wybranych czynników siedliskowych na strukturę populacji oraz opracowanie modelu wyjaśniającego zależność struktury populacji *Pulsatilla patens* od czynników siedliskowych. Większość populacji (10) występowała na stanowiskach leśnych, cechowała się niewielką liczbą osobników, średnio na 1 stanowisku odnotowano ok. 9 rozet, kwitnących – średnio 5, łącznie – 89 osobników sasanki. Zdecydowanie więcej osobników liczyły populacje na stanowiskach nieleśnych – rosło tam 206 rozet *Pulsatilla patens*, średnia liczba osobników w populacji wynosiła ok. 41, osobników kwitnących było średnio ok. 16. Na siedliskach leśnych osobniki kwitnące miały średnio po 3 kwiaty, natomiast na siedliskach nieleśnych – 5.5. W analizie korelacji wykazano, że na siedliskach leśnych wystąpiła dodatnia korelacja między liczbą osobników owocujących a ocienieniem w warstwie krzewiastej oraz korelacja ujemna między liczbą osobników owocujących a udziałem powierzchni potencjalnie dogodnej do kiełkowania. Na stanowiskach nieleśnych na uwagę zasługuje zależność między liczbą osobników owocujących a ocienieniem w warstwie roślin zielnych. Porównując testem U Manna-Whitneya wszystkie analizowane cechy zarówno populacji, jak i siedliska względem zmiennej stanowisko (leśne lub nieleśne), wykazano istotne statystycznie różnice między liczbą osobników oraz ocienieniem w warstwie drzew. Ordynacja RDA tłumaczy ok. 62,7% ogólnej zmienności populacji.

Introduction

Pulsatilla patens is a rare and endangered component of the European flora. It is a species listed in the in Bern Convention (*Convention on the conservation*... 1979) and in Annex II and IV of the European Habitats Directive (*Council Directive 92/43/EEC*... 2004). In Poland, it is protected by law and listed as a low risk (LR) taxon in the Red Data Book of Poland, (WÓJTOWICZ 2001) or as a species to be critically endangered in the Red List of Vascular Plants in Poland (ZARZYCKI, SZELĄG 2006).

Most stations *P. patens* (over 80%) are located in northern Poland (within the Podlaskie, Warmia and Mazury and Kuyavian-Pomeranian) (WOJTOWICZ 2004) must be underlined that the species is gradually disappearing in the

whole country – in central Poland both the number of sites and abundance of particular populations have decreased, while in the west and south this species has lost most of its habitats over the past ten years (CIOSEK 1999, NOWAK et al. 2000, CHMURA 2003, WÓJTOWICZ 2004, ZYCH 2007). Also in north-eastern Poland, there are fewer sites occupied by *Pulsatilla patens* (JUŚKIEWICZ-SWACZYNA 2010a, ŁASKA and SIENKIEWICZ 2010). A similar tendency towards disappearance of sites and drastic decrease in the size of existing populations has been observed in other European countries (RÖDER and KIEHL 2006, UOTILA 1996, KALLIOVIRTA et al. 2003). For example, in central Germany the number of *Pulsatilla patens* rosettes has declined by 60% in the last 12 years (RÖDER and KIEHL 2006). The reasons why populations of the *Pulsatilla patens* are threatened include low competitiveness against other undergrowth plant species, lack of natural disturbances (fires, windblows) in forest ecosystems (UOTILA 1996, KALLIOVIRTA et al. 2006), destruction of flowers and fruit-bearing shoots by animals, hybridization with other species belonging to the genus *Pulsatilla* (UOTILA 1996), reduced seed production due to locally decreasing numbers of pollinating insects (ZYCH 2007), and unfavourable weather conditions such as long and freezing cold winters (CHMURA 2003). According to the life cycle stages, KALLIOVIRTA et al. (2003) distinguished three types of *Pulsatilla patens* populations:

i) increasing, in which relatively many rosettes were in the two smallest size classes, the proportion of seedlings were high and the proportions of generative plants averaged 10%;

ii) stable, in which the proportion of the rosettes in various life-cycle stages remained almost unchanged;

iii) decreasing, in which vegetative adults accounted for the vast majority (96 %) of all rosettes and seedlings were usually completely absent.

In Poland, *Pulsatilla patens* was covered by a nature monitoring project run in 2010–2011. Studies carried out at 34 locations then showed that the principal area occupied by the species is in north-eastern Poland (PAWLIKOWSKI 2012). Considering the continual tendency for populations of *Pulsatilla patens* to grow less abundantly, it is essential to conduct comprehensive research on the biology and ecology of this species, which will contribute to possible development and implementation of effective protection methods.

The study presented in this paper was performed in order to gain better understanding of the ecology of populations of *Pulsatilla patens* in north-eastern Poland, where the species is most common. Two nature reserves called the Myszynieckie Bory Sasankowe and the Military Training Grounds in Orzysz, where Easter pasque flower grows relatively numerously, belong to the Nature 2000 network. This means that we should be to protect both the species and its habitats, because in Poland, populations of *P. patens* are disappearing

from many sites (CIOSEK 1999, CHMURA 2003, WÓJTOWICZ 2004). The purposes of the study have been:

1. to analyze the structure of populations of *Pulsatilla patens* growing in two types of habitats: forest and non-forest ones;
2. to analyze the influence of selected habitat-specific characteristics on the structure of *Pulsatilla patens* populations;
3. to work out a model which will explain the dependence of the structure of a population of *Pulsatilla patens* on environmental conditions.

Material and Methods

Study species

Pulsatilla patens has a circumpolar distribution (HULTÉN and Fries 1986). In Europe, it occurs in the central and central-eastern parts (*Atlas florae europaeae*... 1989). In Poland, *P. patens* appears in boreal forests *Vaccinio-Piceetea* (MATUSZKIEWICZ 2001), xerothermic and psammophilous grasslands (CEYNOWA 1968, CIOSEK 1999, JUŚKIEWICZ-SWACZYNA 2010b), dry heath (JUŚKIEWICZ-SWACZYNA 2010b). In other regions of Europe, this species exists in calcareous grasslands in Germany (RÖDER and KIEHL 2006); open, dry and pine-dominated forests in Finland, mainly on eskers and adjacent sandy areas, pastures, off paths and roads, and at the edges of yard areas (UOTILA 1996, KALLIOVIRTA et al. 2006); in steppe and wood communities in Russia (RYSINA 1981); and in pine-dominated boreal heath forests of the *Cladonia* or *Calluna* site type and in dry boreal forests of the *Vaccinium vitis-idaea* site type, occasionally also in more humid *Vaccinium myrtillus* site type habitats in Estonia (PILT and KUKK 2002).

The *Pulsatilla patens* is a hemicryptophyte and blooms from March to early May. The number of flowers produced by one individual is varied, e.g. from 1 to 12 in north-eastern Poland (JUŚKIEWICZ-SWACZYNA 2010b) up to 50 in Finnish populations (KALLIOVIRTA et al. 2006). Leaves do not develop until the end of flowering and remain on plants until autumn; fruits ripen from April to June (WÓJTOWICZ 2000, 2004).

According to KALAMEES et al. (2005), important factors that affect germination and seedling establishment of *Pulsatilla patens* are cyclic, natural, or controlled wood fires, which change light intensity conditions and destroy moss and litter layers. The seeds are dispersed by wind in June and July over short distances. In good conditions (warm and moist weather) germination occurs in late summer, but if the weather is cold and dry, it is delayed until the next spring, or seeds may remain in the transient seed bank (PILT and KUKK 2002). Effective germination takes place only in the gaps in the vegetation

cover, where other plants circuit is negligible (PILT and KUKK 2002, WÓJTOWICZ 2004, KALLIOVIRTA et al. 2006, RÖDER and KIEHL 2006, JUŚKIEWICZ-SWACZYNA 2010b, JUŚKIEWICZ-SWACZYNA and CHOSZCZ 2012). Intensity of flowering and fruit production is affected by climatic conditions, e.g. snow cover, winter temperature or insolation. Mild winters with warmer periods act adversely, but abundance of *P. patens* populations may increase in years when the continental climate prevails (UOTILA 1996, WÓJTOWICZ 2004).

Study area

The study of the *Pulsatilla patens* populations was conducted in 2011–2012, in the Natura 2000 wildlife refuges: the Myszynickie Bory Sasankowe (PLH140049) and the Military Training Grounds in Orzysz (PLB280014) – Figure 1. The Myszynieckie Bory Sasankowe lies in the mesoregion called Kurpiowska Plain, which belongs to the macroregion of Northern Masovian Lowland. The refuge lies in the southern part of the masurian sandur. The landscape consists of glacial lakes, sandy dunes, moraine hills composed of loams and gravel, and waterlogged terraces. The area called the Military Training Grounds in Orzysz lies in the mesoregion of the Great Masurian Lakes, which belongs to the macroregion named the Masurian Lake District. The landscape consists of sandy plains and moraine hills. This vast and open area is overgrown with well-developed xeric sand calcareous grassland and dry heaths. The northern part of the area is overgrown by pine forest (KONDRACKI 2001).

Fig. 1. Location of the research sites in the Natura 2000 wildlife refuges in NE Poland: *1* – Military Training Grounds in Orzysz, *2* – Myszynieckie Bory Sasankowe

North-eastern Poland lies within the climatic region of Mazury and Podlasie, which encompasses the eastern part of the Masurian Lakes and Podlasie (WOŚ 1996). The weather is characterized by strong affinity to the continental climate with its typical duration of seasons, such as long and freezing winter (110 days), long summer (90 days), but shorter spring and autumn. The snow cover remains here for a long time (85–96 days) and can be up to 10 cm thick. The average annual temperature is low (7°C) and the growing season is short, lasting about 200 days (GÓRNIAK 2000).

Data collection and treatment

The research sites were identified according to the authors' knowledge and documentation of the location of *P. patens* sites supplied by the Forest District Office in Myszyniec. The 15 sites were divided into two groups: forest habitats in boreal forests *Peucedano-Pinetum* and non-forest ones located in open areas, such as dry heath and roadsides. Because of the phenology of the species (KALLIOVIRTA et al. 2003, PAWLIKOWSKI 2011), field observations were carried out twice: during the inflorescence stage (in April) and in the summer (in July). For determination of the number of rosettes in a population, a rosette was considered as a unit (a specimen) (PAWLIKOWSKI 2011), while leaf rosettes closer than 10 cm from each other were assuemd to belong to the same individual (KALLIOVIRTA et al. 2006). In order to assess the condition of a population, the following indicators were determined: total number of rosettes, number of flowering rosettes, number of fruiting rosettes. The following habitat-specific characteristics which could potentially have considerable influence on populations of *Pulsatilla patens* were evaluated: shading (assessed separately percentage for layers – *a* – layer of trees, *b* – layer of shrubs and *c* – layer of understory), average height of understory in the summer season within the area covered by *P. patens*, share of expansive herbaceous plants and cover of bare soil area available for germination accordance with the methodology PAWLIKOWSKI 2011.

Spearman's rank correlations at a significance level of $\alpha = 0.05$ were applied for testing statistical significance of relationships between characteristics of the populations and parameters of the habitats. Comparisons of populations growing in forest and non-forest habitats were completed with U Manna-Whitney's test. All statistical analyses were performed using STATISTICA 10.

A preliminary detrended correspondence analysis (DCA) revealed a first gradient length of 0.16 SD, inferring that models based on linear species response models were appropriate for the data structure (TER BRAAK and SMILAUER 1998, LEPŠ and ŠMILAUER 2007).

The data on the environment and morphometryof *Pulsatilla patens* was explored using redundancy analyses (RDA) (VAN DEN WOLLENBERG 1977), a constrained form of principal components analysis (HOTELLING 1933) in CANOCO (TER BRAAK and ŠMILAUER 2002).

Statistical significance tests were carried out using Monte Carlo permutation tests. The Monte Carlo test was used to test the significance of the axis eigenvalues generated in the analysis and species-environmental correlation (using 5 000 unrestricted iterations).

Results

In the Natura 2000 network wildlife refuge called the Myszynieckie Bory Sasankowe, populations of *Pulsatilla patens* grew mainly at 10 forest sites, with just 2 populations located in open area: on a sandy bank of a road and on a roadside. The total number of Easter pasque flower specimens was 133. In the other refuge, named the Military Training Grounds, studies comprised 5 populations growing in non-forest habitats: 3 on dry heath and 2 near forest roads; the total number of rosettes was 162.

The forest populations were less numerous. They consisted of 10 sites, with an average of 9 rosettes at a single site. In total, the 10 sites comprised 89 rosettes of *P. patens*. Two populations had only vegetative rosettes and the other 8 populations presented on average 5 flowering specimens. The number of fruiting rosettes was small, no more than 6. Much better results were obtained from observations on the non-forest populations, where in total

Table 1

Pulsatilla patens – population and environmental characteristics

Characteristic	Forest sites $n=5$			Non-forest sites $n=10$		
	min.	max.	X ± SD	min.	max.	X± SD
Total number of rosettes	4	28	8.9 ± 7.42	8	145	41.2 ± 58.69
Number of flowering rosettes per population	0	9	4.8 ± 2.78	0	38	15.8 ± 16.24
Number of flowers per population	0	38	14.6 ± 10.58	0	221	87.6 ± 103.82
Number of fruiting rosettes per population	0	6	1.9 ± 1.85	0	41	14.2 ± 18.78
Shading in layer of trees [%]	20	70	45.0 ± 21.60	0	5	1.0 ± 2.24
Shading in layer of shrubs [%]	0	60	20.5 ± 20.88	0	25	9.4 ± 12.10
Shading in layer of understory [%]	20	95	52.5 ± 26.69	10	90	42.0 ± 39.62
Height of understory [cm]	20	45	30.0 ± 10.00	25	40	30.0 ± 6.12
Share of expansive herbaceous species [%]	0	80	18.0 ± 28.69	5	25	14.0 ± 8.94
Place for germination [%]	2	40	11.4 ± 15.12	5	30	15.0 ± 10.00

206 rosettes of *P. patens* were counted. The average number of rosettes in a population was about 41; the average number of flowering rosettes reached *ca* 16. On forest sites, flowering rosettes had on average 4,8 flowers, whereas on non-forest sites the analogous number was 15.8 (Table 1).

Correlation analysis has shown numerous statistically significant relationships present in forest habitats (Table 2). Noteworthy is the positive correlation between the number of fruiting rosettes and the shading in the shrub layer as well as the negative correlation between the number of fruiting rosettes and the share of space potentially suitable for germination. In non-forest habitats, the relationship between the number of fruiting rosettes and the shading in the herbaceous vegetation layer is worth noticing (Table 3).

Table 2
Spearman correlation coefficients (r_s) of the characteristics of the *Pulsatilla patens* populations and environmental characteristics in forest sites ($p \leq 0.05$)

Characteristic	r_s
Total number of rosettes – number of flowering rosettes	0.72
Total number of rosettes – number of fruiting rosettes	0.84
Number of flowering rosettes – number of fruiting rosettes	0.74
Number of flowering rosettes – number of flowers	0.65
Number of fruiting rosettes – shading in layer of shrubs	0.67
Number of fruiting rosettes – place for germination	-0.74
Shading in layer of shrubs – place for germination	-0.65
Shading in layer of understory – share of expansive herbaceous species	0.74

Table 3
Spearman correlation coefficients (r_s) of the characteristics of the *Pulsatilla patens* populations and environmental characteristics in non-forest sites ($p \leq 0.05$)

Characteristic	r_s
Total number of rosettes – number of flowers	0.90
Number of flowering rosettes – number of flowers	0.90
Number of fruiting rosettes – shading in layer of understory	0.89

The comparative analysis accomplished with U Mann-Whitney test on both population and habitat characteristics relative to the habitat as a variable (forest or non-forest) showed statically significant differences between the number of rosettes ($p=0.004$) and shading in layer of trees ($p= 0.003$).

In order to determine the effect of all the analyzed habitat-specific factors on the structure of the *Pulsatilla patens* populations, RDA ordinance was applied (Figure 2). It explains about 62.7% of the total population variability.

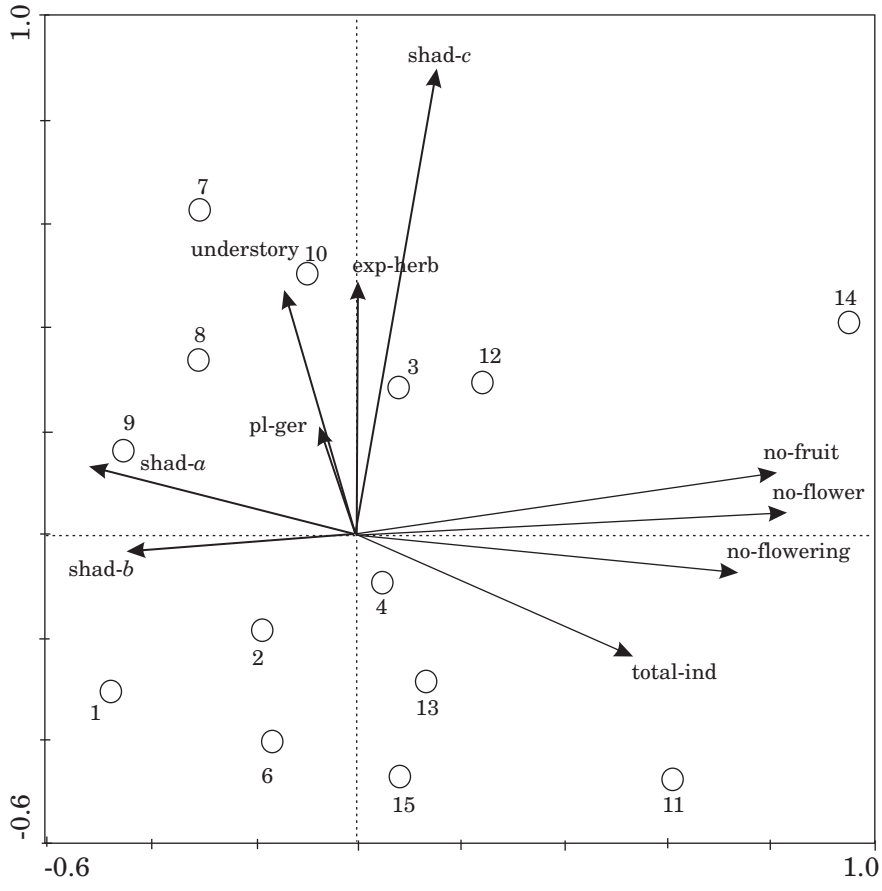

Fig. 2. RDA diagram of variability of the *Pulsatilla patens* populations: total-ind – total number of rosettes; no-flowering – number of flowering rosettes; no-flower – number of flowers; no-fruit – number of fruiting rosettes; shad-*a,b,c* – shading in layer of trees, shrubs, understory; understory – height of understory; exp-herb – share of expansive herbaceous species; pl-ger – place for germination

The first axis explains 97.7% of the total variability and the second one – 2.2%. The results of the significance test for the first canonical axis showed the presence of a gradient which caused variation of the analyzed *P. patens* populations. Correlations with the first and second axis are presented in Table 4. The parameter that proved to be statistically significant (Monte Carlo permutation test $p=0.05$) was the available germination space ($\lambda=0.18$; $p=0.05$; $F=3.85$), which explained 28.7% of the total variability of the analyzed populations. The RDA diagram (Figure 2) shows the variations between sites versus the gradient of habitat-related factors. Group A comprised typical forest habitats, in which Easter pasque flower grew in pine forests. Group B en-

compassed typical non-forest sites (1, 2, 13, 14, 15) as well as the sites located in a forest but near forest roads and stand boundaries (3 ,4, 6) or on slopes on the edge of a forest (11, 12).

Table 4

Correlations of environmental parameters with the first and second axis

Characteristics	AX1	AX2
Shading in layer of trees	-0.4283	0.0481
Shading in layer of shrubs	-0.3723	-0.0122
Shading in layer of understory	0.1266	0.3354
Height of understory	-0.1166	0.1756
Place for germination	-0.0595	0.0797
Share of expansive herbaceous species	0.0049	0.1837

Discussion

The paper presents results of a study on 15 populations of *Pulsatilla patens*. This is a relatively large number of sites, which proves that the two analyzed wildlife refuges, both in the Natura 2000 network, are the principal area in Poland where this species occurs. The monitoring studies covering 34 sites and completed in 2010–2011 showed that most of *P. patens* sites are located in broadly understood north-eastern Poland. The number of Easter pasque flowers at particular sites in Poland ranges from 1 to 939, with most of the populations being very small, holding fewer than 5 rosettes (PAWLIKOWSKI 2012). In the light of these results, the populations presented herein seem quite large since 73% of them contained over 5 rosettes (from 6 to 30). One site, growing on dry heat in the Military Training Grounds in Orzysz, was exceptionally big, consisting of over 100 rosettes of *P. patens*. However, the Polish resources of *P. patens* populations compared with relevant data from the literature are small. For example, the smallest sites studied in Estonia comprise 10 rosettes and the largest ones have up to 10 000 specimens (PILT and KUKK 2002). In Finland, populations are composed of 400 rosettes on average (KALLIOVIRTA et al. 2006).

Pulsatilla patens begin to bloom when they are a few years old (WÓJTOWICZ 2000, UOTILA 2007), so these populations might be composed of very young plants, which have not produced flowers yet. According to KALLIOVIRTA et al. (2003), it is difficult to determine whether a given plant is a juvenile, adult or senile one without analyzing its root system.

Because *P. patens* multiplies mainly generatively, the future of a given population depends on generative rosettes. The share of this fraction

in *P. patens* populations is highly varied. KALLIOVIRTA et al. (2006), who described this species in Finland, report an average 11% of generative rosettes in a population. On Sodowa Mountain in Silesia (south Poland) the share of generative rosettes in the populations was from 3 to 97% (CHMURA 2003). In north-eastern Poland, between 26 to 100% of flowering rosettes were counted (average 44%) in the populations growing in the Myszynieckie Bory Sasankowe and in the Military Training Grounds in Orzysz, of which between 0 to 100% produced fruit (average 21%) (JUŚKIEWICZ-SWACZYNA and CHOSZCZ 2012). Unfortunately, the data on nmber of fruiting rosettes cannot be compared with other populations as such information in lacking in world literature. There is just one mention concerning *P. patens* in Finland, where 62% of flowers were found to produce seeds (KALLIOVIRTA et al. 2006). It seems recommendable to conduct more detailed studies on this speciesthat will not only deal with shares of rosettes in different life stages but also try to determine the role of fruiting rosettes, including their number and the number of seeds. Our comparison of such results indicates that the Polish populations of *Pulsatilla patens* have a large biological potential, while the observed decrease in the number of sites and number of rosettes (CIOSEK 1999, CHMURA 2003, WÓJTOWICZ 2004) may be caused by unfavourable habitat conditions.

At this point, one need to underline that there is dependence between the intensity of flowering and fruiting of *Pulsatilla patens* and climatic conditions, generally mild winters with periods of warming adversely affects the intensity of flowering and fruiting (UOTILA 1996, WÓJTOWICZ 2000). Similar relationships have been described for other species from the family *Ranunculaceae*, e.g. *Delphinium nuttallianum*, where flower production and abundance of flowering plants vary between years and that this variation can be related to the inter-annual variation in the weather conditions (SAAVEDRA et al. 2003). It is therefore essential to conduct steady and long-term studies, which will help to elucidate the role of climatic conditions in the dynamics of fluctuations of *P. patens* population abundance.

Numerous investigations suggest that the structure of *Pulsatilla patens* populations depends on such factors as thick moss layer, most frequently occurring juvenile specimens observed in sites having an average thickness of compactness and moss (UOTILA 1969, KALLIOVIRTA et al. 2006), cover of: phanerogams, cryptogams, litter and bare soil (KALLIOVIRTA et al. 2003, KALLIOVIRTA et al. 2006, RÖDER and KIEHL 2006, JUŚKIEWICZ-SWACZYNA 2010b, JUŚKIEWICZ-SWACZYNA and CHOSZCZ 2012). The present results confirm that the cover of bare soil as a condition for successful seed germination is likewise important – a relationship has been demonstrated between this habitat-specific parameter and the number of fruiting rosettes. The available germination space is a component which, in a statistically significant manner, affects

the variability of populations, a claim verified by the RDA ordination diagram (cf. Figure 2).

Another interesting aspect is the dependence of *Pulsatilla patens* occurrence on light conditions. Studies accomplished in Estonia (PILT and KUKK 2002) a relationship between the number of rosettes in a population and light availability, according to which habitats are divided into open, half-open or closed. Extremely large populations (up to 10 000 rosettes) have been found in half open/open locations with good light availability. On the other hand, KALAMEES et al. (2005) showed that the decrease in germination success with successional age is attributable to some environmental factor other than decreasing light availability. Our study suggests that sunlight conditions have strong influence on populations of *Pulsatilla patens* because populations growing in non-forest habitats were more numerous, contained more flowering and fruiting rosettes and the number of flowers produced by a single plant was also higher (cf. Table 1).

The objective of this study has been to determine the effect of habitat-related conditions on the analyzed Easter pasque flower populations. The role of shading, height of understory, share of expansive plants and cover of bare soil available for germination have been examined. Based on the results, it should be concluded that the above set of factors largely explains (about 63%) the variation of the analyzed populations.

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EFFECT OF ORGANIC POLYMERS ON PROPERTIES OF AGGREGATES OF POST-COAGULATION SLUDGE

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K e y w o r d s: flocculation, polymers, silica suspension.

A b s t r a c t

The study analyzed sizes of aggregates produced in the coagulation process of silica with the use of an inorganic coagulant $Al_2(SO_4)_3$ and three organic polymers: cationic (Z63), anionic (P2540) and non-ionic (N300). Sizes of the formed flocs were measured with the macroscopic photographic method. The greatest size of the flocs (R=0.77 mm) at a fall rate of V=6.45 mm s^{-1} was achieved upon the use of the anionic flocculant P2540, whereas the lowest one (R<0.12 mm, V=2.42 mm s^{-1}) as a result of silica coagulation with the non-ionic polymer N300. The high potential of polymers adsorption makes that the aggregates formed are characterized by high stability and a more open structure.

WPŁYW POLIMERÓW ORGANICZNYCH NA WŁAŚCIWOŚCI AGREGATÓW OSADU KOAGULACYJNEGO

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S ł o w a k l u c z o w e: flokulacja, polimery organiczne, zawiesina krzemionki.

A b s t r a k t

Badano wymiary agregatów utworzonych w procesie koagulacji zawiesiny krzemionki za pomocą koagulanta nieorganicznego $Al_2(SO_4)_3$ oraz trzech polimerów organicznych: kationowego Z63, anionowego P2540 i niejonowego N300. Wielkość utworzonych kłaczków zmierzono, stosując makroskopową metodę fotograficzną. Najwyższe wymiary kłaczków R=0,77 mm dla szybkości opadania

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V=6,45 mm s^{-1} uzyskano po zastosowaniu flokulanta anionowego P2540, a najniższe – R<0,12 mm (V=2,42 mm s^{-1}) na skutek działania polimeru niejonowego N300. Wysoki potencjał adsorpcji polimerów sprawia, że utworzone agregaty charakteryzują się dużą trwałością i bardziej otwartą strukturą.

Introduction

Flocculation is a key element in technological processes using water, as it allows the aggregation of contaminants. Water and wastewater treatment with the coagulation/flocculation method using organic flocculants is effective only when an appropriate degree of particles aggregation is achieved. At the orthokinetic phase of flocculation, sizes of flocs produced in the coagulation process increase as a result of collision and aggregation of particles. The flocculation process that consists in the formation of flocs with specified sizes and properties depends, to a large extent, on the density of a polymer charge and hydrodynamic conditions, i.e. initial fast and slow stirring (LEU and GOSH 1988). Organic polymers are rather used to enhance the action of inorganic coagulants than applied alone.

The mechanism of colloids destabilization and flocs formation is affected by, among other things, conformation of a polymer macromolecule. The polymer macromolecules with a low charge density attain the conformation of a tightly wound ball, whilst the chain of those with a high charge density is highly extended (BRATBY 1980). An increase in the molecular weight of polymers facilitates the formation of more branched conformations of chains. The optimal flocculation proceeds at a high degree of particle surface coverage, at high adsorption force and sufficient thickness of the adsorbed layer of the polymer (BAILEY et al. 1994).

The process of contaminants destabilization follows various mechanisms depending on a polymer charge density. Polymers with a high charge density act accordingly to the mechanism of charge neutralization, whereas these with a low charge density destabilize contaminants as a result of intermolecular bridging. A potential benefit of applying organic polymers is their capability to expand a floccule structure and to enhance sedimentation capability.

$Al_2(SO_4)_3 \cdot 18H_2O$ is a widely applied conventional inorganic coagulant. $Al_2(SO_4)_3$ hydrolysis results in monomeric forms of aluminum. Many authors (Tian et al. 2007) demonstrated the presence of structures with a positive charge like: Al^{3+}, $Al(OH)^{2+}$, $Al(OH)^{4-}$, $Al(H_2O)6^{3+}$, $Al(OH)(H_2O)5^{2+}$, $Al(OH)_2(H_2O)^{4+}$ and AlO_4^{5-} in aqueous solutions of $Al_2(SO_4)_3$. The positively-charged products of $Al_2(SO_4)_3$ hydrolysis are efficient in neutralization of the negatively-charged contaminants of water and wastewaters.

The aim of this study was to determine the characteristics of aggregates produced as a result of coagulation/flocculation of SiO_2 suspension with the use of $Al_2(SO_4)_3$ and anionic (P2540), cationic (Z63) and non-ionic (N300) flocculants.

Methods

Coagulation was applied to a model solution of a silica suspension with the concentration of 300 mg dm^{-3}. $Al_2(SO_4)_3$ was used as the inorganic coagulant. Experiments were carried out with three high-molecular organic polymers: anionic P2540 (produced on the basis of polyacrylamide and sodium acrylate), cationic Z63 (a copolymer of acrylamide and a cationic monomer) and non-ionic N300 (polyacrylamide).

Coagulation was conducted following a standard jar-test procedure: fast stirring (400 rpm) – 1 min, and slow stirring (30 rpm) – 15 min. After 1 h, the sludge was gently collected and let into a column filled with distilled water, and then photographed. Ca. 120 measurements of aggregates at 36x magnification were conducted for one sample of the sludge. Results of these measurements were used to calculate the real distribution of floc sizes and their falling rates.

Results and Discussion

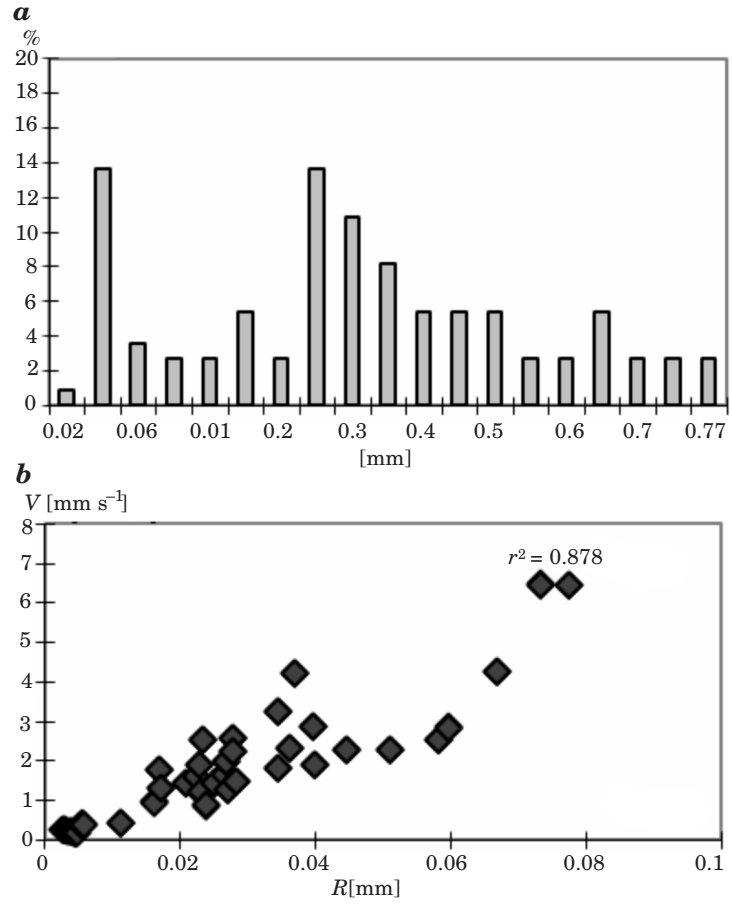
The impact of organic polymers on the size of floccules obtained in the coagulation/flocculation process of a silica suspension is presented in Figures 1–3. Each of the analyzed sludges was characterized by two plots:

a) dependency of the fall rate (V) of aggregates on their size R: $V=f(R)$,

b) % distribution of flocs number depending on size R: $Z(\%)=f(R)$,

where V – fall rate, R – object size, Z (%) – distribution of aggregate sizes.

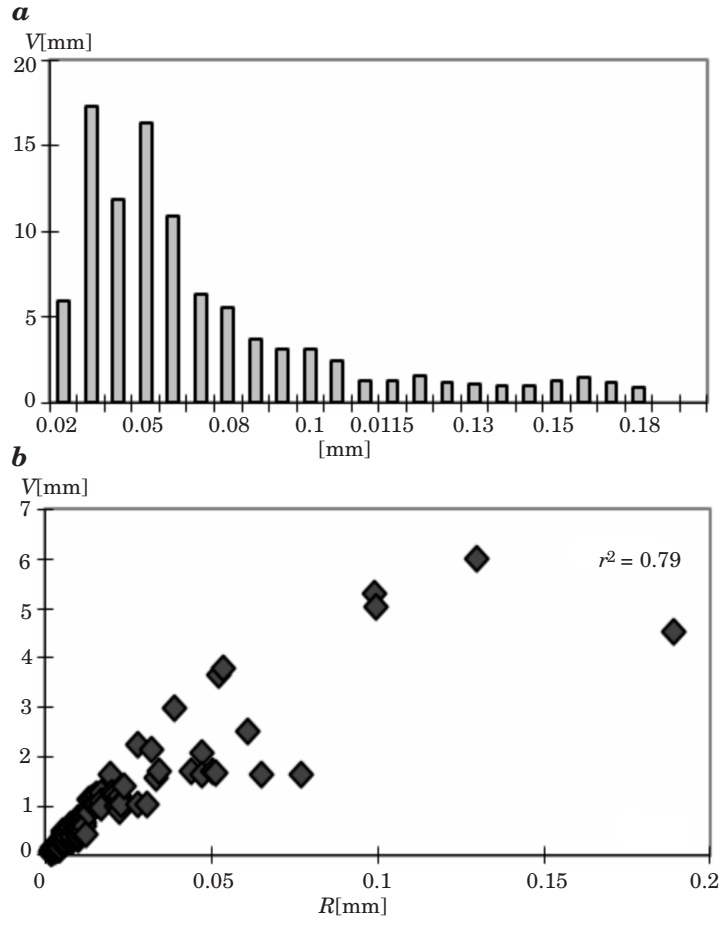
The comparison of sizes of flocs produced upon coagulation/flocculation with various flocculants is presented in figures 1a, 2a and 3a. The sizes of flocs obtained with the anionic polymer P2540 fitted within the range of $R=0.02$–-0.7 mm, whereas flocculation with the cationic polymer Z63 resulted in the flocs with sizes of $R=0.02$–0.2 mm. The figure 1a demonstrate the highest contribution of the flocs with sizes of $R=0.04$ mm (ca. 15%), $R=0.28$ mm (ca. 15%) and $R=0.3$ mm (ca. 12%) in aggregates size distribution. When comparing Figure 1a and Figure 2a it may be noticed that, contrary to the aggregates obtained with the cationic polymer ($R<0.2$ mm), the prevailing part of the flocs (ca. 68%) formed with the anionic flocculent were characterized by the sizes of $R>0.2$ mm. The greatest contribution in the distribution of flocs



R: 0.02–0.77 mm
V: 0.25–6.45 mm s^{-1}

Fig. 1. Characteristics of the aggregates formed with the use of $Al_2(SO_4)_3$ and anionic polymer P2540 (silica suspension)

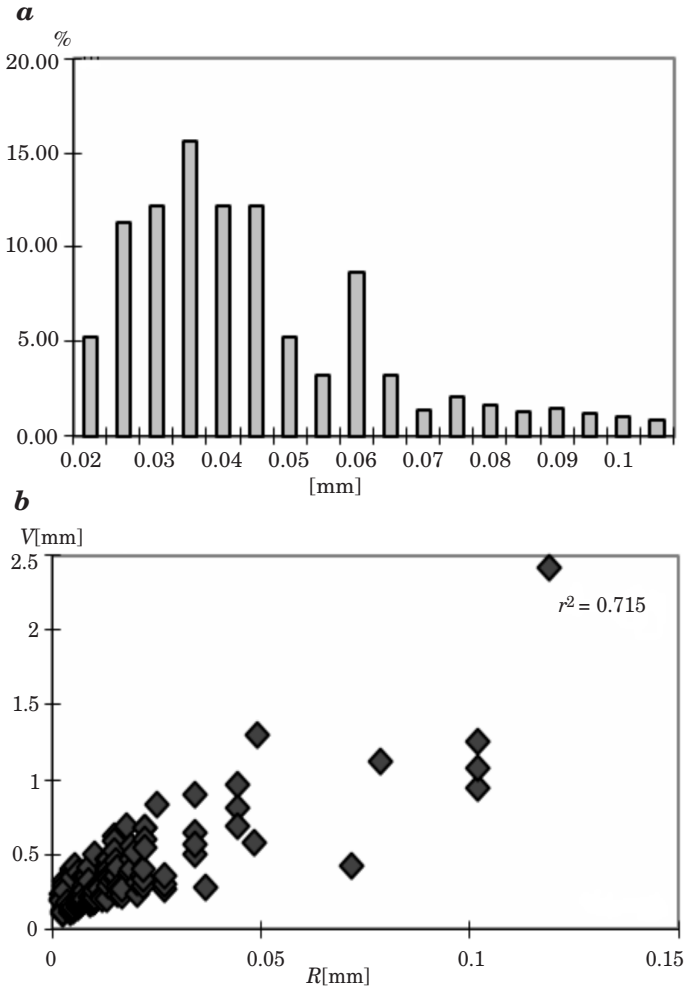
formed with the cationic polymer was reported for the aggregates with sizes of R=0.04–0.06 mm (56%). The increase in flocs size was accompanied by their decreased content (%) in the total size distribution. The smallest sizes were noted for the aggregates produced with the non-ionic flocculant. The greatest contribution was reported for the smallest floccules with sizes in the range of R=0.025–0.045 mm. In turn, the smallest contribution is size distribution was found for the aggregates with sizes of R=0.06–0.1 mm. In comparing the sizes of floccules produced as a result of flocculation with flocculants having various ionic characters it may be concluded that floccule sizes were significantly smaller upon the use of Z63, compared to the anionic flocculant. However,



R: 0.02–0.2 mm
V: 0.6–6.01 mm s^{-1}

Fig. 2. Characteristics of the aggregates formed with the use of $Al_2(SO_4)_3$ and anionic polymer Z63 (silica suspension)

the flocs obtained with the cationic polymer reached nearly twofold greater sizes than these produced with the non-ionic polymer (Figure 3a). Investigations addressing the formation of aggregates demonstrate that the final size of floccules results from some balance between floccules increase and breakage. Polymers taking part in the process of flocculation contribute to the formation of flocs with greater sizes and a more stable structure than these formed with the hydrolyzing coagulant. Explanation of this phenomenon may be searched for in the mechanism, as in the case of high-molecular polymers there proceeds the bridging mechanism. The addition of a small dose of an anionic polymer



R: 0.02–0.12 mm
V: 0.15–2.42 mm s^{-1}

Fig. 3. Characteristics of the aggregates formed with the use of $Al_2(SO_4)_3$ and nonionic polymer N300 (silica suspension)

may cause significant destabilization of the positively-charged sol $(SiO_2)_n$, thus inducing intensive agglomeration. The bridging mechanism assumes that the polymer binds colloidal particles into larger agglomerates. A polymer molecule absorbs on one or a few particles of the system, thereby forming bridges and networks. The system-stabilizing addition of the cationic flocculant can lead to the formation of less stable structures between particles of sol $\{Al(OH)_3\}_n$ and particles. In turn, the non-ionic flocculants that display poor affinity to

particles of the positively-charged sol $(SiO_2)_n$ may lead to the formation of instable structures of aggregates.

The analysis of data obtained for the flocs formed by $Al_2(SO_4)_3$ coagulation with flocculant P2540 (Figure 1a) demonstrated that they were characterized the highest fall rate reaching up to $V=6.45$ mm s^{-1} at a relatively large flocs size of $R=0.77$ mm. Owing to a high fall rate, the sludge produced in this process posed some difficulties while taking photos. The large, stable floccules formed in this way are characterized by good sedimentation properties. This sludge was additionally characterized by the highest value of fractal dimension – $D_A=1.988$ (WIERZBICKA 2000). A similar fall rate reaching $V=6.01$ mm s^{-1} was noted for the flocs formed as a result of $Al_2(SO_4)_3$ coagulation with Z 63 (Figure 2b). However, these flocs were characterized by slightly smaller sizes of $R=0.02$–0.2 mm. Therefore, when analyzing sizes of aggregates (R), their fall rate (V) and fractal dimension (D, in this system reaching $D_K=1.978$) (WIERZBICKA 2000), it may be speculated that owing to a more open structure such this sludge is characterized by a lower degree of hydration than the sludge formed with the addition of P2540. According to YU et al. (2006), high-molecular polymers with a low charge density cause more rapid flocculation than the low-molecular polymers with high charge density do. They demonstrated that the flocs formed upon rapid adsorption of a polyelectrolyte had an open structure, whereas those that were formed upon slow adsorption of a polymer were characterized by a compact structure. The rate of polymers adsorption is assumed to depend on the difference in diffusion rate. The aggregates formed in the process of $Al_2(SO_4)_3$ coagulation with flocculant N300 (Figure 3a) were falling with the rate of 0.15–2.42 mm s^{-1} despite small sizes reaching barely 0.12 mm. The value of the fractal dimension of this sludge was $D_N=1.505$ (WIERZBICKA 2000). Results achieved in this study point to a significant effect of the ionic character of flocculants on the size and structure of aggregates. A small addition of the flocculant in respect of the coagulant leads to the formation of floccules with very diversified properties. The anionic polymer coupled with the inorganic coagulant results in the formation of floccules with greater sizes than those produced with the cationic and non-ionic polymers. As reported by GREGORY (1996), floccules formed with the use of a high-molecular polymer are characterized by a more stable structure than those produced with the low-molecular polymer. Floccules formed in this way have a stable structure and exhibit higher resistance to breaking.

The application of the macroscopic photographic method (DA-HONG and GANCZARCZYK 1989, JULLIEN and BOTET 1987) provides many valuable information on the properties of flocs. The analysis of the mechanism and kinetics of flocculation enables monitoring the status of particles aggregations. In the solid-liquid separation processes one of the key problems includes properties

of aggregates formed as a result of flocculation. The understanding of formation mechanisms of flocs having specified characteristics will enable the optimization of sludge flocculation and dehydration processes.

Conclusions

1. The ionic character of organic polymers has a significant effect on the size of post-coagulation sludge aggregates.

2. The greatest impact on the size and fall rate of flocs was noted for the anionic polymer P2540, which enhanced for formation of flocs with the greatest sizes (R=0.02–0.77 mm) at the fall rate of V=6.45 mm s^{-1}.

3. Aggregates formed with the use of the cationic polymer (R=0.02–0.2 mm, V=6.01 mm s^{-1}) reached nearly twofold greater sizes than those formed with the non-ionic polymer (R<0.12 mm, V=0.15–2.42 mm s^{-1}).

Translated by Joanna Molga

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MOBILITIES AND LIFESTYLE. NEW HORIZONS IN LAKE TOURISM

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K e y W o r d s: tourism, mobilities, lifestyle, slow travel.

A b s t r a c t

Tourism has expanded exponentially, as advances in transportation technology among others have led to enhanced personal mobility worldwide. In effect, the migratory phenomena of tourism and travel have become integral components of lifestyle migration for an ever increasing proportion of the world's population. In 2000, Urry introduced the concept of "mobilities", which is here conceptualised as including not only mobility (the migration of people) but also the movements of capital, information, skills, expertise and knowledge that arise from tourism and travel. In this paper, a preliminary schematic of lifestyle mobilities, including tourism, is presented and is illustrated in a discussion of the emerging area of slow tourism.

PRZEMIESZCZANIE SIĘ I STYL ŻYCIA. NOWE PERSPEKTYWY W TURYSTYCE JEZIOROWEJ

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S ł o w a k l u c z o w e: turystyka, mobilności, styl życia, podróże typu slow.

A b s t r a k t

Turystyka rozwija się bardzo szybko dzięki m.in. postępowi w technologiach transportu umożliwiającemu zwiększoną mobilność ludzi na całym świecie. Zjawiska przemieszczania się ludzi uprawiających turystykę bądź podróżujących dla stale rosnącej części ludzkiej populacji stały się więc nieodłącznymi elementami stylu życia. Urry (2000) wprowadził termin mobilności, który w prezentowanej pracy obejmuje nie tylko przemieszczanie się (migrację ludzi), ale także przepływ kapitału, informacji, umiejętności, doświadczenia i wiedzy związanych są z turystyką i podróżowaniem. W pracy przedstawiono wstępny zarys mobilności związanych ze stylem życia, łącznie z uprawianiem turystyki oraz zobrazowano go rozważaniami na temat nowego nurtu, czyli turystki w powolniejszym tempie, typu „slow".

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Introduction

It is well recognized that advances in transportation and communication technologies, more flexible working arrangements, and increases in discretionary wealth and time have led to enhanced personal mobility world-wide. Although recognising that these benefits are not uniformly distributed among people or countries, a defining characteristic of the late-modern era has been their extension to a widening cross section of the world's population. While the majority of this mobility involves commuting, shopping and other daily circulation, a significant proportion is tourist movements involving journeys of longer duration between home and one or more destinations in search of leisure and amenity and with a view to enhancing lifestyle (MCINTYRE et al., 2006). As a migratory phenomenon, (WILLIAMS and HALL 2002), tourism can be seen as an important aspect of lifestyle migration for an increasingly wider proportion of the world's population (Figure 1). Lifestyle migration not only describes tourists (e.g., passing trade, second-home owners) but also encompass a significant proportion of people engaged in the tourist trade (e.g., entrepreneurs, peripatetic tourism workers), who make lifestyle choices characterised by an emphasis on balancing quality-of-life considerations including the natural environment, family time, freedom, a slower pace of life and community involvement with economic self-sufficiency

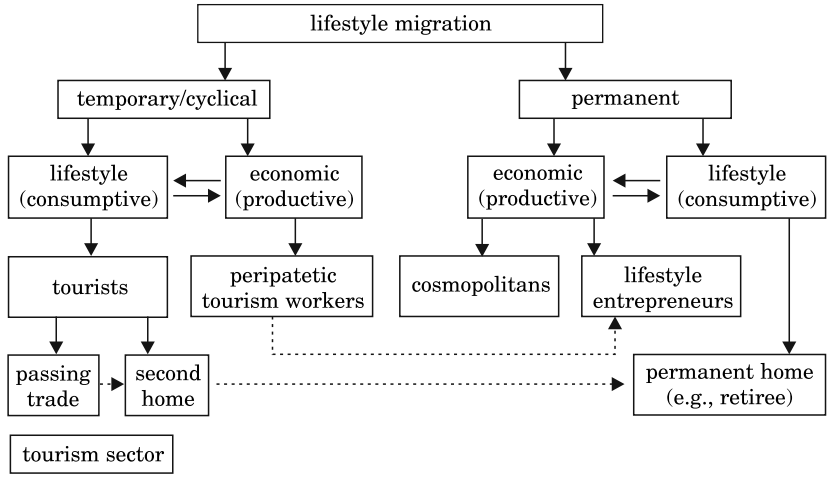

Fig. 1. Tourism and lifestyle migration

Source: McIntyre (2009).

Lifestyle Mobilities

In his book *Sociology beyond Societies: Mobilities for the Twenty-first Century*, URRY (2000) introduced the term "mobilities" to extend the concept of mobility beyond the circulation of people to include the movements of "objects, images, information... and... (their) complex interdependencies... and social consequences" (p. 1). Thus, in the context of tourism, mobilities imply more than simply tourists travelling; they also include the movements of capital, information, skills, expertise and knowledge that arise from these movements.

MCINTYRE (2009) defined lifestyle mobilities as the movements of *people, capital, information and objects* associated with the process of voluntary relocation to places that are perceived as providing an enhanced or, at least, different lifestyle (p. 232).

Using this conceptualisation and the notion of *scapes* from APPADURAI (2008), a preliminary model of lifestyle mobilities was developed (MCINTYRE, in press). This schematic (Figure 2) encapsulates four scalar and fluid dimensions of late-modern global culture: *ethnoscapes* (e.g., tourists and other lifestyle

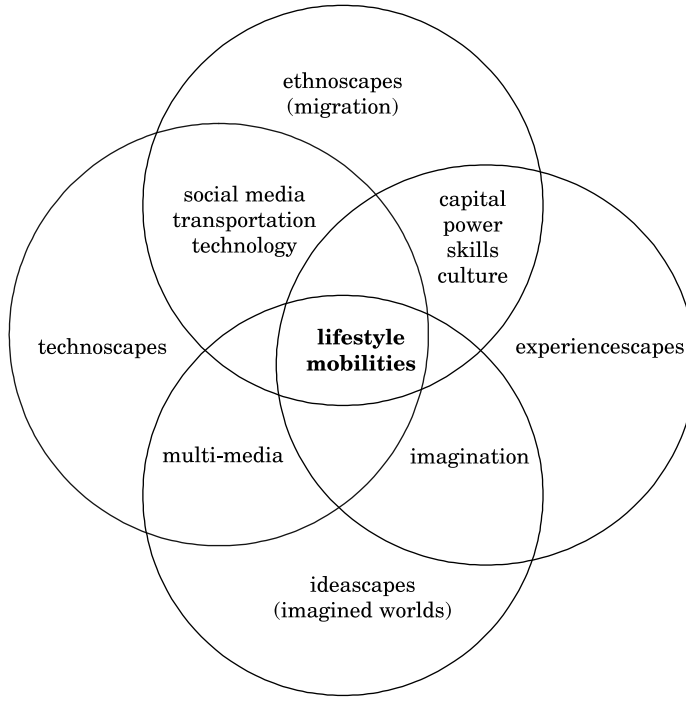

Fig. 2. A conceptual model of lifestyle mobilites (McIntyre in press)

migrants); *technoscapes* (e.g., social media and transportation technology); *experiencescapes* (the various experiential, personal and financial characteristics that potentially accompany the lifestyle migrants) and *ideascapes* (the imagined worlds of the lifestyle migrant) and the intersections between them.

The intersection between the *ethno* and *experience*-scapes represents the financial capital, skills, knowledge, power and influence of migrants. A not uncommon situation in tourist destinations throughout the developed world involves the impacts of capital flow on housing values resulting from second home purchase. The prevalence of this situation has caused the UK government to consider a ban on home purchase for seasonal use, as young people are being priced out of their home towns in such desirable areas as the Lake District in northern England (McCandless 2005).

A new emphasis on mobilities has led to a re-assessment of tourism transportation (intersection of the *ethno* and *techno*-scapes) and its impacts on the global environment, the quality of travel experiences and the contribution of tourism to the lifestyles of residents and tourists.

Slow Mobilities

Today, tourism is based largely on a plentiful and relatively cheap supply of fossil fuels particularly oil. Over 90 percent of tourism transport uses oil, which accounts for between 75 and 90 percent of all carbon emissions resulting from tourism. Many tourism commentators (e.g., Dickinson and Lumsdon 2010, Hall 2009) have argued that the "business as usual scenario" for tourism is no longer tenable. The world is approaching or has already passed "peak oil" and there is a growing consensus on the inevitability of negative impacts of anthropogenic climate change. On this basis, it is argued that the long term sustainability of tourism will hinge upon its ability to decouple from dependence on high-carbon forms of transport. In this context, attention is turning to consideration of "slow mobilities" (Fullager et al. 2012) or "slow travel" as an attractive alternative to tourism faced by increasing geopolitical and ethical constraints.

Sharing many similarities with the "slow food" and "slow cities" movements which emerged in Italy in the 1980's and 1990's, slow tourism is a reaction against the emphasis on speed and convenience of the "fast food" industry which is increasingly dominating service industries worldwide (Ritzer 1993, Nilson et al. 2007). It is characterised by a number of themes which differentiate it from the majority of present-day tourism activity.

– a shift away from car and air travel;

– a behavioural shift to the rediscovery of travel for its own sake to facilitate slower, but more carbon-efficient, journeys;

– journeys that engender engagement with people encountered and places en-route, as well as the destination;

– an increasing expression of environmental concern by tourists conscious of the need to reduce their carbon footprint whilst maintaining the benefits of travel (DICKINSON and LUMSDON 2010: 176).

Examples of slow travel include: walking and cycle tourism, train tourism, bus and coach tourism, and water-based tourism (e.g., ferries, cruising, canoes, kayaks, yachts). This last is of particular interest in lake tourism (e.g., the Lake District in U.K., Mecklenburgischer Seen, Germany, Lake Superior, Canada). Also, walking and cycling routes are often focused on lakes, rivers and coastal areas which provide relatively flat and aesthetic terrain.

Slow travel hearkens back to the early days of tourism to the Grand Tour and pilgrimages (HOWARD 2012), when the main modes of transport were walking or animal drawn vehicles. The advent of the bicycle provided an affordable form of transport which revolutionised mobility in the late 19th and early 20th century enabling people to travel from urban areas and explore the countryside (DICKINSON and LUMSDON 2010). Similarly, the increasing availability of the motor car later in the 20th century progressively extended leisure and travel opportunities to wider and wider sections of the community and ushered in a decline in cycle use both for commuting and more extended travel.

The dual emphasis on the journey and destination embodied in slow travel contrasts with the overriding destination orientation of most tourism today. A key aspect is the valuing of: slowness... embodied in the in the qualities of rhythm, pace, tempo and velocity that are produced in the sensory and affective relationship between the traveler and the world (CRESSWELL 2010: 19).

Arguably, it is these experiential aspects that attract most slow tourists (i.e., soft slow travelers) who see any environmental benefits as an added bonus only.

DICKINSON and LUMSDON (2010) argued that slow travel is potentially a sustainable alternative to current tourism practices, which result in "a degradation of the "public goods" (and)... a loss of diversity (and biodiversity)" (p. 181) and which are recognised by many as unsustainable. While acknowledging that the locked-in institutional processes characterising the tourism system will be extremely difficult to reverse, they point to policy and consumer attitudinal changes, which they argue may be the progenitors of a paradigmatic shift to an alternative, more ethical and environmentally responsible tourism. Additionally, they note that in all countries most tourism is short to medium distance and is principally domestic or cross-border and as such, slow alternatives (e.g., trains, coaches) for the vast majority of tourism may involve less change than we imagine.

On the other hand, FULLAGER et al. (2012) argue that it would be naive and simplistic to argue that slow travel is the answer given the enormous growth in middle-class consumerism and global travel already becoming evident in the newly booming economies of India and China. In addition, a majority of slow tourists use high-carbon modes of transport as a component of the holiday trip (e.g., travel by air or car to access slow travel destination). In effect, such practices nullify or reduce any carbon benefits arising from their choice of slow travel modalities. More generally, the freedom to move is a scarce and unequally distributed commodity, which has become a major stratifying factor of late modern times (BAUMANN 1998). Policies aimed at reducing travel will therefore not be viewed positively due to implications for people's lifestyles and social networks.

Whatever the ultimate fate of slow travel; universal panacea or niche market, an emerging industry is already evident variously emphasizing the avoidance of air and car travel, reducing individual environmental impact and extolling the richness of the travel experience (DICKINSON and LUMSDEN 2010). For example: taking time on the journey to engage with people and place... Slow travel is about enjoying the journey, as well as the destination. Moving away from budget flights and quick getaways to faraway places... moving towards overnight train journeys, lake cruises, ferries, cycle trips and home stays. Why not start a holiday from when you leave your front door, rather than when you enter your hotel room? (www.slowmovesblog.blogspot.com/) and with our philosophy of slow travel for women, the perceptive and sensitive traveler will be exposed to a "full immersion" of sensations, from the visual to the audio, to the tactile, the olfactive and the gustative. In this way the memories are deeply imbedded into our minds. (www.slow-travel-for-women.com.

Conclusions

In this short review, I have focused first on elaborating the concept of "mobilities" in the context of tourism and its links to the broader concept of lifestyle. As an example, I then explored the evolving concept of slow tourism and its potential in focusing tourism development beyond high-carbon transport dependency.

The mobilities concept provides a novel framework for understanding the impacts of tourism on people's lifestyle and critiquing current tourism practices. However, its potential contribution goes much beyond this, as FRANKLIN and KRANG (2001) suggest:

The excitement of mobilities in these highly mobile times, structured as they are by the language and practice of tourism, is that they generate new

social relations, new ways of living, new ties to space, new places, new forms of consumption and leisure and new aesthetic sensibilities (p. 12).

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INFLUENCE OF ANTIOXIDANTS ON SPERMATOZOA IN THE SHORT-TERM STORAGE OF SALMONIDAE MILT*

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K e y w o r d s: rainbow trout, Arctic chair, milt, motility, CASA.

A b s t r a c t

Short-term storage of semen in cooling conditions (+4°C) is one way to perfect artificial fish reproduction. In this experiment, we attempted to add antioxidants (vitamins C and E, glutathione and cysteine) during the storage of Arctic char (*Salvelinus alpinus*) and rainbow trout (*Oncorhynchus mykiss*) semen. An analysis of the CASA parameters showed that addition of the antioxidants to semen during storage did not benefit spermatozoa motility of studied salmonids. An analysis of the parameters showed that added vitamins C and E did not influence the sperm motility of Salmonidae during semen storage. The addition of glutathione and cysteine significantly worsened the vitality of Arctic char and rainbow trout sperm.

WPŁYW DODATKU ANTYOKSYDANTÓW NA KRÓTKOOKRESOWE PRZECHOWYWANIE NASIENIA RYB ŁOSOSIOWATYCH

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S ł o w a k l u c z o w e: pstrąg tęczowy, palia alpejska, mlecz, ruchliwość, CASA.

A b s t r a k t

Krótkookresowe przechowywanie nasienia w warunkach chłodniczych (+4°C) jest jednym ze sposobów doskonalenia metod sztucznego rozrodu u ryb. W pracy podjęto próbę zastosowania dodatku antyoksydantów (witaminy C, witaminy E, glutationu i cysteiny) w trakcie przechowywania

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nasienia palii alpejskiej (*Salvelinus alpinus*) oraz pstrąga tęczowego (*Oncorhynchus mykiss*). W analizie parametrów ruchu plemników wykazano, że dodatek witaminy C oraz witaminy E nie wpływa istotnie na ruchliwość plemników ryb łososiowatych w trakcie przechowywania nasienia. Dodatek glutationu i cysteiny w istotnym stopniu pogorszył żywotność plemników palii alpejskiej i pstrąga tęczowego.

Introduction

In recent years, the possibility of short- and long-term sperm storage in fish reproduction has become more and more important. The formation of free oxygen radicals, which occurs during cryopreservation and semen storage in chilling conditions (+4°C), is responsible for decreased stability of the cell membrane, impaired functioning of the mitochondria and DNA fragmentation. These processes influence the lowering of sperm motility parameters, and thus as a result decrease in sperm fertility (BILLARD et al. 2004, SANOCKA and KURPISZ 2004, LAHNSTEINER et al. 2011).

In natural conditions, seminal plasma protects sperm from free oxygen radicals. Semen storage in chilling conditions however must be diluted with various buffers, which demonstrates the insufficiency of seminal plasma's protective role. Experiments by BUCAK et al. (2007) and THUWANUT et al. (2008) showed that supplementing antioxidants to buffers used during cryopreservation aids prevention of sperm damage due to the presence of free radicals. The positive effect of enriching fish feed with antioxidants such as vitamins C and E on fertility has been known for a while (CIERESZKO and DABROWSKI 1995 – *Oncorhynchus mykiss*; MANSOUR et al. 2006 – *Salvelinus alpinus*; METWALLY and FOUAD 2009 – *Ctenopharyngodon idellus*).

Results by GLOGOWSKI et al. (2008) demonstrated the detrimental effect of oxygen on the survival of rainbow trout sperm stored *in vitro*. The addition of albumin, a protein with antioxidant properties, significantly lengthened the period of trout semen storage (*Oncorhynchus mykiss*) (KOWALSKI et al. 2009). In this paper, we attempted to improve the buffer composition for short-term storage of Arctic char (*Salvelinus alpinus*) and rainbow trout (*Oncorhynchus mykiss*) semen, by the addition of selected antioxidants to the extender solution, such as vitamins A and E, glutathione and cysteine.

Material and Methods

Semen from Arctic char (*Salvelinus alpinus*) and rainbow trout (*Oncorhynchus mykiss*) were obtained during the autumn mating season in 2011 from the Pstrąg Tarnowo Fish Hatchery (Wielkopolski Voivodeship). The fish were put

under anesthesia and then semen was gathered through abdominal massage. The semen from males ($n=5$) was mixed with Morisawa's buffer with 1% albumin at a proportion 1:10, the control sample. In order to determine antioxidants' influence on semen storage, we used Morisawa's buffer, composed of 0.1 M NaCl, 0.04 M KCl, 0.03 M $CaCl_2$, 0.0015 M $MgCl_2x6H_2O$, 0.05 M Tris, 0.5% albumin, pH 8.5 (MORISAWA and MORISAWA 1988) with a supplement of 1 mM of vitamin C, 1.5 mg ml^{-1} of vitamin E, 5 mM of glutathione and 5 mM of cysteine. After two days of storage in chilling conditions (+4°C), an antibiotic was added to every sample (penicillin and streptomycin in proportions of 100 U ml^{-1} and 100 μg ml^{-1}, respectively). The samples were mixed every 24 h to avoid the negative effects of sperm sedimentation. In the prepared samples, the sperm parameters were marked every two or three days with the CASA system. Motility activating fluids were used for the activation of sperm movement, Billard fluid with an addition of 0.5% albumin (BILLARD 1985). Activation was done in the small eppendorf tube where sperm samples were mixed with activation solutions supplemented. 0.5 μl of sperm samples were mixed with 200 μl of activation solution. After activation 1 μl of each sample was transferred to the 12 wells Teflon coated slide glass (Tekdon INC. 40521 State Road 64 Myakka City, Florida 34251). Motility analysis was carried out using the Crismas equipment (Image House CRISMAS Company Ltd.). Sperm movement was documented 6 second after activation with a Basler 202K digital camera integrated with an Olympus BX51 microscope. Total sperm velocity, VCL (μm s^{-1}), and the percentage of motile sperm, MOT (%) were analysed.

The statistical analysis was made using the GraphPad Prism program (GraphPad Software Inc., USA), incorporating the ANOVA two-way analysis of variance. The differences between particular test subjects were established by the Bonferroni post-test.

Results

Among the four applied antioxidants, adding vitamins C and E to the semen of Arctic char brought the best results, as well as control samples, diluted in Morisawa buffer without any additions (Table 1). The other two antioxidants, glutathione and cysteine, caused a complete loss of movement after ten days of storage, whereas motile sperm was still observed in the control sample and the samples with vitamins C and E after fifteen days of storage. Rainbow trout semen diluted with Morisawa buffer (control) or with added vitamins C and E maintained motility longer than Arctic char semen. After eighteen days of storage at +4°C, 28–35% of trout sperm was observed to

be motile (Table 1). The motile spermatozoa characterized the similar VCL values for every day of experiment.

Table 1
Arcic char and rainbow trout sperm motility parameters during 15 or 18 days of storage at +4°C

Time (day)	Control	Vit. C 5 mM	Vit. E 1.5 mg ml^{-1}	Glutathione 5 mM	Cysteine 5 mM
Arctic char MOT [%]					
1	89^{ax}	72^{ax}	87^{ax}	69^{ax}	84^{ax}
3	91^{ax}	48^{bx}	87^{ax}	45^{bxy}	34^{by}
5	83^{ax}	46^{bxy}	65^{abx}	24^{cy}	23^{cy}
8	64^{axy}	26^{byz}	29^{by}	21^{by}	14^{by}
10	43^{ayz}	30^{abyz}	42^{ay}	14^{by}	11^{byz}
12	35^{ayz}	21^{ayz}	17^{ay}	0^{bz}	0^{bz}
15	12^{az}	7^{az}	14^{ay}	0^{az}	0^{az}
VCL [μm s^{-1}]					
1	271^{ax}	272^{ax}	280^{ax}	255^{ax}	255^{ax}
3	294^{ax}	261^{ax}	300^{ax}	246^{ax}	291^{ax}
5	300^{ax}	172^{ax}	231^{ax}	187^{ax}	172^{ax}
8	295^{ax}	147^{ax}	154^{ax}	154^{ac}	143^{axy}
10	271^{ax}	164^{ax}	217^{ax}	128^{abx}	69^{by}
13	229^{axy}	141^{axy}	142^{ax}	0^{by}	0^{bz}
15	91^{ay}	108^{ay}	134^{ax}	0^{by}	0^{bz}
Rainbow trout MOT [%]					
1	96^{ax}	93^{ax}	88^{ax}	78^{ax}	78^{ax}
3	94^{ax}	86^{axy}	84^{ax}	55^{bx}	32^{by}
5	84^{ax}	68^{ay}	89^{ax}	31^{by}	50^{bz}
8	77^{axy}	58^{ayz}	85^{ax}	12^{bz}	23^{bq}
10	65^{ay}	56^{ayz}	75^{ax}	4^{bz}	18^{bq}
12	62^{ayz}	48^{az}	68^{axy}	0^{bz}	10^{bq}
15	51^{az}	38^{az}	53^{ay}	0^{bz}	0^{bq}
18	35^{az}	28^{az}	29^{ay}	0^{bz}	0^{bq}
VCL [μm s^{-1}]					
1	244^{ax}	261^{ax}	251^{ax}	262^{ax}	288^{ax}
3	308^{ax}	289^{ax}	280^{ax}	263^{ax}	231^{ax}
5	280^{ax}	268^{ax}	292^{ax}	175^{bx}	233^{bx}
8	270^{ax}	253^{ax}	290^{ax}	66^{by}	216^{xy}
10	285^{ax}	244^{ax}	289^{ax}	20^{byz}	170^{cy}
12	273^{ax}	233^{ax}	250^{ax}	0^{bz}	89^{by}
15	227^{ax}	177^{ax}	234^{ax}	0^{bz}	0^{bz}
18	234^{ax}	164^{ax}	241^{ax}	0^{bz}	0^{bz}

Control sample was diluted 1:9 with Morisawa buffer (Morisawa and Morisawa 1988). Vit. C – diluted samples with vitamin C addition, vit. E – diluted samples with vitamin E addition, glutathione – diluted samples with glutathione addition, Cysteine – diluted samples with cysteine addition. Data shows the percentage of motile spermatozoa (MOT) and curvilinear velocity (VCL). Data represent mean values (n=5). Different letters (*a, b, c*) indicate statistically significant differences between the buffers at the same time; the letters *x, y, z, q* indicate statistically significant differences between time points for each buffer ($p \leq 0.01$).

Discussion

Our previous experiments on antioxidant supplements' effect on sperm survival of ide (*Leuciscus leuciscus*) after cryopreservation demonstrated that supplements of vitamins C and E as well as glutathione and cysteine improve some of the sperm motility parameters (SAROSIEK et al. 2011):

VCL – curvilinear velocity of sperm;

VSL – straight-line velocity;

VAP – average path velocity of sperm (SAROSIEK et al. 2011). We also concluded that a supplement of antioxidants improves the condition of sperm after freezing and increases the percentage of ide eggs fertilization by 20% in comparison with the fertility of frozen sperm without antioxidants. However, contrary to cryopreservation results, the antioxidants supplementation did not significantly lengthen the period of short-term storage for salmonid semen compared to the control sample.

In mammalians several negative effects of reactive oxygen species on sperm viability have been recorded. They may cause lipid peroxidation of sperm cell membranes, damage of midpiece structure or loss of motility and infertility (SIKKA et al. 1995, SIKKA 2004). KOWALSKI et al. (2009) observed shortened time of Rainbow trout spermatozoa viability during storage at +4°C in oxygen atmosphere. On the other hand, high concentration of uric acid (strong antioxidant) in fish seminal plasma have been related to protection of spermatozoa against oxygen free radicals (CIERESZKO et al. 1999).

Our latest experiment showed (SAROSIEK et al. 2013), that a supplement of antioxidants (vitamins C and E, glutathione and cysteine) during the storage of perch semen (*Perca fluviatilis*) did not benefit its vitality. The results on perch and salmonid semen indicated that the semen dilution with immobilization buffer was sufficient for short-term storage. The antioxidants addition did not improve the sperm motility parameters. It should be investigated wheatear it might be a cause of high antioxidant potential of their seminal plasma (SŁOWIŃSKA et al. 2013) and therefore any further supplementation did not change overall effectiveness of protection against free radicals.

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