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**MATERNAL WEIGHT GAIN IN GILTS AND CHANGES
IN THE BODY WEIGHT AND BACKFAT THICKNESS
OF SOWS FED DIETS CONTAINING NAKED OATS
(*AVENA NUDA*)**

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Key words: feeding, gilts, naked oats, sows.

Abstract

The optimal body condition of gilts and sows significantly influences their future reproductive results. The aim of the study was to determine the effect of naked oats used in feed rations for gilts on their diets and on changes in body weight and backfat thickness during gestation and lactation. The study was carried out on Polish Landrace gilts raised on a breeding farm. Three gilts weighing about 30 kg were selected from each of 60 litters and assigned to three groups – two experimental and one control – with 60 individuals each. The ration to the experimental groups contained 40% (D_1) and 20% (D_2) naked oats of the Akt variety. In the sows, body weight and backfat thickness were measured in each reproductive cycle on the day before parturition, the first day after parturition and day 42 of lactation. During the period from the 91st day of pregnancy and during the mating period the gilts and sows were fed the same mixed feed as during lactation. The study showed that the naked oats used in the diet of the gilts and sows had a beneficial effect on their body condition and fat reserves measured as backfat thickness. The naked oats included in the diets to the gilts and sows of both experimental groups increased body weight gain in the sows during gestation, and the lowest weight loss during lactation was noted in these groups as well.

ZMIANY MASY CIAŁA I GRUBOŚCI SŁONINY U LOSZEK I LOCH ŻYWIONYCH MIESZANKAMI Z UDZIAŁEM OWSA NAGOZIARNISTEGO (*AVENA NUDA*)

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Słowa kluczowe: żywienie, loszki, owies nagoziarnisty, lochy.

Abstrakt

Stopień otluszczenia loszek i loch znacząco wpływa na ich wyniki rozrodcze. Celem badań było określenie wpływu diety zawierającej nasiona owsa nagoziarnistego, stosowanej w żywieniu loszek, na ich otluszczenie oraz na zmiany masy ciała i grubości słoniny loch podczas ciąży i laktacji.

Badanie przeprowadzono na zwierzętach rasy Landrace utrzymywanych w gospodarstwie hodowlanym. Z 60 miotów wybrano po trzy loszki o masie ciała około 30 kg i metodą analogów przydzielono je do trzech grup: dwóch doświadczalnych i jednej kontrolnej po 60 osobników w każdej. Mieszanka pełnoporcjowa podawana grupom doświadczalnym zawierała 40% (D_1) i 20% (D_2) owsa nagoziarnistego odmiany Akt. U loch zmierzono masę ciała i grubość słoniny w każdym cyklu reprodukcyjnym w dniu przed porodem, pierwszego dnia po porodzie i w 42 dniu laktacji. Loszki i lochy od 91 dnia ciąży i w okresie krycia były żywione taką samą mieszanką, jak podczas laktacji.

W badaniu wykazano, że owies nagoziarnisty stosowany w diecie loch i loszek miał korzystny wpływ na stan ich ciała i rezerwy tłuszczu mierzone grubością słoniny. Mieszanki paszowe z udziałem owsa nagoziarnistego, podawane loszkom i maciorom obu grup doświadczalnych, przyczyniły się do zwiększenia przyrostu masy ciała u loch podczas ciąży. W grupach doświadczalnych odnotowano też najniższą utratę masy ciała loch podczas laktacji.

Introduction

Nutrition is the main factor influencing reproductive performance and longevity sows (KARPIESIUK et al. 2018). Normal functioning of the body requires an appropriate balance to be maintained between the amount of energy supplied by the diet and the amount of energy expended. The degree of diets in gilts significantly influences their future reproductive results (BOCIAN et al. 2010, MATYSIAK et al. 2010, REKIEL et al. 2016).

Rational nutrition for pregnant and nursing sows affects their body weight in individual phases of the reproductive cycle, as well as the thickness of the backfat. The diet of pregnant sows should ensure appropriate body condition in the sow during lactation. According to YOUNG et al. (2004), backfat thickness is a reliable indicator that is simple to assess.

Proper nutrition is also necessary to prevent excessive weight loss in sows during lactation and excessive fat storage during gestation, which affects reproductive performance. According to FANDREJEWSKI et al. (1994), in order to protect sows from excessive loss of body weight and reserves of fat and protein, they should be ensured a suitable level and quality of nutrition. The appropriate level of energy, protein and amino acids in fodder given to sows is discussed by WILLIAMS and MULTAN (1989), JOHNSTON et al. (1993), COFFEY et al. (1994), PETTIGREW and YANG (1997) and GRELA et al. (2005).

Nevertheless, losses in body weight during lactation are inevitable. During lactation the sow exploits her accumulated energy reserves in the form of subcutaneous fat and internal fat. When these reserves are exhausted, the organism of the sow draws energy from other tissues, leading to a significant decrease in body weight. The magnitude of the loss of body weight components depends on the quantity and type of fodder given in each period of the reproductive cycle (particularly lactation), on the size of the litter that the sow is nursing, and on the length of the nursing period (WIELBO 1995, REKIEL 2002).

Restoration of fat tissue and maintenance of optimal body weight in successive cycles are very difficult, and in consequence the reproductive potential of sows is not fully exploited (KOCZANOWSKI 1986). During the rearing of each litter, fat content in the body of the sow gradually decreases (YANG et al. 1989). High-energy feeding during the lactation period reduces weight loss and the decrease in backfat thickness, so that sows fed in this manner exploit the energy reserves of their own bodies to a lesser degree (COFFEY et al. 1994, DOURMAD et al. 1994, MIGDAŁ 1996).

Naked oat grain is an excellent fodder for pigs because it has the highest protein and fat content of all cereals, as well as beneficial fibre composition (PELTONEN-SAINIO 1997, PETKOV et al. 2001, SEMENIUK 2001, PISULEWSKA et al. 2011). Naked oats have higher energy value and protein content than other cereals regarded as the most beneficial for feeding monogastric animals (PETKOV et al. 2001). An important component of oats with and without hulls is fat, the content of which ranges from 6% to 8% of dry matter (PIECH et al. 2003, PISULEWSKA et al. 2011). The fat of naked oats is dominated by unsaturated fatty acids (UFA), which account for 80% of the fat (PISULEWSKA et al. 1999). The lipids in oats have been found to contain compounds with strong antioxidant properties, such as tocopherols, ferulic acid, caffeic acid, polyphenolic compounds, their esters and amides, alkylphenols, flavonoids and avenanthramides (PETERSON 2001).

The positive effect of oats on the animal organism is also linked to their high mineral content (CYRAN 1997).

The aim of the study was to determine the effect of naked oats used in feed rations for gilts on their diets and on changes in body weight and backfat thickness during gestation and lactation.

Material and Methods

The study was carried out on Polish Landrace gilts raised on a breeding farm. Three gilts weighing about 30 kg were selected from each of 60 litters and assigned to three groups – two experimental and one control – with 60 individuals each. The animals were fed complete mixed rations in amounts consistent with the requirements given in *Swine Feeding Standards [Normy żywienia świń]* (1993). The mix fed to the experimental groups (3) contained *C* (control) 0%, *D*₁ 40% and *D*₂ 20% naked oats of the Akt variety. The composition and nutritional value of the diets is presented in Table 1.

Table 1

Composition of diets for gilts and for pregnant and lactating sows

Feed [%]	Gilts and sows up to 90 th day of gestation			Lactation		
	<i>C</i>	<i>D</i> ₁	<i>D</i> ₂	<i>C</i>	<i>D</i> ₁	<i>D</i> ₂
Naked oats meal	–	40.00	20.00	–	40.00	20.00
Wheat meal	40.00	–	20.00	40.00	–	20.00
Barley meal	48.40	48.50	48.45	40.00	40.10	40.05
Soybean meal	9.00	9.00	9.00	17.00	17.00	17.00
2-Ca phosphate	0.90	0.90	0.90	1.00	1.00	1.00
Fodder chalk	1.30	1.30	1.30	1.30	1.30	1.30
<i>L</i> -lysine 50% premixture	0.10	–	0.05	0.30	0.20	0.25
NaCl	0.30	0.30	0.30	0.40	0.40	0.40
Content [g/kg of diet]						
ME MJ	12.75	13.03	12.89	12.72	13.01	12.87
Crude Protein [g]	136.32	138.03	137.18	159.00	160.71	159.85
Crude Fat [g]	20.32	39.98	30.17	20.39	40.10	30.24
Lysine [g]	5.96	5.98	5.97	8.56	8.58	8.57
Methionine + cystine [g]	4.84	5.06	4.95	5.40	5.62	5.51
Ca [g]	7.78	7.69	7.73	8.53	8.43	8.48
P [g]	5.55	5.73	5.64	6.09	6.27	6.18
Na [g]	1.35	1.37	1.36	1.77	1.79	1.78

Following the onset of the second and third oestrus, the gilts were weighed and backfat thickness was measured with a Renco PREG-ALERT apparatus in four places: I – above the scapula, 3 cm from the back line, II – behind the last rib 3 cm from the back line, III – behind the last rib 8 cm from the back line and IV – on the lumbar region, 8 cm from the back line. Gel was used to better contact the tube with the skin.

In the sows, body weight and backfat thickness were measured in each reproductive cycle on the day before parturition, the first day after parturition and day 42 of lactation.

During the period from the 91st day of pregnancy and during the mating period the gilts and sows were fed the same diets as during lactation.

The results obtained were analysed statistically using one-way analysis of variance (group effect). Differences between means from each group were determined by Tukey's test.

Results and Discussion

Body condition and fat reserves in gilts at first mating affect the length of their reproductive life. The mean thickness of the fat tissue was highest in the group D_1 gilts; depending on oestrus number (second or third) it ranged from 15.6 to 17.7 mm. The backfat thickness (mean of the 4 measurements) is related to the fat content in the diets: 0, 40, 20 of CF/kg of diets C , D_1 and D_2 corresponds to linear decrease in the backfat thickness of gilts (Table 2).

Table 2

Backfat thickness in gilts during the second and third oestrus

Oestrus	Group	n	Backfat thickness [mm]				
			meas- urement 1	meas- urement 2	meas- urement 3	meas- urement 4	mean of 4 meas- urements
II	C	60	19.3	14.1	10.8	09.8	13.5 ^B ± 2.80
	D_1	60	21.9	16.4	12.8	11.2	15.6 ^A ± 3.20
	D_2	60	20.6	14.7	11.6	10.2	14.3 ± 2.90
III	C	30	20.6	15.4	12.3	11.3	14.9 ^B ± 3.30
	D_1	30	23.6	19.1	14.6	13.3	17.7 ^A ± 4.10
	D_2	30	23.1	16.8	13.2	11.7	16.2 ±3.80

n – number of gilts evaluated

^{A,B} – significant differences within groups of gilts in the second or third oestrus at $p \leq 0.01$

These values differed significantly ($P < 0.01$) from the backfat thickness noted in the control group. It can be stated that the diets containing naked oats fed to the gilts in this study beneficially influenced the increase in backfat thickness. According to many authors (WHITTEMORE et al. 1995, SINCLAIR et al. 1998), backfat thickness exceeding 16 mm determines good reproductive results. The average backfat thickness of currently grown Polish Landrace gilts is approximately 10 mm. The obtained genetic progress of this feature did not adversely affect their reproductive performance (BLICHARSKI and SNOPIKIEWICZ, 2017). High-energy 'flush feeding' a few days before the onset of oestrus in gilts has a beneficial effect on the intensity of ovulation. This effect is much less perceptible in sows (HOFFMANN 1994, VAN DEN BRAND et al. 2001).

Changes in body weight and backfat thickness in sows mated during the second oestrus are presented in Table 3. The data show that the increase in body weight during pregnancy was lower in primiparous sows than in multiparous sows. Body weight and backfat thickness during gestation increased more in the experimental groups than in the control. Body weight loss after parturition ranged from 17.90 to 20.14 kg. Sows in the experimental groups lost less body weight during lactation, although no statistically significant differences were found. Fat reserves measured as backfat thickness at the time of mating were greater in gilts and sows whose diets included naked oats. A similar tendency persisted in the case of the increase in backfat thickness during gestation.

Table 3
Changes in body weight and backfat thickness in sows during gestation and during lactation

Reproductive cycle	Experimental group	Gross body weight gain during gestation [kg]	Body weight loss after parturition	Body weight loss during lactation [kg]	Backfat thickness at mating [mm]	Increase in backfat thickness during pregnancy [mm]	Decrease in backfat thickness during lactation [mm]
I	C	57.90±5.55	17.90	17.00	13.6	4.7	4.6
	D ₁	59.36±4.93	18.33	16.36	16.0	4.9	4.3
	D ₂	58.78±5.10	18.01	16.85	14.4	4.8	4.5
II	C	63.30±6.23	18.97	25.11	13.7	5.5	5.6
	D ₁	64.94±5.47	19.81	23.72	16.6	5.7	5.6
	D ₂	64.09±5.90	19.43	24.39	14.7	5.6	5.8
III	C	63.96±6.64	19.42	25.72	13.6	5.5	5.6
	D ₁	66.72±5.63	20.14	25.90	16.7	6.0	5.9
	D ₂	65.23±6.20	19.88	26.86	14.5	5.8	5.7

A measure of rational nutrition in gilts and sows is the change in body weight and backfat thickness in each stage of the reproductive cycle. During the productive life of the sow, periods of increased body weight during gestation, weight loss after parturition and weight loss during lactation occur in succession (WIELBO 1995, REKIEL 2002). The use of naked oats had a beneficial effect on body weight and fat gain, and also slightly reduced weight loss during lactation. During lactation a 10 kg loss of body weight in sows is a normal process of mobilization of energy and protein reserves needed for intensive milk production. Losses exceeding 10% of body weight may prolong the weaning-to-mating period, which occurs most often in primiparous sows (KAMYCZEK 2006).

Conclusions

The study showed that the naked oats used in the diet of the gilts and sows had a beneficial effect on their body condition and fat reserves measured as backfat thickness.

The naked oats included in the diets to the gilts and sows of both experimental groups increased body weight gain in the sows during gestation, and the lowest weight loss during lactation was noted in these groups as well.

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**FOOD SELECTIVITY OF EUROPEAN BEAVER
(*CASTOR FIBER* L.) OCCURRING IN THE AREA
LUBACZÓW AND CHOTYLÓW FOREST DISTRICTS**

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Key words: *Castor fiber*, food, chew trees.

Abstract

The aim of the study was to comparison of food selectivity of the European beaver (*Castor fiber*) occurring in the area Lubaczów and Chotyłów Forest Districts. Analyzed percentage distribution of damaged trees in relation to their species and diameter at the chewing site, correlations between the diameter of damaged trees and their distance from beavers' lodges, and correlations between the number of damaged trees and the hardness of the wood of the tree species. Two peaks of beavers' activity were noted in the analysed period (from October 2016 to May 2017), i.e. in October and November when the animals prepared for the winter and stored food, and in January and February when winter stocks are over and vegetation has not started and no herbaceous vegetation. The aspen poplar (*Populus tremula*) was mostly preferred by the rodents (59%), especially in the Lubaczów Forest District, whereas the bird cherry, alder, or willow species (25%) were most frequently chosen by the rodents in the Chotyłów Forest District. The observations also revealed that a majority of the chewed trees (36%) had a diameter of 21–30 cm, but these preferences differed between the study areas. In the Lubaczów Forest District, beavers damaged trees with a larger diameter, compared to those in the Chotyłów Forest District, and the difference was statistically significant ($t = 5.560$; $p = 0.000$).

The relationship between the number of damaged trees and the hardness of wood of the tree species was inversely proportional and negative but statistically insignificant ($r = -0.4$). There was a similar statistically insignificant ($r = 0.47$) correlation between the diameter of the damaged trees and their distance from beavers' lodges. The diameter of damaged trees decreased with the increasing distance from the lodges.

WYBIÓRCZOŚĆ POKARMOWA BOBRA EUROPEJSKIEGO (*CASTOR FIBER* L.) BYTUJĄCEGO NA OBSZARZE NADLEŚNICTW LUBACZÓW I CHOTYLÓW

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Słowa kluczowe: *Castor fiber*, pokarm, zgryzane drzewa.

Abstrakt

Celem badań był porównanie wybiórczości pokarmowej bobra europejskiego występującego na terenie Nadleśnictwa Lubaczów i Nadleśnictwa Chotyłów. Przeanalizowano rozkład procentowy zgryzionych drzew ze względu na gatunek, ich średnicę w miejscu ścięcia, zależność między średnicą uszkodzonych drzew a ich odległością od źeremi i korelację między liczbą uszkodzonych drzew a twardością drewna danego gatunku. Na terenach, na których przeprowadzono obserwacje, występowały dwa szczyty działalności bobrów – od października 2016 do maja 2017 r. W październiku i w listopadzie zwierzęta przygotowywały się na zimę i magazynowały pożywienie, z kolei w styczniu i w lutym, kiedy zapasy się skończyły, a wegetacja nie rozpoczęła się jeszcze, brakowało roślinności zielonej. Najczęściej wybieranym gatunkiem drzew przez gryzonie była osika (59%), zwłaszcza w Nadleśnictwie Lubaczów, natomiast w Nadleśnictwie Chotyłów – czeremcha, olsza czy wierzba (25%). W przeprowadzonych obserwacjach wykazano również, że najczęściej zgryzanych drzew (36%) miało średnicę 21–30 cm. W Nadleśnictwie Lubaczów bobry uszkadzały drzewa o większej średnicy w porównaniu z Nadleśnictwem Chotyłów i różnica ta była statystycznie istotna ($t = 5,560$; $p = 0,000$).

Zależność między liczbą uszkodzonych drzew a twardością drewna danego gatunku była odwrotnie proporcjonalna do tej cechy i była ujemna, lecz statystycznie nieistotna ($r = -0,4$). Podobnie kształtowała się zależność między średnicą uszkodzonych drzew a ich odległością od źeremi i również była statystycznie nieistotna ($r = 0,47$). Wraz ze wzrostem odległości drzew od źeremi spadała średnica drzew zgryzanych.

Introduction

Until recently, the beaver was a rare species in our country. Currently, as a result of reintroduction initiated in the 70^s of the 20th century and species protection, beavers have become a common species. The population size of *Castor fiber* in Poland is systematically increasing; the inventories carried out in 1998 indicated that its population size was 12 thousand animals. Subsequently, it increased to 35 thousand in 2009 and approximately 55 thousand in 2014 (DZIĘCIOŁOWSKI 1999, DZIĘCIOŁOWSKI AND GOŹDZIEWSKI 2011, GUS 2014). At present, beavers have spread virtually

across the country with the greatest abundance in the north-eastern part of Poland. As reported by the Central Statistical Office (2016), the number of beavers in Poland was estimated at 121 624 individuals, with the greatest abundance noted in Mazowieckie (16 thousand), Podlaskie (15 thousand), Podkarpackie (14 thousand) Wielkopolskie (12 thousand), and Warmińsko-Mazurskie (11.5 thousand) Provinces and the lowest numbers in Pomorskie and Śląskie Provinces (2 thousand in each) as well as Dolnośląskie (1 thousand) and Opolskie (450 individuals) Provinces.

Beavers are characterised by a unique ability to adapt the environment to their needs. The activity of this species can be beneficial for nature, especially in forest complexes. Beavers play an important role in renaturalisation of forest ecosystems and contribute to water retention in periods of summer droughts and cleaning of water. Areas flooded as a result of beavers' activity are inhabited by many species of invertebrates, amphibians, fish, and waterfowl, and the backwater environs of provide refugia for many species of mammals and birds of prey. Simultaneously, the role of the beaver in shaping the environment and in the enhancement of the attractiveness and aesthetic, recreational, and educational assets of an area should be emphasised (KONOPKA and ERENC 1993). The benefits of beavers' activities are often underestimated and difficult to evaluate.

Yet, beavers can cause losses in the human economy. An increase in the population of beavers in a given area is accompanied by a greater number of reports of damage caused by their activity. The most frequent problems include flooding of fields, grassland, and forest crops, felling and chewing trees in forests, tree stands, and fruit orchards, damage to pond banks, flood embankments, or road and railway embankments, and destruction of agricultural crops, e.g. maize, carrots, and beets etc. The greatest damage is noted in the initial period of beaver family settling down. Its extent declines with stabilisation of the population.

The aim of the study was to determine beavers' food selectivity in the analysed areas, percentage distribution of damaged trees according to their diameter at the chewing site, correlations between the diameter of damaged trees and their distance from beavers' lodges, and correlations between the number of damaged trees and the hardness of the wood of the tree species.

Material and Methods

The observations were carried out in two Forest Districts: Lubaczów and Chotyłów between October and May at the turn of 2016 and 2017.

The Lubaczów Forest District is located in Lubaczów County in the north-eastern part of Podkarpackie Province. The area of the Forest District is dominated by fertile and very fertile forest habitats, including mixed and riparian forests. The largest share of tree-forming species is noted for pine (51%), oak and birch (11%), and beech (10%) as well as alder, hornbeam, larch, and spruce. The observations were carried out in the Opaka Forestry branch 395 with an area of approximately 15 ha, in which there are 6 ha of meadows and the rest is a pre-felling tree stand (*Plan urządzania lasów Nadleśnictwa Lubaczów...* 2009).

The other area of observation was the Chotyłów Forest District located in Bialsk County in the north-eastern part of Lubelskie Province. The Forest District has 13.3 thousand ha of forest area. Fresh coniferous forest and fresh mixed deciduous forest, with pine (72%), birch, alder, and oak are the main forest-forming components. Beavers were observed in the Lutnia and Wólka Dobryńska Forestry areas (*Plan Urządzania Lasów Nadleśnictwa Chotyłów...* 2014).

The observations were carried out once a week together with an inventory of damage. The tree species and the distance from beavers' lodges were determined and the diameter of all trunks was measured at the felling height with an accuracy of 1 cm using a timber diameter meter (caliper). Additionally, photographic documentation of the animal habitats and transformations induced in the environment was prepared.

The statistical analysis of the results was performed using statistical package *Statistica* 13.1. The normality of the distributions of the analysed traits was assessed with the use of the Shapiro-Wilk test. Since the distributions of the variables were normal, the *t*-test for independent samples was employed to assess the significance of the differences between the means. Pearson's correlation coefficients were calculated to determine the relationships between the analysed values.

Results

The greatest number of damaged trees in both Forest Districts was noted in February (24%), October and November (22%), and January (19%). There were two peaks of beavers' food activity during the observation periods: in October and November when the animals prepared for the winter and stored food and in January and February in the mating period and beginning of pregnancy. Early spring is the time when animals are probably hungry due to the shrinkage of winter stockpiles and lack of herbaceous vegetation. They also probably forced to repair destroyed during the winter of the lodge. In the subsequent months, the number of damaged trees declined (Figure 1).

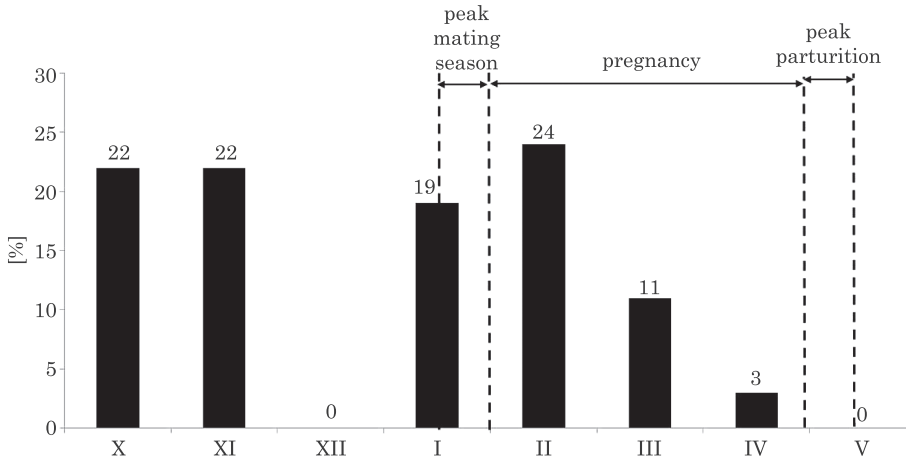


Fig. 1. Percentage distribution of damaged trees in the subsequent months of the study period

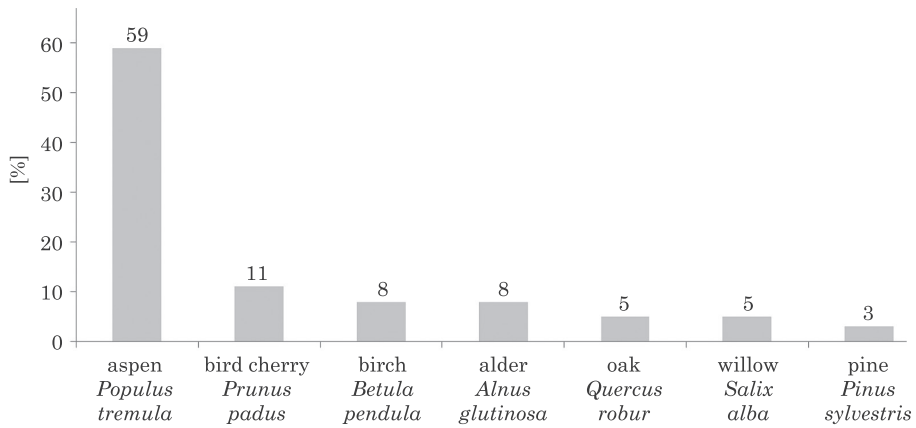


Fig. 2. Percentage share of damaged species in the total number of damaged trees



Fig. 3. Percentage distribution of damaged species in the compared Forest Districts

The aspen poplar (*Populus tremula*) was mostly preferred by the rodents (59%), especially in the Lubaczów Forest District, where it accounted for 88% of damaged plants. The beavers were less inclined to chew the bird cherry (*Padus avium*), black alder (*Alnus glutinosa*), or silver birch (*Betula pendula*). Noteworthy is the fact that bird cherry, alder, or willow trees were chewed by the animals most frequently (25%) in the Chotyłów Forest District (Figures 2–3). In the winter period, damage to pine trees (*Pinus sylvestris*) was noted as well (8%).

The observations indicate the highest percentage (36%) of chewed trees with a diameter of 21–30 cm (Figure 4); however, this preference differed between area the Forest Districts. In the Lubaczów Forest District, beavers damaged trees with a larger diameter than that of the trees observed in the Chotyłów Forest District (Figure 5); the difference estimated with the *t* test for independent samples was statistically significant ($t = 5.560$; $p = 0.000$).

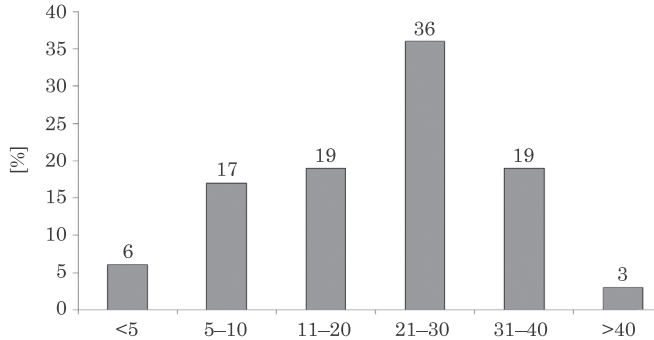


Fig. 4. Percentage distribution of damaged trees according to their diameter measured at the chewing site

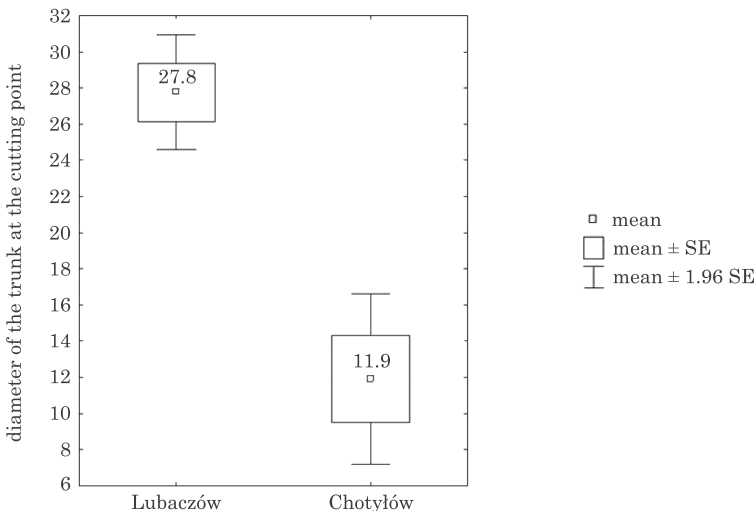


Fig. 5. Distribution of the mean diameters of damaged trees in the compared Forest Districts (test *t* for independent samples: $t = 5.560$, $p = 0.000$)

The relationship of the number of damaged trees was inversely proportional to the hardness of the wood of the analysed tree species. The calculated correlation between the number of chewed trees and wood hardness [kg cm^{-2}] was negative and statistically insignificant ($r = -0.4$). The number of damaged trees decreased with the increasing hardness of the wood (Figure 6). There was a similar correlation between the diameter of damaged trees and their distance from beavers' lodges, which was statistically insignificant as well ($r = 0.47$). The diameter of the damaged trees decreased with the increasing distance from the lodges (Figure 7).

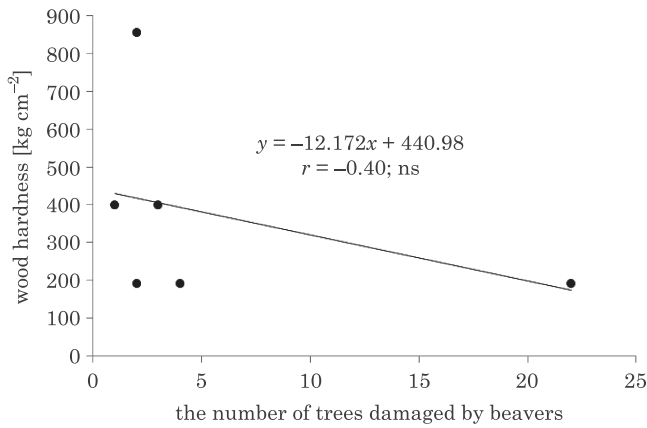


Fig. 6. Correlation between the number of damaged trees and hardness of the wood of the analysed species [kg cm^{-2}] (Kokociński 2004)

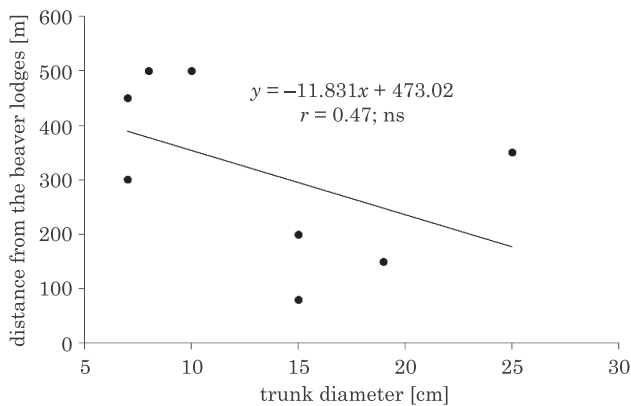


Fig. 7. Correlation between the diameter of damaged trees and their distance from beavers' lodges

Discussion and Conclusions

Beavers are herbivores eating almost all species of littoral and aquatic plants; their menu comprises over 200 species of herbaceous and 100 woody plants (CZECH 2000a). In general, this diversity is limited by the availability of food, as beavers feed in a relatively narrow 20-meter littoral zone. Besides the depth of the water reservoir, also the abundance of woody food required for winter survival determines the site of settlement. From mid-October, shrubs and deciduous trees stored for the winter time are the diet of beavers. The animals do not eat wood but leaves, young shoots, thin twigs, phloem, and bark. They especially prefer aspen and other poplar species as well as willow, followed by birch, hazel and other deciduous species (CZECH 2000b). As shown by the present observations, the feeding preferences of beavers depended mainly on the abundance of vegetation growing in the living habitat. They most often chose quick-growing tree species, i.e. aspen poplar, common willow or bird cherry in the first and second age class. This is confirmed by research carried out in the Czech Republic, where a strong preference for poplar and willow has been demonstrated as well (FICEK 2003, JOHN 2001, BELOWSKI 1984, FUSTEC et al. 2001, BRENNER 1962, DERWICH 2001). Aspen is digested by the beaver faster than other tree species, and the daily consumption of other plants was inversely proportional to their digestibility. Other studies (NOLET et al. 1994, DZIĘCIOŁOWSKI 1996) have proposed a hypothesis of the preference for willow associated with the low content of resin and essential oils, which beavers try to avoid. Additionally, the number of tree species in the diet increases at the end of the growing season, when beavers collect reserves for the winter. To meet this need, beavers consume species from the genera *Populus spp.* and *Salix spp.* (KOSTKAN 2000, FICEK 2003, NOLET et al. 1994, DANIŁOW and KANSHIEV 1983, KROJEROVÁ et al. 2010). In the absence of these species, beavers eat *Betula spp.*, *Acer spp.* as well as *Tillia cordata*, *Corylus avellana*, and *Quercus robur* (DOUCET and BALL 1994, DOUCET et al. 1996).

Similar observations were reported from the Poleski National Park, where an interesting fact of consumption of alder buckthorn (26%) and pedunculate oak (17%) was described (JANISZEWSKI et al. 2017). The study conducted in the Chotyłów and Lubaczów Forest Districts showed the bird cherry (11%), oak (5%), and pine (3%) as components of beavers' diet. As reported by NOLET et al. (1994), beavers can choose plant species to supplement specific nutrients whose content in the preferred species is low.

The present study has shown that the beavers chose species of trees with a trunk diameter in the range of 21–30 cm (36%) as well as 11–20 cm

and 31–40 cm (19%). In contrast, a Czech report has demonstrated preferences for species with a trunk diameter below 20 cm (78%). The diameter of the most damaged tree species ranged from 2.6 to 6 cm, the second diameter chosen most frequently was in the range from 6.1 to 12 cm, and the third most preferred group comprised trees with a diameter below 2.5 cm (DVORAK 2013). The observations conducted in the Lubaczów and Chotyłów Forest Districts have indicated that beavers may also be interested in trees with larger diameters. It should be borne in mind that a larger diameter of a felled tree requires a longer time spent by beavers on the land, which increases the potential danger e.g. from predators. Therefore, beavers select trees in terms of their species and trunk diameter. The investigations conducted by JANISZEWSKI et al. (2017) described preferences for trees with a diameter up to 15 cm with a mean 88% percentage share of such trees (from 79.0% to 90.9%).

In the Lubaczów and Chotyłów Forest Districts, there was a negative but statistically insignificant ($r = -0.4$) correlation between the number of chewed trees and wood hardness (kg cm^{-2}). Similar results were presented by CZYŻOWSKI et al. (2009) in their research carried out in Lublin and Nadwieprzański Landscape Park ($r = -0.3211$). The number of damaged trees decreased with the increasing hardness of wood. There was a similar inversely proportional correlation between the diameter of damaged trees and their distance from beavers' lodges.

In areas that are intensively used by beavers, forests temporarily disappear and non-forest flora and fauna encroach (VALACHOVI AND GÍMEŠ 2003). The strong preference for willow or poplar trees can slow down the succession of these species on wet floodplains. Simultaneously, the fact that beavers willingly chew these species contributes to transformation of the tree stand and enhances the development of other vegetation species thus creating favourable living conditions for aquatic environment fauna (JANISZEWSKI et al. 2014). Some shoots and branches from felled trees are used by beavers to build dams. However, it has been shown that the selection of constructions materials depends on the occurrence and availability of woody vegetation in the littoral zone. No selection of tree or shrub species in acquisition of building material has been reported (JANISZEWSKI et al. 2006).

The presence of beaver families in the studied area does not interfere seriously with human activities and forest management, although periodic flooding can be the greatest nuisance. Damage to the forest environment caused by waterlogging is not estimated to be high. Areas flooded by beavers are usually an inconsiderable part of the entire production area and have no economic significance, since the habitats in river and stream

valleys are characterised by the presence of trees with low quality of raw material. These tree stands have low mass reserves, which also makes the economic losses in forest management negligible (Brzuski and Kulczycka 1999). Furthermore, this type of small retention increases the biodiversity and creates a specific microclimate.

In the present situation related to the occurrence and increasing numbers of this species on the territory of Poland, these studies may be used in practice, for example when planning trees plantings in coastal watercourses and water reservoirs.

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**THE RISK ELEMENTS BIOMONITORING
IN THE AMBIENT AIR OF AN UNDERGROUND
PARKING LOT***

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Key words: moss and lichen bags, RAF value, traffic pollution, air pollution, vehicles.

Abstract

Moss and lichen bag technique with use of three mosses (*Pleurosium* spp., *Rhytidiadelphus* spp., *Polytrichum* spp.) and one lichen *Pseudevernia furfuracea* taxon was used to monitor air quality in underground parking lot in the city of Prešov, Slovakia. Moss and lichen bags were exposed for 6 weeks at different distances from the entrances/exits. Accumulation ability was expressed by relative accumulation factor. The study aims to detect the level of pollution by the risk elements using relative accumulation factor (RAF) and compare accumulation abilities of different moss and lichen taxa. Accumulation ability of exposed taxa decreased in order: *Pleurosium* spp. > *Rhytidiadelphus* spp. > *Pseudevernia furfuracea* > *Polytrichum* spp. Comparing the evaluated elements, Zn and Ni reached the highest RAF values. The distance from the entrance/exit did not affect the level of elements. It is not being given much attention to the air quality assessment in enclosed parking lots in the world, and this is the first such kind of research in Slovakia.

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BIOMONITORING SZKODLIWYCH ZWIĄZKÓW W WENTYLOWANYM POWIETRZU PARKINGU PODZIEMNEGO

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Słowa kluczowe: pakiety mchów i porostów, wartość RAF, zanieczyszczenie powietrza, pojazdy mechaniczne.

Abstrakt

Do monitorowania jakości powietrza na podziemnym parkingu w Preszowie na Słowacji użyto techniki biomonitoringu za pomocą tzw. pakietów mchów i porostów z wykorzystaniem trzech mchów (*Pleurosium* spp., *Rhytidiadelphus* spp., *Polytrichum* spp.) i jednego taksonu porostów *Pseudevernia furfuracea*. Pakiety mchów i porostów były eksponowane przez 6 tygodni w różnych odległościach od wejść/wyjść. Zdolność akumulacji wyrażono względnym współczynnikiem akumulacji. Celem badania było wykrycie poziomu zanieczyszczenia szkodliwymi związkami za pomocą względnego współczynnika akumulacji (RAF) i porównanie zdolności akumulacyjnych różnych taksonów mchów i porostów. Zdolność akumulacji narażonych taksonów zmniejszyła się w kolejności: *Pleurosium* spp. > *Rhytidiadelphus* spp. > *Pseudevernia furfuracea* > *Polytrichum* spp. Porównując oceniane zanieczyszczenia, Zn i Ni osiągnęły najwyższe wartości RAF. Odległość od wejścia na parking i od wyjścia z niego nie miała wpływu na poziom zanieczyszczeń.

Ocenie jakości powietrza w zamkniętych parkingach na świecie nie poświęcono do tej pory wiele uwagi. Jest to pierwsze tego rodzaju badanie również na Słowacji.

Introduction

The urbanisation and continuous increase in the number of vehicles cause a great pressure to the environment. Industrial activities, domestic fuel burning, agricultural activities, natural dust, and salt, are together with the traffic the most involved in air pollution in urban areas. Traffic related emission originates predominantly from exhaust fumes, tyre wear, asphalt erosion, wear of braked and engines, rusting of the car body, as well as sand and silt transported by wind and other means (THROPE and HARRISON 2008). Numerous studies have confirmed significant relationship between car intensity and the trace metals concentrations (LUILO and OTHMAN 2008, WEI et al. 2015). There has been found that the biggest

volume of risk elements is released from the cars during cold-starts (CHEN et al. 2011, REITER and KOCKELMAN 2016) and at lower speeds typical for driving into or out of car parking lots (ZIELINSKA et al. 2012). Underground parking lots hold a lot of cars and additionally, they are partially closed what causes accumulation of emissions. Ventilation systems to remove contaminated air out of the parking lot is usually inadequate or irregularly controlled (CHITHRA and NEGENDRA 2012). Accumulation of the risk elements leads to the cancer risk (VUKOVIĆ et al. 2015), respiratory or cardiovascular diseases (POPE 2000) and nervous system and reproductive systems defects (PAPANIKOLAOU et al. 2005). VUKOVIĆ et al. (2015), who evaluated the concentration of heavy metals in the underground parking areas in Belgrade have found, concentrations lower than acceptable limits set by the US environmental Protection Agency (EPA). The higher values of Zn and Cr at the exits comparing entrances were found in the underground garages in Wuhan (China) (LI and XIANG 2013).

There are many traditional methods for air pollution monitoring, but they are limited by the high costs or difficulties of carrying out extensive sampling (SZCZEPANIAK and BIZIUK 2003). Mosses and lichens, naturally occurred or transplanted, are very often used for air biomonitoring purposes in various types of the environment such as forest areas (KŁOS et al. 2018), indoor environment of the buildings (TONG et al. 2016), environment along roads and/or urban areas (VUKOVIĆ et al. 2013), or near the mines (BALABOVA et al. 2014) or tailing ponds (DEMKOVÁ et al. 2017). The high accumulation capacity makes mosses and lichens suitable organisms for biomonitoring studies (VUKOVIĆ et al. 2013).

The aim of the study was i) to assess the level of risk elements at underground parking lot in the city of Prešov (Slovakia) using relative accumulation factor (RAF), ii) compare the accumulation ability of mosses and lichen (M/L) taxa and iii) evaluate the influence of the presence/absence of the daylight on the accumulation ability of selected taxon.

Research activities devoted to the assessment of the content of hazardous elements in the air due to transport are still insufficient. Moreover, this is the first research focused on air quality in underground parking lots in Slovakia.

In this study, we hypothesise high volumes of the risk elements in the underground parking lot, due to combustion of fossil fuels and car body corrosion. Additionally, we hypothesise increasing volumes of the risk elements from outside to the inside of the parking lot. We also suppose that different mosses/lichens show different ability to accumulate different elements, that's why is suitable to use them together.

Material and Methods

Three mosses (*Pleurosium* spp., *Polytrichum* spp., *Rhytidiadelphus* spp.) and one lichen taxon (*Pseudevernia furfuracea*) were sampled in June 2016 in Slanske vrchy hills (the Eastern Slovakia). Sampling locations were selected at least 1 km from main road and 500 m from the forest road. Around 500 g of each taxon was sampled, stored in plastic bags, and transported to the laboratory. The samples were manually cleaned from needle, parts of the bars and residues of other plants. Moss/lichen (M/L) taxa were washed three times, lasting 20, 15 and 10 minutes (with 10 L of distilled water per 100 g M/L dry weight), hand squeezed and dry out (60°C for 24 h) according to the procedures described in ADAMO et al. (2007). 5 g of each taxon was wrapped into the nylon mosquito net cut at pieces 10 x 10 cm. Underground parking lot (Figure 1), with an area 10.148 m² and 356 parking spots, containing two similar parts with the entrance/exit localized at opposite (Figure 2).



Fig. 1. Localization of the underground parking lot in the Prešov city (Slovakia)

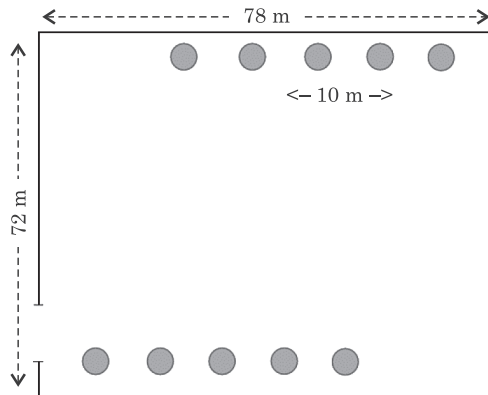


Fig. 2. Exposure position of the M/L taxa bags in underground parking lot

Ten exposition positions (EP), five at each side of the parking area, directed from the main entrance/exit inside the parking lot (48°59'59,71"N; 21°16'14,34"E) were selected. At each EP (approximately 3 m height), two samples of each taxa (a total 80 samples) were exposed for 6 weeks. The experiment was carried out twice, in May 2017 and again in October 2017. After exposure, the M/L samples were stored in closed plastic bags (-18°C) prior to analysis.

The homogenized M/L samples were prepared by milling in a laboratory grinder IKA A10 basic (IKA Works, Wilmington, USA). The homogenized samples were stored in closed plastic bags until the next treatment step. For pressure microwave digestion, approximately ~0.20 g (with a precision to 4 decimal places) of samples was weighed into PTFE digestion tubes. Consequently, 5 mL of HNO₃ and 1 mL of H₂O₂ (trace purity) were purchased from Lambda Life spol. s r.o., Bratislava, Slovakia (producer: Sigma-Aldrich Chemie GmbH, Steiheim, Germany), was added directly to the PTFE vessels. The digestion procedure was carried out using pressure microwave digestion system ETHOS-One (Milestone, Srl., Italy). Elemental analysis was carried out on an Agilent ICP-OES spectrometer 725 (Agilent Technologies Inc., Santa Clara, CA, USA) with axial plasma configuration and with an auto-sampler SPS-3 (Agilent Technologies, GmbH, Germany). Detailed experimental conditions were set as follows: RF power 1.45 kW; plasma gas flow 16.0 L min⁻¹; auxiliary gas flow 1.50 L min⁻¹ and nebulizer gas flow 0.85 L min⁻¹ and CCD detector temperature -35°C. Signal accumulation time 3 s for 3 replicates. In total, 80 M/L samples were analysed for concentration of 14 elements (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, Sb and Zn). Calibration of the analytical method ICP-OES was realized using mixed standard TraceCert ICP 5 (Sigma Aldrich, GmbH, Steiheim, Nemecko), which was diluted to the three calibration levels (I – 0.0475 mg kg⁻¹; II – 0.950 mg kg⁻¹; III – 0.190 mg kg⁻¹). Argon and carbon were used as internal standard elements. ERM[®]-CE278k (mussel tissue; IRMM, Belgium) was used for quality measurements control. Following spectral lines were used for quantitative and qualitative elements determination: Al – 167,019 nm; As – 188,980 nm; Ba – 455,403 nm; Cd – 226,502 nm; Co – 228,615 nm; Cr – 267,716 nm; Cu – 324,754 nm; Fe – 234,350 nm; Li – 670,783 nm; Mn – 257,610 nm; Ni – 231,604 nm; Pb – 220,353 nm; Sb – 206,834 nm and Zn – 206,200 nm. All risk elements data were calculated to the mg kg⁻¹ DW. Total content of the risk elements was determined also in the reference material – M/L samples not exposed. The final values of risk elements in M/L bags were computed as the measured (exposed) valued minus reference values. The same procedure was used in both experimental seasons. The final values

of risk elements content were computed as the average values of both (May, October) measurements.

Relative accumulation factor - RAF (Equation 1) was calculated, to assess the risk element accumulation in the evaluated M/L taxa, as follows:

$$\text{RAF} = (C_{\text{exposed}} - C_{\text{initial}}) / C_{\text{initial}} \quad (1)$$

The differences in risk elements RAF values between taxa were analysed using One-way ANOVA test, followed by the Tukey's multiple comparison test. Cluster analysis (CA)Wards method was used to find out the similarities between taxa to accumulate evaluated risk elements. Spearman's correlation coefficient was used to determine significant correlation of risk elements RAF and the EP in the underground parking lot. One-way ANOVA test the same as Spearman's correlation coefficient were considered statistically significant if P-value was less than 0.05 and/or 0.01. Principal Component Analysis (PCA) was used to distinguished taxa based on the risk elements concentrations. All statistical analyses were performed using R studio (R Studio Team, Boston). All data were log-transformed prior to analysis.

Results and Discussion

The descriptive statistics of the risk elements RAF in M/L taxa in the underground parking lot is shown in the Table 1. The average RAF concentrations decreased in the following order: Zn > Ni > Mn > Fe > Cr > Cd > Co > Pb > Sb > Li > Al > As > Cd > Cu (Table 2). High RAF values of Zn

Table 1

The descriptive statistics of the risk elements RAF values in the different M/L taxa

		<i>Pleurosimium</i> spp.	<i>Polytrichum</i> spp.	<i>Pseudevernia furfuracea</i>	<i>Rhytidiadelphus</i> spp.
Al	min.–max	1.21–1.83	0.98–1.42	2.04–2.95	0.68–1.03
	median±std	0.24±1.57	0.20±1.20	0.39±2.25	0.15±0.86
As	min.–max	1.16–2.22	0.00–4.23	0.00–2.61	0.27–1.97
	median±std	0.48±1.22	1.69±0.99	0.97±0.77	0.61±1.04
Ba	min.–max	0.42–1.56	1.00–1.14	0.37–0.63	1.27–1.49
	median±std	0.46±0.61	1.12±0.07	0.46±0.11	1.38±0.09
Cd	min.–max	0.88–1.84	0.72–1.14	1.01–1.83	5.69–9.00
	median±std	1.53±0.36	0.81±0.16	1.31±0.31	6.7–1.31

Co	min.–max	0.19–1.00	0.24–1.05	0.05–0.46	3.98–14.9
	median±std	0.62±0.33	0.85±0.31	0.26±0.15	7.69±4.00
Cr	min.–max	1.39–7.17	1.16–2.05	2.44–4.72	2.17–4.28
	median±std	3.19–2.39	1.74±0.34	3.66±0.86	2.50±0.68
Cu	min.–max	0.22–0.27	0.57–0.66	0.66–0.96	0.99–1.48
	median±std	0.27±0.02	0.59±0.04	0.86±0.12	1.23±0.21
Fe	min.–max	2.02–3.93	1.97–2.48	2.81–5.32	2.76–3.59
	median±std	3.49±0.82	2.30±0.19	4.36±0.96	1.18±0.31
Li	min.–max	1.29–2.50	1.71–2.24	1.01–1.37	0.87–1.42
	median±std	1.82±0.54	2.08±0.23	1.20±0.13	1.01±0.22
Mn	min.–max	2.47–6.57	1.83–2.37	2.54–4.10	8.25–9.64
	median±std	4.62±1.45	2.10±0.20	3.77±0.61	8.96±0.55
Ni	min.–max	3.80–9.43	3.80–5.01	6.19–12.1	5.17–5.90
	median±std	5.43±2.31	4.06±0.51	7.96±2.25	5.62±0.30
Pb	min.–max	1.37–13.3	1.35–1.76	0.96–1.68	1.46–2.03
	median±std	2.08±5.15	1.68±0.16	1.48±0.28	1.83–0.24
Sb	min.–max	0.75–1.62	2.30–3.20.39	1.11–1.8	1.31–2422
	median±std	1.36±0.32	2.87±0.39	1.24±0.29	1.86±0.42
Zn	min.–max	32.9–157	9.57–12.6	16.3–24.3	45.6–51.0
	median±std	47.1±51.9	11.7±1.13	21.4±2.96	47.4±2.2

Table 2

RAF values of risk elements in M/L samples after the 6 weeks of exposure in underground parking lot

RAF	Element
≤0	–
0–1	Al, As Ba, Cu
1–5	Cd, Co, Cr, Fe, Li, Mn, Pb, As, Sb
<5	Zn, Ni

were confirmed in several studies focused on the traffic pollution or urban air pollution (VUKOVIĆ et al. 2015). Except zinc, in the surrounding of the heavy traffic places serious concentrations of Cd, Cu, Ni and Pb were found (ALSOUBOU and AL-KHASHMAN 2018, BUDAI and CLEMENT 2018). In our case, the pollution by Cd and Cu wasn't as serious. Comparing taxa, the highest RAF values of Cr, Pb and Zn were found for *Pleurosiium* spp.; As, Li and Sb for *Polytrichum* spp., Ba, Cd, Co, Cu and Mn for *Rhytidiadelphus* spp., and Al, Fe and Ni for *Pseudevernia furfuracea*. *Pleurosiium* spp. was found as the best accumulator of Zn in a study focused on ambient air quality assessment around petrol stations (DEMKOVA' et al. 2017). One-way ANOVA followed by Tukey post-hoc test was used to detect significant differences in accumula-

tion abilities between taxa. Significantly highest ability to accumulate Sb was found for *Polytrichum* spp. *Rhytidiadelphus* spp. was statistically confirmed as the best accumulator of Co, Cd and Mn ($p < 0.01$) – Table 3.

Table 3

The results of one-way ANOVA for the comparison the risk elements RAF values between different taxa and between EP

Risk element	Factor	Df	F-value	p	Factor	Df	F-value	p
Al	between taxa	3	34.59	3.18e-07**	between EP	4	0.155	0.958
As		3	0.361	0.782		4	3.884	0.023*
Ba		3	14.46	8.09e-05**		4	0.218	0.924
Cd		3	120.8	3.35e-11**		4	0.08	0.987
Co		3	59.18	7.01e-09**		4	0.073	0.989
Cr		3	3.58	0.0376*		4	1.754	0.191
Cu		3	79.87	7.65e-10**		4	0.081	0.987
Fe		3	8.56	0.00123**		4	0.106	0.978
Li		3	2.74	0.000164		4	0.100	0.981
Mn		3	44.19	5.75e-08**		4	0.145	0.962
Ni		3	6.63	0.00406**		4	1.488	0.255
Pb		3	1.488	0.256		4	0.398	0.807
Sb		3	14.38	8.36e-05**		4	0.374	0.823
Zn		3	25.62	2.39e-06**		4	0.083	0.986

* $P < 0.05$; ** $P < 0.01$

Highest Cd and Mn accumulation ability of *Rhytidiadelphus* spp. was confirmed in the study focused to the air pollution monitoring around tailing pond (DEMKOVA et al. 2017). *Pseudevernia furfuracea* and *Polytrichum* spp. were significantly different in their ability to accumulate Cr and Ni. All evaluated taxa significantly differ in their ability to accumulate Cu. No differences in Pb, Zn and As RAF values were confirmed between taxa. Comparing taxa (average risk element RAF value for each taxon), the decrease in order *Pleurosium* spp. > *Rhytidiadelphus* spp. > *Pseudevernia furfuracea* > *Polytrichum* spp. was detected. *Pleurosium schreberi* is very often used for bioaccumulation studies in Europe, because of its wide spread distribution (HARMENS et al. 2015) and excellent ability to accumulate not only heavy metals but also organic pollutants (KOSIOR et al. 2017). VINGIANI et al. (2015) have found, that in the same exposure conditions, the element accumulation in moss-bags was higher than in lichen-bags. Because moss and lichen taxa differ in accumulation ability of various risk elements, it is suitable to use them together to obtain more thorough results (LOPPI and BONINI 2000). In our case, *P. furfuracea* did not stand

out in accumulation capacities compared to the mosses. M/L bags were deployed in different distances from the main entrance/exit (Figure 2). Reduction of the daily light in the inward direction could disrupt the lichen taxon accumulation because they need sunlight and humidity for their good functionality (SHUKLA et al. 2014). On the other hand, bright sunlight and high temperature have an inhibiting effect on the lichen growth (SHUKLA et al. 2014).

Mean RAF values (average value for each taxon at each EP), the same as partial RAF values (RAF values of individual risk element for each taxon at each EP) were computed to determine correlation between EP and the average RAF value of the individual taxon. EP has no influence on the risk element accumulation in case of *P. furfuracea*, *Rhytidiadelphus* spp., and *Pleurosium* spp. Significant negative correlation ($p < 0.05$) was found between average RAF value and EP in case of *Polytrichum* sp.

Specifically, Mn and Zn gave significant negative correlation ($p < 0.01$) and Al significant positive ($p < 0.05$) correlation with the EP in case of *Polytrichum* spp. The average RAF (regardless the taxon) are listed in the Figure 3.

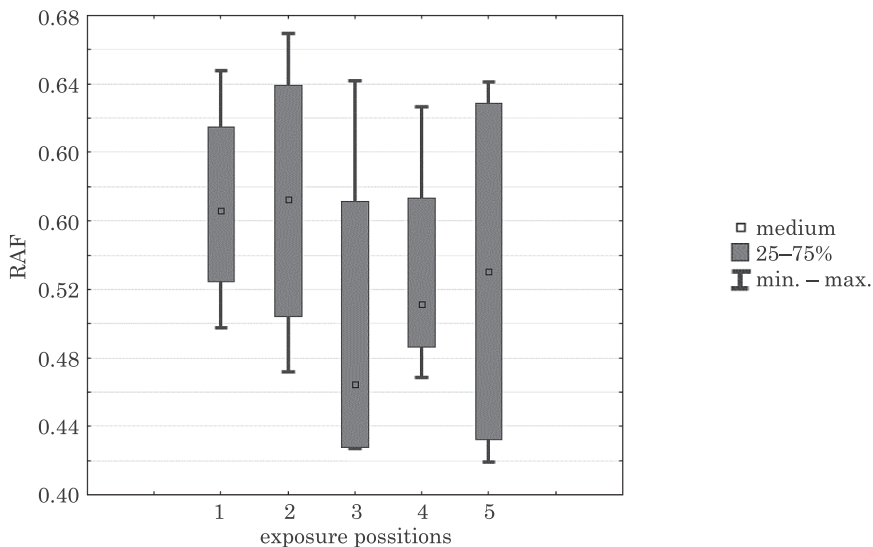


Fig. 3. The median values of RAF at the various EP regardless the M/L taxa

The highest average RAF value was determined at the second EP, then fell slightly. The highest RAF variation was confirmed for the last (5th) EP. Air flow, car movement, and ventilation system affected the distribution of metals within the parking lot (YAN et al. 2017, ZHAO AND ZHAO 2016). One-way ANOVA test (Table 3) was used to determine the

differences in the taxa's risk elements accumulation ability between EP. In our case, no significant differences were found for all evaluated risk elements RAF values except As. The As values were significantly highest in the EP located next to the entrances/exits. Some studies confirmed that fuels, oils, brake pads and tyres have traces of As (LUILO et al. 2014). But, on the other hand, the main As pollution comes from industrial activities or agricultural activities (herbicides or insecticides using), what could affect their accumulation in the EP located next to the entrance (KABATA-PENDIAS 2011).

It has been found that the risk elements originating from the same source correlate with each other (LI et al. 2008). Spearman's correlation coefficient was used to find out correlation relationships between RAF values of evaluated risk elements. Based the Spearman's correlation coefficient, no significant correlation between risk elements RAF values and the EP were found (Table 4). Only arsenic RAF gave significant negative correlation with the EP. The values of arsenic increase towards the exists/entrances. Zinc gave significant positive correlation ($p < 0.01$) with the Cd, Mn, Pb and significant negative correlation with Sb ($p < 0.05$).

Table 4
The Spearman's correlation analysis, between risk elements RAF values themselves, and between risk elements RAF values and the EP

	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Li	Mn	Ni	Pb	Sb	Zn
EP	0.18	-0.57**	0.11	-0.08	-0.09	-0.36	-0.06	-0.12	-0.02	-0.07	-0.37	0.12	-0.18	0.04
Al		-0.2	-0.7	-0.55*	-0.72	0.44*	-0.25	0.63**	0.13	-0.36	0.52*	0.03	-0.4	-0.13
As			0.03	-0.02	-0.06	0.04	-0.25	-0.04	0.17	0.07	0.12	0.09	0.03	0.10
Ba				0.58**	0.72**	-0.49*	0.35	-0.28	-0.04	0.44*	-0.50*	0.41	0.48*	0.29
Cd					0.92**	0.16	0.67**	0.18	-0.55*	0.91**	0.09	0.03	-0.03	0.56**
Co						-0.01	0.66**	-0.02	-0.46*	0.77**	-0.11	0.05	0.22	-0.06
Cr							0.01	0.64**	-0.01	0.36	0.84**	-0.18	-0.3	0.24
Cu								0.24	-0.71**	0.44*	0.18	-0.29	0.23	-0.26
Fe									-0.22	0.33	0.75**	0.14	-0.39	0.33
Li										-0.45*	-0.20	0.12	0.26	-0.23
Mn											0.29	0.12	-0.26	0.73**
Ni												-0.19	0.26	0.11
Pb													-0.08	0.65**
Sb														-0.48*

* $P < 0.05$; ** $P < 0.01$

Arsenic gave significant negative correlation with Cd, Co and Fe. As mentioned above, arsenic originates predominantly from anthropogenic activities related to agricultural (the use of pesticides and fungicides)

(CHUNG et al. 2014). Large quantities of As are released into the environment through industrial activities, what causing its increased concentrations, especially in the urban areas (BHATTACHARYA et al. 2007).

Significant positive correlation was found between Al, Fe, Ni and Cr themselves. Additionally, all of them gave negative (significant for Ni and Cr) correlation with Ba. The presence of these elements in the underground parking lot could be attributed to the vehicle bodywork corrosion. Chromium is very often used in refractory steels (FURINI 2012), Ni in alloys and metal processing industries, and Al as corrosion inhibitor (KABATA-PENDIAS 2011). Close relationship was found between Cd, Ba, Co, Cu, Mn and Zn, which gave positive correlation (almost significant) between themselves, except Cu-Zn and Co-Zn. Cu, Cd and Zn are attributed to tyre abrasion (LI and XIANG 2013), lubrication oils (CHRISTOFODIS and STAMATIS 2009), and galvanized automotive parts (BLOK 2005). Ba and Mn are mainly released from fuel combustion (KABATA-PENDIAS 2011). Significant but negative correlation gave Li with Cd, Co, Cu and Mn. Lithium is usually used in batteries. Significant positive correlation was confirmed between Pb-Zn ($p < 0.01$) and Sb-Ba ($p < 0.05$). The presence of lead in gasolines has been banned in many countries for its toxicological effects, but it is still used in some types of fuel additives (SANTOS et al. 2015).

The results of the cluster analysis (Figure 4), based on the RAF risk element contents, indicate two clusters: (1): Zn and (2): Al, As, Ba, Cd, Cu, Co, Cr, Fe, Li, Ni, Mn, Pb, Sb. Inside the group 2, we can differentiate two smaller groups a) Al, Fe, Cr, Ni and b) As, Ba, Co, Cd, Cu, Li, Mn, Pb, Sb.

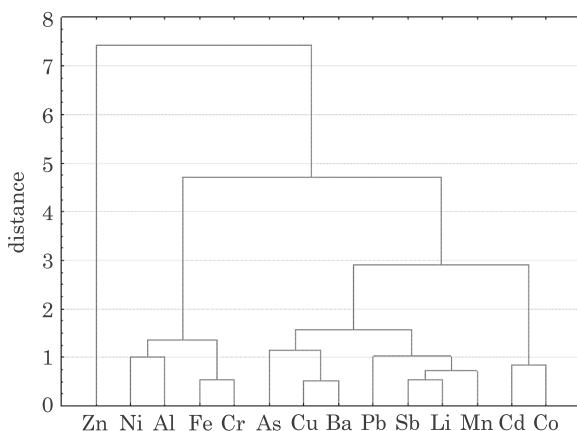


Fig. 4. Dendrogram of the RAF risk elements values determined in the M/L bag samples

Compared to the other risk elements, zinc reached the highest RAF values in moss and lichen insoles, what explain their separate position in cluster. Close relationship between Fe, Ni, Cr and Al was identically determined by Spearman's correlation. PCA distinguished evaluated taxa based on risk elements (Figure 5). The total variance explained by PCA was 74.51% (component 1: 55.79%; component 2: 18.72). The position and the direction of the risk elements towards the evaluated taxa, reflect the accumulation capacities of individual taxon values determined in the M/L bag samples.

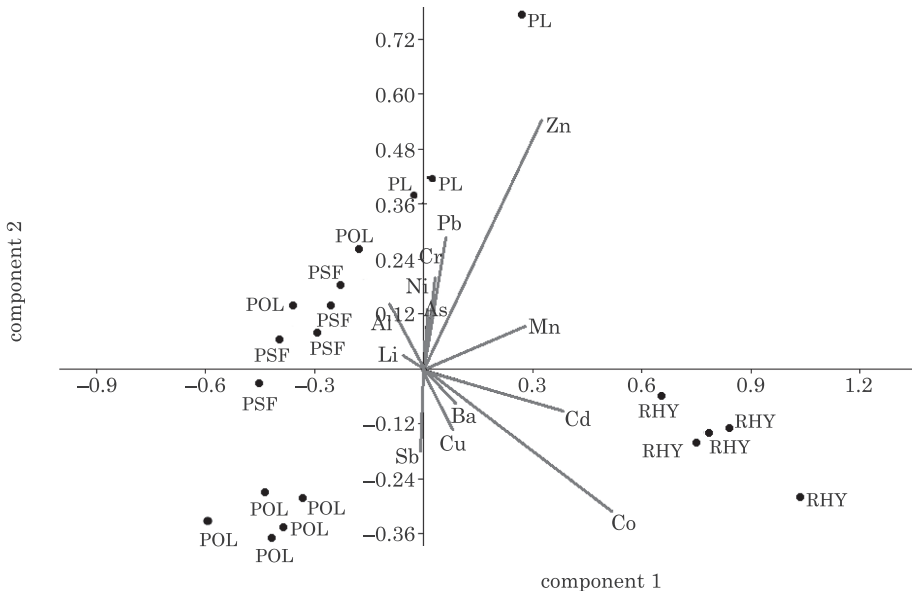


Fig. 5. The PCA biplot showing separation of different risk elements based on various taxa

As we hypothesised, serious pollution was determined in the underground parking lot, and more detailed statistical analysis pointed to the origin of the individual elements. The distance from the entrance/exit did not influence the concentrations of individual elements (with exception of arsenic), what is probably caused by a ventilation system that influences the airflow. As we supposed, mosses/lichens differ in their ability to accumulate different risk elements, what supports the theory of using them together to obtain complex results.

Conclusion

Zn, Ni, Mn and Fe were found as the most serious pollutants in underground parking lot. Accumulation abilities of individual taxa decreased in

order *Pleurosium* spp. > *Rhytidiadelphus* spp. > *Pseudevernia furfuracea* > *Polytrichum* spp. *Rhytidiadelphus* spp. was found to be the best accumulator of Co, Cd and Mn, *Polytrichum* spp. as the best accumulator of Sb. Close relationship between Al, Fe, Ni and Cr, which originate from car body corrosion, was confirmed by cluster analysis as well as Spearman's correlation relationship. Fuel combustion, releasing of the lubricate oils, or tyre abrasion are the main sources of risk elements such as Cd, Ba, Co, Cu, Mn and Zn. M/L bags were found as the suitable bioindicator of the air pollution conditions in underground parking lot. Because M/L taxa differ in accumulation ability of various risk elements, it is suitable to use them together to obtain more thorough results. No comprehensive work was until now focused on the risk elements evaluation in the underground parking lots in Slovakia. Based on the obtained results we can conclude, there is a serious accumulation of the toxic substances in underground parking lots, but regular maintenance could improve the air quality.

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**STIMULATION OF PATHOGENICITY
OF ENTOMOPATHOGENIC FUNGI WITH WATER
TREATED WITH LOW-TEMPERATURE,
LOW-PRESSURE GLOW PLASMA
OF LOW FREQUENCY**

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Key words: *Beauveria bassiana* (Bals.) Vuill, biopesticides, *Isaria fumosorosea* (Wize), plazmed water, *Tenebrio molitor* (Col., Tenebrionidae).

Abstract

Similarity of the structure and physicochemical properties of water treated with low-temperature, low-pressure, low-frequency glow plasma and water treated with static magnets of high induction suggests that like magnetically treated water, also plazmed water could stimulate the growth and pathogenicity of entomopathogenic fungi. It was found that distilled water and tap water treated with low-temperature, low-pressure, low-frequency glow plasma for 30 min stimulated pathogenicity of entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *Isaria fumosorosea* (Wize). The total number of dead *Tenebrio molitor* larvae contacted with those stimulated biopesticides and the rate of their mortality significantly increased. Both kinds of water did not influence the linear growth of the fungi. Thus, plazmed water is suitable for the stimulation of pathogenicity of tested biopesticides.

STYMULOWANIE PATOGENICZNOŚCI ENTOMOPATOGENNYCH GRZYBÓW WODĄ PODDANĄ DZIAŁANIU NISKOTEMPERATUROWEJ, NISKOCIŚNIENIOWEJ PLAZMY JARZENIOWEJ O NISKIEJ CZĘSTOŚCI

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Słowa kluczowe: *Beauveria bassiana* (Bals.) Vuill, biopestycydy, *Isaria fumosorosea* (Wize), plazmowana woda, *Tenebrio molitor* (Col., Tenebrionidae).

Abstrakt

Podobieństwo struktury i właściwości fizykochemicznych wody poddanej działaniu niskotemperaturowej, niskociśnieniowej plazmy jarzeniowej o niskiej częstotliwości i wody poddanej działaniu statycznego pola magnetycznego o wysokiej indukcji sugeruje, że podobnie jak woda poddana działaniu magnezu, woda plazmowana mogłaby stymulować wzrost i patogenność entomopatogennych grzybów. Woda destylowana i kranowa poddana działaniu niskotemperaturowej i niskociśnieniowej plazmy jarzeniowej przez 30 min stymulowała patogenność entomopatogennych grzybów *Beauveria bassiana* (Bals.) Vuill. i *Isaria fumosorosea* (Wize) wyrażoną całkowitą liczbą martwych larw *Tenebrio molitor*, które zetknęły się z tymi biopestycydami, oraz szybkością giniecia. Oba rodzaje wody nie wpływały na liniowy wzrost grzybów. Plazmowana woda nadaje się do stymulowania badanych biopestycydów.

Introduction

Biopesticides, for instance, fungi, attracted attention already in XX century (GUL et al. 2014, ROBERTS and HAJEK 1992, SHAH and PELL 2003). These preparations offer alternative solutions providing meeting restrictive regulations for sustainable maintaining environment and ecological standards. Currently, several preparations containing biopesticides are available on the market. They are used for protection of land and horticulture plantations, orchards, lawns, forests as well as plantations of champignons and urban trees. For their high virulence (epizootics) (AUGUSTYNIUK-KRAM and KRAM 2012), among over 1000 biopesticides entomopathogenic fungi pay considerable attention. For their breeding, storage and applications their growth and pathogenicity are key properties (STRASSER et al. 2010, FERNANDES et al. 2012, KHAN et al. 2012).

In our recent patent and paper (JAWORSKA et al. 2014, 2016) a novel approach to breeding of *Beauveria bassiana* and *Isaria (Paecilomyces) fumosorosea* fungi was demonstrated. It involved stimulation of pathogenicity and growth of those entomopathogenic fungi with a magnetically treated water. These organisms are among those the most commonly used in the plant protection. Thus, the fungi were either cultured on the medium composed of glucose and potato dissolved in water treated with permanent magnets (MW) of ~ 0.5 T induction or their cultures were exposed to the magnetic field of such induction. In both cases, the magnetic field provided an increase in both the linear growth of the fungal colonies and their pathogenicity against the *Tenebrio molitor* (Col., Tenebrionidae) larvae. The observed effects depended on the fungus species – *I. fumosorosea* fungus better responded to both ways of treatment than did the *B. bassiana*, induction of applied magnets and the exposure time of either water or culture to magnets. The extent of evoked by the magnetic field changes in MW could be controlled by the Raman spectroscopy.

Recently (BIALOPIOTROWICZ et al. 2017), preparation and properties of water treated with low-pressure, low-frequency, low-temperature glow plasma (PW) were presented.

Spectral Raman analysis of PW showed that it was identical with MW. It suggested that like MW also PW might be useful in promoting linear growth and pathogenicity of entomopathogenic fungi.

This paper presents a study on a suitability of PW as the stimulant of the growth, and pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Isaria fumosorosea* (Wize) entomopathogenic fungi.

Materials and Methods

Materials

Fungi

Beauveria bassiana and *Isaria fumosorosea* isolated from Polish soils were stored under no. 13 in the collection of Department of the Agricultural Environment Protection of the University of Agriculture in Cracow.

Water

Either distilled water or tap water from Bolesławiec of total hardness $129 \text{ mg l}^{-1} \text{ CaCO}_3$; pH 7.1; conductivity $334 \text{ }\mu\text{S cm}^{-1}$; Fe $< 50 \text{ }\mu\text{g l}^{-1}$; Mn $< 5 \text{ }\mu\text{g l}^{-1}$; dissolved oxygen 6.93 mg l^{-1} were used.

Methods

Plazming water

Either distilled or tap water (1000 ml) was placed in the chamber of the reactor (OSZCZĘDA et al. 2009) and exposed to plasma for 30 min. Plasma of 38°C was generated at $5 \cdot 10^{-3}$ mbar, 600 V, 50 mA and 280 GHz frequency. The produced water was stored at ambient temperature in 100 ml closed Teflon containers.

Culturing fungi

The procedure followed that described by Jaworska et al. (JAWORSKA et al. 2016). Thus, the solution of PDA (glucose and potato medium purchased from Biocorp. Warsaw, Poland) in 100 ml of either control distilled water (CDW), control tap water (CTW), plazmed distilled water (DPW) or plazmed tap water (TPW) was sterilized in an autoclave at 120°C for 30 min followed by its cooling to room temperature. That solution was poured into Petri dishes (10 dishes for each broth and 10 control dishes) and after 20 min since it solidified the fungi were inoculated on the medium. Dishes inoculated with fungi were incubated in 25°C. Every experiment was run in 10 replicates.

Test for linear growth and test for pathogenicity

The linear growth was measured with a caliper from the reversed side of the dish.

The test was performed against last development stage larvae of *Tenebrio molitor* from their own cultures. The tests were run in 10 replications involving always 10 larvae. The procedure followed the method published by JAWORSKA et al. (2016). Thus, larvae were completely sunk for 5 s in the fungus colony and transferred into Petri dishes lined with filter paper. Once a day up to the completion of the tests, the filter paper was moisturized with 1 mL of distilled water. Tests were performed after 2nd, 4th, 6th and 8th day of the experiment.

Statistics

The results of the linear growth were statistically elaborated with the single-factor ANOVA method. The means followed by the same letters are not significantly different at $p = 0.05$ according to the Duncan test. Statistical treatment of the results for pathogenicity employed analysis of variance preceded with the Freeman–Tukey transformation.

Results and Discussion

Comparison of the time dependent changes in the Raman spectra of MW and PW showed that production of PW proceeded much faster than production of MW. Moreover, construction of the generating plasma device (OSZCZĘDA et al. 2009) provided a massive production of PW. Therefore, the use of PW for the stimulation of entomopathogenic fungi seemed advantageous over the use of MW. However, identical Raman spectra of MW and PW (BIAŁOPIOTROWICZ et al. 2017) did not rationalize assumption that both waters would have the same macrostructure and, hence, the same functional properties, particularly these suitable for stimulation the fungi growth and entomopathogenicity of the fungi. Indeed, it has appeared that the functional properties of MW and PW were not identical. Figure 1 demonstrates that both fungi grew practically identically on the media prepared with either CDW, CTW or PDW and on the medium prepared with PTW a slight inhibition of the growth could be observed. This behavior contrasted with the formerly reported stimulating effect of MW upon the growth of those fungi (JAWORSKA et al. 2014, 2016).

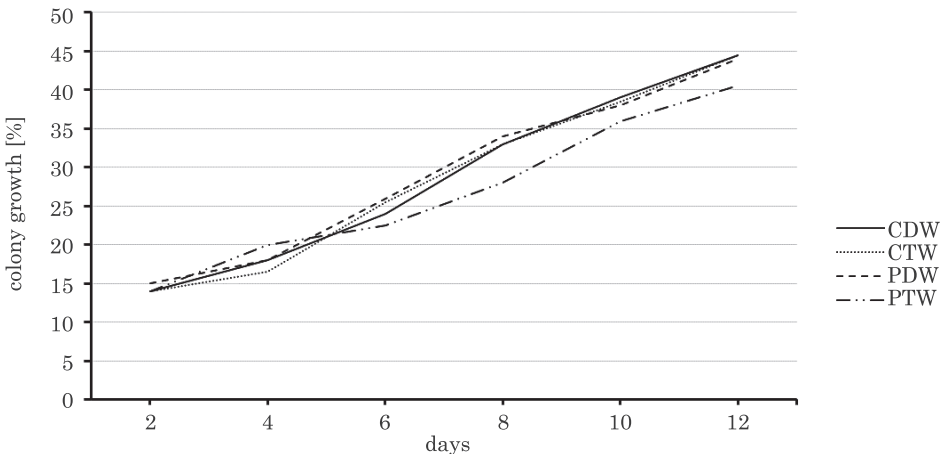


Fig. 1. The lack of the stimulation of the linear growth of *B. bassiana* and *I. fumosorosea* with CDW, CTW, PDW and PTW within 8 day experiments. The course of the curves are identical for both fungi

However, the pathogenicity of *I. fumosorosea* and *B. bassiana* was nicely stimulated by PW and, as shown in Table 1, *I. fumosorosea* was more sensitive to stimulation than *B. bassiana*.

The same difference was noted in the sensitivity of those fungi to stimulation when MW was applied (JAWORSKA et al. 2014, 2016).

Table 1

Observed within 8 day experiments mortality [%] of *Tenebrio molitor* larvae caused by entomopathogenic fungi stimulated in various aqueous media^{a,b}

Water ^c	Fungus											
	<i>B. bassiana</i>						<i>I. fumosorosea</i>					
	days						days					
	3	4	5	6	7	8	3	4	5	6	7	8
CDW	0 ^a	0 ^a	0 ^a	20 ^a	52 ^a	76 ^a	0 ^a	20 ^a	64 ^a	92 ^a	96 ^a	100 ^a
CTW	0 ^a	0 ^a	8 ^{ab}	35 ^a	56 ^a	72 ^a	0 ^a	16 ^a	48 ^a	96 ^a	100 ^a	100 ^a
DPW	0 ^a	4 ^a	20 ^b	48 ^a	75 ^a	81 ^{ab}	4 ^a	40 ^{ab}	64 ^a	96 ^a	100 ^a	100 ^a
TPW	0 ^a	4 ^a	4 ^{ab}	51 ^a	84 ^a	100 ^b	8 ^a	64 ^b	96 ^b	100 ^a	100 ^a	100 ^a

^a means of five repetitions with five larvae in each experiment. The presented data are related to the mortality of 100 larvae of *Tenebrio molitor*

^b the means followed by the same letters in particular columns are not significantly different at $p = 0.05$.

^c CDW – control distilled water; CTW – control tap water; DPW – plazmed distilled water; TPW – plazmed tap water

Regardless PW stimulated or non-stimulated, *B. bassiana* did not kill any larva within 3 first days of experiment. First larvae were killed by stimulated fungus just in the fourth day. PW stimulated *I. fumosorosea* appeared to be more toxic for the larvae. First larvae died in contact with PW stimulated fungus already in the third day. An insight in the time dependent mortality of larvae (Table 1) both fungi performed better when stimulated with TPW.

Although non-stimulated, *I. fumosorosea* also killed all *T. molitor* larvae. The kinetics of the pathogenic effect (Figure 2a and Figure 2b) shows benefits of the stimulation of the fungi with plazmed water. *I. fumosorosea* stimulated with TPW provided a significant pathogenic effect already in the fifth day of experiment that is by one day earlier that did non-stimulated fungus. The total annihilation of the test larvae by *B. bassiana* stimulated with TPW was possible in the 8th day of experiment whereas non-stimulated fungus in that day left around 25% of the alive larvae population.

Above findings indicated an advantage in the stimulation of the fungi with PW over their stimulation with MW. PW prepared by 30 min plazming performed more efficiently than MW treated for 2 h with static neodyme magnets of 0.1 and 0.2 T induction (JAWORSKA et al. 2016).

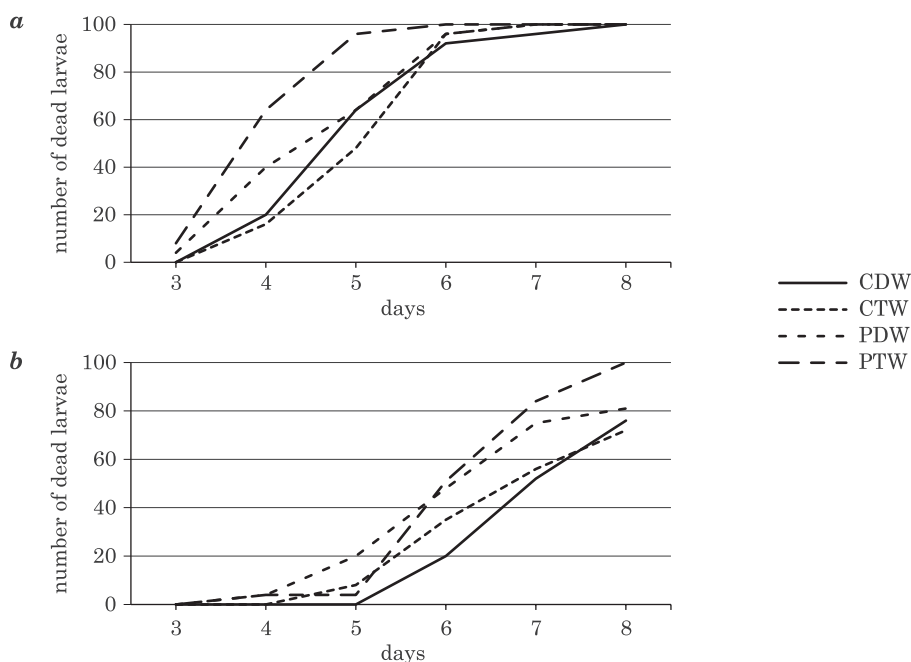


Fig. 2. Kinetics of the development of pathogenicity: *I. fumosorosea* (a) and *B. bassiana* (b) and against *T. molitor* larvae (mortality in %)

Conclusions

1. Low-temperature, low-pressure, low-frequency glow plasma treated water stimulates pathogenicity of entomopathogenic *Beauveria bassiana* and *Isaria fumosorosea*.

2. Low-temperature, low-pressure, low-frequency glow plasma treated water does not stimulate the linear growth of entomopathogenic *Beauveria bassiana* and *Isaria fumosorosea*.

3. *Isaria fumosorosea* is more sensitive to the stimulation of their pathogenicity than *Beauveria bassiana*.

4. Low-temperature, low-pressure, low-frequency glow plasma treated water provides more efficient stimulation of the fungi pathogenicity than formerly presented water treated with static neodyme magnets.

5. Plazmed tap water provides higher efficiency of the stimulation than plazmed distilled water.

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THE INFLUENCE ON NONTHERMAL, LOW-PRESSURE PLASMA ON MICROBIOLOGICAL CONTAMINATION OF FOOD

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Key words: nonthermal plasma, food, *Salmonella* sp., *Enterococcus* sp., *Escherichia coli*.

Abstract

Among many methods for food decontamination, high hopes are associated with nonthermal, low-pressure plasma technology. This study aimed to estimate the survival rate of *Escherichia coli*, *Salmonella* Senftenberg W775 and *Enterococcus faecalis* exposed to the action of plasma for the time from 0 to 800 s. As carriers there were used the laurel leaves. An analogous experiment was performed with the use of UV-C radiation. The results show that the plasma technology is more effective in bacteria reduction than ultraviolet radiation. The cells of *E. coli*, were the most susceptible to the action of nonthermal plasma and their numbers after 800s decreased by 7 log, after the same time the amount of enterococci decreased by about 6 log. *Salmonella* cells were characterized by a very high resistance to low-pressure plasma. Results of this study show that the plasma technology should be used for a longer time and in a higher dose to guarantee the full decontamination of food.

WPŁYW NISKOTEMPERATUROWEJ, NISKOCIŚNIENIOWEJ PLAZMY NA MIKROBIOLOGICZNE ZANIECZYSZCZENIE ŻYWNOSCI

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Słowa kluczowe: niskotemperaturowa plazma, żywność, *Salmonella* sp., *Enterococcus* sp., *Escherichia coli*.

Abstrakt

Spośród wielu metod dekontaminacji żywności duże nadzieje wiąże się z technologią zimnej, niskociśnieniowej plazmy. Celem pracy było zbadanie przeżywalności bakterii *Escherichia coli*, *Salmonella* Senftenberg W775 oraz *Enterococcus faecalis* poddanych działaniu plazmy w czasie od 0 do 800 s. Jako nośniki bakterii wykorzystano liście laurowe. Wykonano także analogiczne doświadczenie z wykorzystaniem promieniowania UV-C, aby ocenić skuteczność metody.

Wyniki wskazują, że technologia plazmowa jest efektywniejsza w redukcji liczby bakterii niż promieniowanie ultrafioletowe. Na działanie zimnej plazmy najwrażliwsze były bakterie *Escherichia coli*, których liczebność po 800 s zmalała o 7 log, po tym samym czasie liczba paciorkowców kałowych obniżyła się o ok. 6 log. Pałeczki z rodzaju *Salmonella* charakteryzowały się bardzo dużą opornością na niskociśnieniową plazmę. Wyniki badań wskazują, że produkty spożywcze powinny być poddawane działaniu niskotemperaturowej plazmy przez dłuższy czas lub generatorem o większej mocy, by uzyskać ich pełną dekontaminację.

Introduction

Consumers more often look for fresh or low-processed products, therefore it is essential to ensure their high quality and microbiological purity. Unfortunately, the growing number of food borne diseases and poisonings indicates that traditional decontamination technologies are insufficient (BIBEK 2004, *Microbiological hazards...* 2008). Pasteurization and sterilization considerably reduce the number of undesirable microorganisms, but they change the nutritive and sensory properties of food products. Competition on the market and the growing level of consumer knowledge and legal regulations force the producers to develop new technological solutions which could be used in food processing. One of more modern methods is nonthermal plasma (AFSHARI and HOSSEINI 2014). It is effec-

tive at the ambient temperature and does not increase the product temperature, which is of utmost importance, especially in the case of fresh food products. Apart from the high effectiveness of inactivation of many pathogens and spores, plasma is also environment friendly and it does not change the organoleptic and sensory values of products (ZHANG et al. 2014). Nonthermal plasma may be used to purify fresh and processed fruit and vegetables, meat, eggs or dried food products. It also gains recognition in the package industry, due to providing the appropriate storage conditions (KNOERZER et al. 2012, SCHNABEL et al. 2015). After treatment with nonthermal plasma, the nutritive and sensory values of food remain unchanged, also no presence of toxic structures was observed, which affects the maintenance of consumer safety (FERNÁNDEZ and THOMPSON 2012, KNOERZER et al. 2012). High reactivity of chemical particles being plasma components have destructive effect of biological structures of microorganisms. The highest lethality is provided by the hydroxyl radical and atomic oxygen, which affect the oxidation of protein and lipids of the cell membrane (WIKTOR et al. 2013). Pores are generated through which charged particles penetrate into the microbial cell and induce disturbances of enzyme and protein activity. Cytoplasm leaking from the generated pores may be the cause of cell death. The study conducted over nonthermal plasma has confirmed the ability to eliminate microorganisms, even those resistant, such as: bacterial spores, fungi, *Vibrio parahaemolyticus*, *Salmonella* spp., EHEC strains of *Escherichia coli*, *Staphylococcus aureus* or *Listeria monocytogenes* (PIGNATA et al. 2014, GUO et al. 2015).

The aim of this study was to evaluate the effect of nonthermal low-pressure plasma on the survival rates of the bacteria: *Escherichia coli*, *Enterococcus faecalis*, and the strain *Salmonella* Senftenberg W775, known for its resistance to various physicochemical factors. Carriers imitating dry food products were inoculated with microorganisms, they were subjected to the action of plasma and the number of bacteria after different times of exposure was determined.

Materials and Methods

Microorganisms used in this experiment, *Escherichia coli*, *Enterococcus faecalis*, and *Salmonella* Senftenberg W775, are derived from pure culture collections of the Institut für Umwelt und Tierhygiene sowie Tiermedizin, Universität Hohenheim in Stuttgart. The first two mentioned are the faecal, commensal bacteria, while *Salmonella enterica* ssp. *enterica* serovar Senftenberg strain W775 (*Salmonella* Senftenberg W775) is very

common in animals and food. Additionally, it is known for its resistance to many physicochemical factors, such as high temperature, gamma radiation, dessication or disinfectants. Therefore, this bacterium is often used for the estimation of the effectiveness of different antimicrobial methods. All bacteria represent the wide range of microorganisms that may contaminate food. The bacteria were inoculated on liquid media (enriched broth, Merck 7882) and incubated for 24 hours at 37°C. Laurel leaves were used as bacteria carriers imitating food products. They were rinsed twice in distilled water, dried, cut into squares of 1 cm, and then attached to the microscopic slides with double-sided adhesive tape. Fragments of leaves were contaminated with the bacterial suspension (100 µl each), dried in the laminar chamber and exposed to the action of nonthermal plasma. The plasma was generated in a cylindrical chamber of the diameter 100 mm and the length of 270 mm between two parallel electrodes using plasma activator FEMTO (Diener Electronic, Germany). Discharges frequency and nominal power of this activator were 40 kHz and 100 W, respectively. The chamber was evacuated to a pressure of about 0.2 mbar over approximately 5 min, before admitting the gas (air) with the rate of 5 sccm for 5 min until the desired pressured was reached and stabilized. The plasma was then ignited, at the required power, and exposure was controlled with a build-in electronic timer. To recover the bacteria, the carriers were transferred to 9 mL 0.85% NaCl and shaken for 5 min (Vortex Shaker QL-866, 2250 r/min). Suspensions were diluted (10^{-2} - 10^{-8}), inoculated on suitable selective media and incubated at 37°C for 24 hours. The experiment was conducted in five replications. To perform microbiological analyses, we used the following culture media: ENDO (Merck 104044) for *Escherichia coli*, BPLA acc. to Kaufmann (Merck 7236) for bacteria from the genus *Salmonella* and kanamycin, esculine and azide agar for *E. faecalis* (Merck 5222).

To compare the efficacy of the nonthermal plasma technology with the effect of more commonly used in food industry ultraviolet radiation, analogous carriers were irradiated with UV-C (in a device equipped in the Philips TUV 36W/G36 T8 lamps, 253.7 nm) every 100 s (100–800 s) in doses from 2,32 to 18,56 kJ m⁻². The results of experiments were subjected to the statistical analysis. The theoretical time needed for microbial inactivation was calculated based on regression lines.

Results

The results of the study comparing the effect of nonthermal plasma and UV-C radiation on selected bacteria were presented both in form of

graphs illustrating the number of microorganisms at different times of exposure to the factor, and as regression line equations in tables. The study indicated a varied sensitivity of bacteria to the action of both physical factors. Figure 1 shows that the number of *E. coli* was gradually decreased along with the time of action of nonthermal plasma. After 800 s the number of these bacteria was reduced by 6 log. UV-C radiation was characterized by a much lower effectiveness, as after the maximal time of exposure it reduced the number of the rods only by 1 log. From regression line equations (Table 1) it follows that the total survival time of *E. coli* in the environment of UV-C was almost five times longer than in plasma.

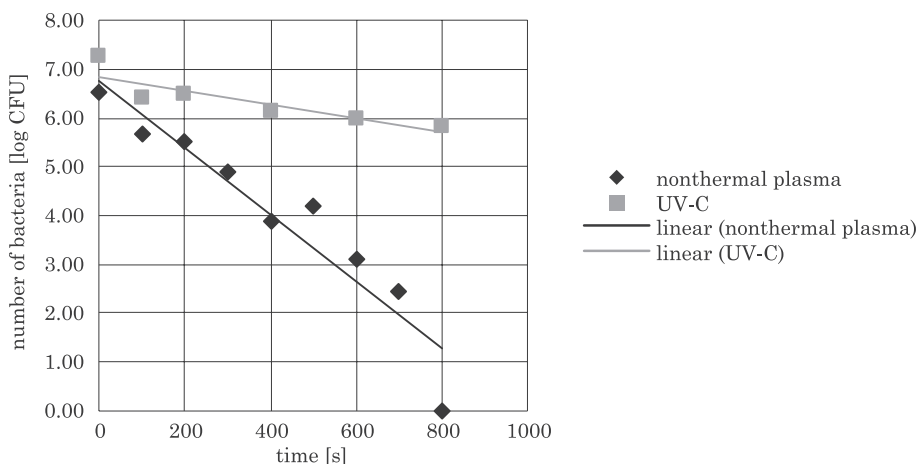


Fig. 1. Changes in the number of *Escherichia coli* as a result of action of nonthermal plasma and UV-C radiation

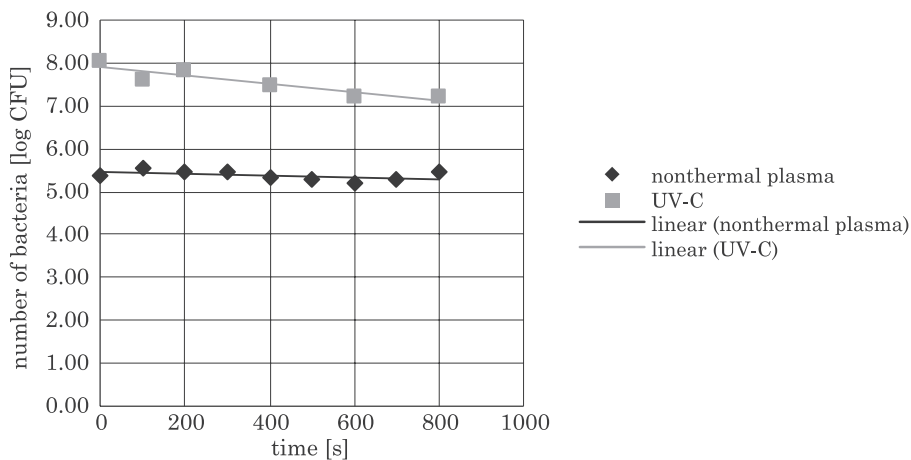


Fig. 2. Changes in the number of *Salmonella Senftenberg W775* as a result of action of nonthermal plasma and UV-C radiation

The number of rods of *Salmonella* Senftenberg W775 did not change substantially either under the influence of plasma or UV-C and after 800 s it was comparable with the control sample (Figure 2). Regression equations indicate that UV-C radiation was more effective in their elimination. A decrease in the number of bacteria was more than two times faster than in the environment of plasma. This affected the total survival time of the cells, which was longer also after the use of UV-C (Table 1).

Table 1

Regression line equations describing the inactivation rate of bacteria under the influence of nonthermal plasma and UV-C radiation

Factor	Regression equations	Total survival time [min]	Fall of bacteria number [log/min]
<i>Escherichia coli</i>			
Plasma	$y = -0.0069x + 6.7704$	16.4	0.4
UV-C	$y = -0.0014x + 6.8589$	81.7	0.08
<i>Salmonella</i> Senftenberg W775			
Plasma	$y = -0.0002x + 5.4598$	455.0	0.01
UV-C	$y = -0.001x + 7.913$	132.0	0.06
<i>Enterococcus faecalis</i>			
Plasma	$y = -0.0047x + 6.3881$ $y = -0.0002x + 6,1504$	22.7	0.3
UV-C	$y = -0.0002x + 6.1504$	512.5	0.01

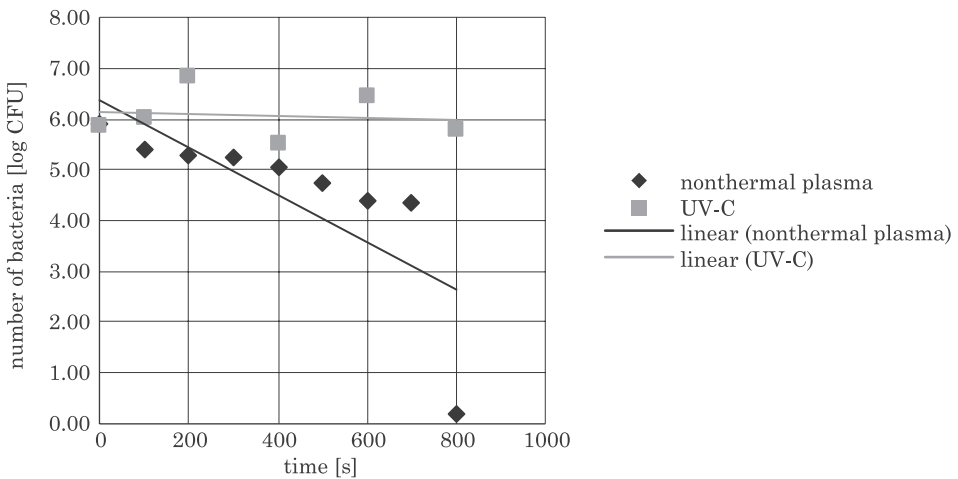


Fig. 3. Changes in the number of *Enterococcus faecalis* as a result of action of nonthermal plasma and UV-C radiation

Like *E. coli*, streptococci were effectively eliminated under the influence of nonthermal plasma. However, as long as until the 700th s their number fall much slower. Only during the last 100 s there was a reduction in the number of *E. faecalis* by 4 log as compared with the control sample. No bacteria inactivation was observed under the influence of UV-C radiation. As it follows from regression lines, streptococci were the most resistant to the action of UV-C radiation of all the bacteria. The total time of their survival was as long as 512 min, whereas in the environment of nonthermal plasma it was only slightly more than 22 min (Figure 3, Table 1).

Discussion

The possibility of using nonthermal plasma in the food industry is currently at the stage of experiments. Their results indicate wide possibilities for the future application of this technique for decontamination of surfaces of grains, seeds, meat or packages. This study tested the effect of low-pressure nonthermal plasma on the survival of faecal indicator bacteria *Escherichia coli* and *Enterococcus faecalis* as well as *Salmonella* Senftenberg W775.

DENG et al. (2007) tested the possibility of using nonthermal plasma (2 kHz) for inactivation of *Escherichia coli* on the surface of almonds. Already after 30 s they noted a decrease in the number of microorganisms by 5 logs. In the present study a similar decrease in the number of *E. coli* was obtained only after 700 s, which probably resulted from the use of a device with a much less power. A type of gas seems to have a large effect on the elimination rate of microorganisms in the plasma generator. PIGNATA et al. (2014) subjected *E. coli* adsorbed on membrane filter to the action of plasma in the environment of pure oxygen and argon for 15, 30, 60 and 180 s. Oxygen destroyed all bacteria after 60 s, and argon already after 30 s. In the present study, after 60 s of treating with plasma, the number of *E. coli* decreased only by more than 1 log, which indicates the fact that plasma generated in the atmospheric air is less effective in eliminating microorganisms than argon and pure oxygen.

ZHANG et al. (2013) studied the survival rate of *Salmonella* Typhimurium LT2, using spinach, lettuce, tomato and potato as carriers. Plasma was generated in conditions of lowered pressure with participation of oxygen. The device generated nonthermal plasma with the use of 13.56 MHz of radio frequency, pressure 34 Pa and power 150 W. After 600 s of the process, bacteria on spinach leaves were eliminated most efficiently, since

a decrease in the number of cells by 3 logs was recorded. On the surface of lettuce, the number of microorganisms decreased by 2.75 log, whereas in tomato and potato, by 2.12 log. After 800 s, a very good effect of disinfection was obtained for spinach. In the present study, *Salmonella* Senftenberg W775 even after 800 s did not undergo too much reduction, as compared with the control sample. The used strain showed a high resistance to the action of nonthermal plasma. The result of the experiment could also be dependent both on a lower power and intensity of gas flow, different type of gas, and different types of bacterial carriers.

To compare the effectiveness of nonthermal plasma on microorganisms contaminating food, an experiment was made using the same carriers, but they were subjected to the action of more commonly used in food industry UV-C radiation. Relationship between the effectiveness of pathogenic microorganisms' inactivation and the UV wavelength was studied by YIN et al. (2015). They conducted experiments on cow milk, irradiating samples contaminated with *E. coli* O157:H7 with waves of different length and energy. Apart from the wavelength, also delivered energy had an impact on a reduction in the number of microorganisms. Both for 5 and 20 mJ cm⁻² the highest reduction in the number of bacteria was observed at the wavelength 254 nm. BIRMPA et al. (2013) analysed the effect of ultraviolet radiation with the wavelength 254 nm on decontamination of the surface of fresh food products – Roman lettuce and strawberries contaminated with *Escherichia coli* and *Salmonella* Enteritidis. Samples were irradiated for 10, 20, 30, 45 and 60 min and a gradual reduction in the number of both bacteria species were recorded. After 13 min it was smaller by 0.4 log. In the case of strawberries, *Salmonella* sp. turned out to be more susceptible to the action of UV-C, after 13 min the number of microorganisms decreased by 0.8 log. In the present study after the same time of the action of UV-C the number of *Salmonella* and streptococci remained at the same level as the control sample, and the number of *E. coli* decreased by 1 log, that is over two times more than in the study by BIRMPA et al. (2013). Divergence in the study results could result from the use of different bacterial strains and carriers. The method of microorganisms' inactivation with UV-C radiation was also used by MUKHOPADHYAY et al. (2014). Their experiment aimed to assess the effectiveness of elimination of *Salmonella enterica* and *Escherichia coli* O157:H7 on the surface of fresh tomatoes. The device emitting radiation was equipped with lamps of a power 15 W, generating waves with a length of 254 nm. Tomatoes were irradiated by 100 s, in doses from 0.6 to 6 kJ m⁻². The number of both bacteria decreased along with an increase in dose of ultraviolet radiation. The rods of *Salmonella* sp. were characterized by a higher resistance.

The results of the present study also show a lower susceptibility of *Salmonella* to the action of ultraviolet radiation. The time of 800 s is insufficient to eliminate all microorganisms, including also the pathogens that occur in food products. To increase the effectiveness of decontamination, it would be necessary to increase the time and dose of irradiation.

It has been reported that many stressful conditions, including plasma treatment, or irradiation can lead bacteria to change into the VBNC state. The cells lose their ability to be cultivated, but still are alive and therefore may display pathogenicity. For this reason, the evaluation of decontamination processes of food might not be credible when only the classical cultivation methods are used (DOLEZALOWA, LUKES 2015, DING et al 2017, SAID et al 2010, ZHANG et al 2015). Further research should include some fluorescent techniques.

Conclusions

The plasma technology is more effective in bacteria reduction than ultraviolet radiation but nevertheless does not guarantee the full effectiveness of food decontamination. The treatment time and power of non-thermal plasma should be chosen so that the number of every indicator bacteria will be reduced. The real decontamination effectiveness of non-thermal plasma remains to be demonstrated by further research supplemented by VNBC analysis.

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**CHANGES IN THE CONTENT OF CLA AND OTHER
TRANS ISOMERS IN THE KORMORAN CHEESE
DURING SIX WEEKS OF RIPENING**

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Key words: conjugated linoleic acid (CLA), *trans* fatty acids, cheese, ripening time.

Abstract

The aim of this study was to evaluate the effect of first six weeks of ripening on the content of *cis9,trans11* CLA and *trans* isomers of C18:1 and C18:2 acids in Kormoran cheese. The experimental material included rennet, ripening Swiss – type Kormoran cheeses made of cow's milk. The cheeses were obtained from a dairy plant located in the Warmia and Mazury voivodeship. Analyses were conducted for normalized milk prepared for cheese production, freshly made cheeses and cheeses after 1, 2, 4 and 6 weeks of ripening. Cheeses were ripening at the manufacturing plant.

The study showed that the time of ripening had a significant impact on the content of conjugated linoleic acid *cis9,trans11* CLA as well as on the content of *trans* C18:2 isomers in cheeses. In the analyzed cheeses, the content of CLA was significantly higher ($P < 0.05$) after 6 weeks of ripening than the content of those isomers in fresh cheeses and those analyzed after 1, 2 and 4 weeks of ripening.

ZMIANY W ZAWARTOŚCI CLA I INNYCH IZOMERÓW *TRANS* W SERZE KORMORAN PODCZAS SZEŚCIU TYGODNI DOJRZEWANIA

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Słowa kluczowe: sprzężony kwas linolowy (CLA), izomery *trans*, ser, czas dojrzewania.

Abstrakt

Celem pracy była ocena wpływu czasu dojrzewania serów na zawartość kwasu *cis9,trans11* CLA oraz izomerów *trans* kwasu C18:1 i C18:2 w serach Kormoran. Materiałem badanym były sery kwasowo-podpuszczkowe typu szwajcarskiego wyprodukowane z mleka krowiego. Sery pozyskano z zakładu mleczarskiego zlokalizowanego w województwie warmińsko-mazurskim. Analizie poddano mleko znormalizowane przygotowane do produkcji serów, świeże sery oraz sery po 1, 2, 4 i 6 tygodniach dojrzewania. Sery dojrzewały w zakładzie produkcyjnym.

W badaniach wykazano, że czas dojrzewania miał istotny wpływ na zawartość sprzężonego kwasu linolowego *cis9,trans11* CLA, jak również na zawartości izomerów *trans* C18:2 w serach. W objętych badaniem serach Kormoran zawartość CLA była istotnie wyższa ($P < 0,05$) po 6 tygodniach dojrzewania w porównaniu z zawartością tego izomeru w świeżych serach oraz badanych po 1, 2 i 4 tygodniach dojrzewania.

Introduction

The term conjugated linoleic acid (CLA) describes a group of polyunsaturated fatty acids that are isomers of linoleic acid with double conjugated bonds, mainly at carbons 9 and 11 or 10 and 12. For each positional isomer, four pairs of geometric isomers are possible. In fat of the ruminants, the main representative of this group of isomers is *cis9,trans11* CLA which in milk fat constitutes from 76 to over 80% of total isomers of C18:2 acids with conjugated bonds (PRECHT and MOLKENTIN 2000, PARK 2009, KEE et al. 2010). The *cis9,trans11* CLA displays a variety of health-promoting properties, including: antioxidative, anticarcinogenic and antimutagenic ones (KEE et al. 2010, MOLKENTIN 1999, PARODI 2003, PARK 2009, AYDIN 2005).

One of the main sources of CLA for humans is milk and dairy products (butter, fermented milk drinks, soft and hard cheeses). The content of CLA in milk fat fits within a very wide range and depends on the feeding period, lactation period, breed and individual determinants of cows. Out of these

factors, the greatest significance is attributed to the period of feeding. Higher contents of this acid occur in milk fat originating from pasture feeding, whereas lower ones in milk fat from the period of stall feeding (PRECHT and MOKKENTIN 1997, KELSEY et al. 2003, ŹEGARSKA et al. 2006, ZUNONG et al. 2008, FRELICH et al. 2012). In the case of dairy products (cheeses or fermented drinks), apart from the animal diet, the level of CLA was shown to be significantly affected by conditions occurring during technological processing and by the activity of the starter cultures added (SIEBER et al. 2004, SHANTHA et al. 1992, JIANG et al. 1998, LIN et al. 1998, LIN 2000, KIM and LIU 2002, BZDUCHA and OBIEDZIŃSKI 2007, DOMAGAŁA et al. 2009, MURTAZA et al. 2014). KIM et al. (2009) demonstrated that after aging for 4 months, the cheese made of milk obtained from pasture-raised cows contained relatively higher levels of CLA compared to the cheese made of milk obtained from indoor-raised cows (8.12 mg g⁻¹ fat vs 6.76 mg g⁻¹ fat, respectively), but the difference was not observed in 7-month-aged cheeses. However, both from pasture and indoor feeding, the 7-month-aged cheeses had a higher CLA content than the 4-month-aged ones, which indicates that the time of ripening is another important factor affecting CLA content in cheeses.

The aim of this study was to evaluate the effect of first six weeks of ripening on the content of *cis*9, *trans*11 CLA and *trans* isomers of C18:1 and C18:2 acids in rennet, ripening Swiss – type Kormoran cheese.

Materials and Methods

Experimental material

The experimental material was rennet, ripening Swiss-type Kormoran cheese, made of cow's milk. The cheese was obtained from a dairy plant located in the Warmia and Mazury voivodeship. The production process was as follows: pasteurization (72°C, 15s), normalization of milk, addition of sourdough of pure cultures (mesophilic, thermophilic and propionibacteria) and calcium chloride, mixing and heated to 32–34°C, addition of rennet, coagulation and cutting a clot, reheating (53–54°C), forming and pressing cheese, salting in brine (18–22% NaCl, 12–14 °C, 8–10 days), dripping, ripening: first two weeks (13–14°C), next two weeks (19–21°C), further ripening period (8–10°C). The content of *cis*9, *trans*11 CLA and *trans* isomers of C18:1 and C18:2 acids was determined in normalized milk used for

the cheese production, freshly made cheese and the cheese after 1, 2, 4 and 6 weeks of ripening. The cheeses were ripening at the manufacturing plant. Three samples of the cheese were taken for analyses each week. All determinations were conducted in two parallel replications.

Analytical Methods

Fat content

Fat content in milk was determined with the Roesse-Gottlieb's method according to the Polish norm *Milk-Determination...* PN-EN ISO 1211:2011. Fat content in cheese was determined with the Schmidt-Bondzyński-Ratzlaff's method according to the Polish norm *Cheese and processed...* PN-EN ISO 1735:2006.

Lipid extraction

To determine CLA and *trans* C18:1 and C18:2 isomers, fat was isolated from normalized milk and chesses with the Folch method (CHRISTIE 1973).

Preparation of fatty acid methyl esters

Methyl esters of fatty acids were prepared according to the International Dairy Federation (IDF) method, using a methanol solution of KOH (according to *Milkfat. Preparation...* ISO 15884:2002).

Gas chromatography (GC) analysis

Determinations of CLA and *trans* isomers of unsaturated fatty acids were carried out with a gas chromatography with flame ionization detector (GC-FID) method using the HP 6890 chromatograph (Palo Alto, CA, USA). Chromatographic separation of fatty acid methyl esters was carried out on a capillary column (100 m x 0.25 mm i.d., film thickness 0.20 μm) with CP Sil 88 phase (Varian, USA). Separation conditions were as follows: column temperature: 60°C (1 min) – 180°C, $\Delta t = 5^\circ\text{C min}^{-1}$; detector temperature: 250°C; injector temperature: 225°C; carrier gas: helium, flow rate: 1.5 ml/min, and injector: split 50:1.

The *cis-9,trans-11* CLA isomer was identified using a mixture of CLA methyl esters (Sigma-Aldrich). The positional *trans* isomers of C18:1 were identified using the following methyl ester standards of these isomers:

*trans*₆ (Supelco, Bellefonte, USA; *trans*₉ and *trans*₁₁(Sigma-Aldrich, St. Louis, USA) and literature data (CONTARINI et al., 2013). The *trans* isomers of C18:2 acid (*cis,trans* and *trans,cis*) were identified with the use of a standard mixture of these isomers (Supelco, Bellefonte, USA).

Quantitative computations of *cis*₉,*trans*₁₁ C18:2 acid and *trans* isomers of C18:1 and C18:2 acids were made against the introduced standard (methyl ester of C21:0 acid).

Statistical analysis

The statistical analysis was carried out using STATISTICA software ver.10. One-way analysis of variance (ANOVA) was used to determine differences among time periods of ripening at a significance level of $\alpha = 0.05$. Differences between each mean values were evaluated with the Duncan's *post hoc* test. The Pearson's correlation coefficients between the content of *cis*₉,*trans*₁₁ CLA, *trans* C18:1 and *trans* C18:2 isomers were evaluated using a STATISTICA software ver.10.

Results and discussion

The content of fat in milk intended for cheese production was 3.10%. Freshly produced cheese contained 26.4% of fat.

The content of *cis*₉,*trans*₁₁ CLA and C18:1 and C18:2 *trans* isomers in milk intended for cheese production, as well as in fresh cheese and in cheeses after 1, 2, 4 and 6 weeks of ripening, is presented in Table 1. The average content of *cis*₉,*trans*₁₁ CLA was 3.50 mg g⁻¹ fat. The content of this acid in fresh cheese was similar (3.65 mg g⁻¹ fat, $P > 0.05$). The study showed that CLA content in the cheese analyzed after 1, 2 and 4 weeks of ripening did not differ significantly from that found in normalized milk and fresh cheeses ($P > 0.05$). A significantly higher content of *cis*₉*trans*₁₁ CLA was found in the cheese analyzed after 6 weeks of ripening (4.17 mg g⁻¹ fat, $P < 0.05$). BIAŁEK and TOKARZ (2009) analysed 44 different kinds of cheeses available on polish market, which included: soft-ripened cheeses (10) – camembert (7) and brie (3), blue cheeses (10), yellow cheese (17), goats cheeses (5), oscypek and home-made cottage cheese. The study has shown that blue cheeses were characterized by the lowest content of CLA (1.66 to 2.39 mg g⁻¹ of fat), oscypek cheese contained CLA on the level goats cheeses ranged from 1.96 to 2.5 mg g⁻¹ of fat. The most amounts as well as the biggest variety of the content of *cis*₉*trans*₁₁ C18:2 acid was of 1.7 mg g⁻¹ of fat and cottage cheese 3.0 mg g⁻¹ of fat. In analysed soft-ripened cheeses the content of CLA ranged from 1.74 to 3.07 mg g⁻¹ of fat and

Table 1
Changes in the content of CLA and C18:1 and C18:2 *trans* isomers in the Kormoran cheese during ripening [mg g⁻¹ fat]

Isomers	Normalized milk $\bar{x} \pm s$ /SD	Ripening time [weeks]				
		fresh $\bar{x} \pm s$ /SD	1 week $\bar{x} \pm s$ /SD	2 weeks $\bar{x} \pm s$ /SD	4 weeks $\bar{x} \pm s$ /SD	6 weeks $\bar{x} \pm s$ /SD
<i>cis9,trans11</i> CLA	3.50^b ± 0.06	3.65^b ± 0.05	3.79^b ± 0.22	3.45^b ± 0.21	3.60^b ± 0.18	4.17^a ± 0.03
Σ <i>trans</i> C18:1	17.13^b ± 0.62	19.10^a ± 0.07	19.63^a ± 0.62	18.19^{a,b} ± 0.93	18.43^{a,b} ± 1.05	19.54^a ± 0.46
<i>t6 - t9</i>	3.26 ^b ± 0.15	3.74 ^a ± 0.05	3.86 ^a ± 0.14	3.60 ^a ± 0.19	3.60 ^a ± 0.23	3.82 ^a ± 0.08
<i>t10 + t11</i>	9.55 ^c ± 0.33	10.27 ^{a,b,c} ± 0.05	10.67 ^{a,b} ± 0.30	9.82 ^{b,c} ± 0.56	10.05 ^{a,b,c} ± 0.54	10.78 ^c ± 0.39
<i>t12</i>	2.01 ^b ± 0.05	2.41 ^a ± 0.03	2.39 ^a ± 0.10	2.23 ^a ± 0.13	2.30 ^a ± 0.15	2.38 ^a ± 0.06
<i>t16</i>	2.32 ^b ± 0.09	2.67 ^{a,b} ± 0.10	2.77 ^a ± 0.16	2.55 ^{a,b} ± 0.09	2.48 ^a ± 0.13	2.56 ^{a,b} ± 0.07
Σ <i>trans</i> C18:2	4.67^c ± 0.28	5.14^b ± 0.07	4.25^d ± 0.06	5.14^b ± 0.17	5.08^b ± 0.29	5.65^a ± 0.24
<i>c9 t13</i>	1.55 ^a ± 0.30	1.78 ^a ± 0.02	1.10 ^b ± 0.11	1.75 ^a ± 0.10	1.68 ^a ± 0.24	1.62 ^a ± 0.03
<i>c9 t12</i>	2.15 ^{b,c} ± 0.04	2.32 ^{a,b} ± 0.02	1.85 ^c ± 0.15	2.28 ^{a,b} ± 0.13	2.34 ^{a,b} ± 0.16	2.42 ^a ± 0.12
<i>t11 c15</i>	0.97 ^d ± 0.01	1.04 ^{c,d} ± 0.03	1.20 ^b ± 0.10	1.11 ^c ± 0.05	1.06 ^{c,d} ± 0.04	1.61 ^a ± 0.13

Explanation: $\bar{x} \pm s$ /SD – mean value ± standard deviation; *n* = 3, *a*, *b*, *c*, *d* – values in the rows denoted by different letters differ statistically significantly (*P* < 0.05)

in yellow cheeses – from 0.5 to 6.26 mg g⁻¹ of fat. CLA content in Canadian chesses analysed by MA et al. (1999) ranged from 2.7 mg g⁻¹ fat in goat cheese to 4.7 mg g⁻¹ in Imperial Cheddar cheese and farmer cheese. As reported by LIN et at. (1995), the content of conjugated linoleic acid in 15 tested cheeses ranged from 3.59 to 7.96 mg g⁻¹ fat. Blue, Cottage, Monterey Jack, Brie, Edam and Swiss cheeses had a higher CLA content than the other cheeses: Cheddar-medium, Cheddar-sharp, Cougar Gold, Cream, Mozzarella, Proccesed cheese, Proccesed American, Parmesan and Viking. The content of CLA in Italian commercial cheeses from cow's milk: Alpine cheeses (9 samples), Swiss Emmental (8 samples) Fontina Valdostans (8 samples) and Grana/Parmigiano (5 samples) ranged from 3.85 to 8.11 mg g⁻¹ fat (PRANDINII et al. 2007). CLA contents of most popular Turkish hard cheeses, that is Tulum, Teneke Tulum, Aged Kasha and Fresh Kasha studied by GÜRSOY et al. (2003) were in the range of 3.38–4.88 mg g⁻¹ fat, 2.700–5.390 mg g⁻¹ fat, 3.120–12.610 mg g⁻¹ fat and 0.082–5.390 mg g⁻¹ fat, respectively. The same authors found that the content of CLA in Turkish White Pickled cheeses was in the range of 0.010 to 5.470 mg g⁻¹ fat. In Italian and French cow cheeses studied by PRANDINI et al. (2011), the average content of CLA was 5.66 mg g⁻¹ fat. In fresh cheeses made of cow's milk studied by these authors, the CLA content was 6.10 mg g⁻¹ fat. According to CICOGNINI et al. (2014), the content of CLA in Italian cheeses made of cow's milk that were studied from January to December 2011 ranged from 4.32 mg g⁻¹ fat in Gouda cheeses to 10.21 mg g⁻¹ fat in Gruyere cheeses. In fact, the CLA content may increase in some dairy products subjected to processing, such as heat treatment (GARCIA-LOPEZ et al. 1994), aeration (LIN et al. 1995) and ripening. Many authors reported an increase in the CLA levels in cheeses as the ripening progressed (LAVILLONNIERE et al. 1998, LIN et al. 1999, TALPUR et al. 2008, KIM et al. 2009, LOBOS-ORTEGA et al. 2012, DOMAGAŁA et. al. 2013, CICOGNINI et al. 2014). According to LAVILLONNIERE et al. (1998), the content of CLA in French cheeses with a long ripening time (>4 month) ranged from 9.9 to 15.8 mg g⁻¹ fat, whereas in cheeses ripening for a shorter period of time (<1 month) it ranged from 5.3 to 6.0 mg g⁻¹ fat. The studies of CICOGNINI et al. (2014) showed that the highest content of CLA (8.45 mg g⁻¹ fat) was found in long-ripening cheeses (>180 days). The average content of CLA in fresh cheeses (<45 days of ripening) was 5.70 mg g⁻¹ fat, in cheeses ripened for >45 and <90 days, it was 4.85 mg g⁻¹ fat and in cheeses ripening for 90 to 180 days, it was 5.45 mg g⁻¹ fat. There are also authors who indicated that the ripening time of cheeses did not significantly affect the content of CLA (LIN et al. 1998, MURTAZA et al. 2014, LUNA et al. 2007). On the one hand, a longer exposure to bacterial action cause an increase in CLA contents,

but on the other hand, oxidation reactions could lead to the destruction of the double bonds, thus decreasing CLA levels (LIN et al. 1995, SHANTHA 1995, YANG et al. 2000).

LIN et al. (1999) evaluated the content of CLA in Cheddar, Cougar Gold and Viking cheeses after 1, 3 and 6 months of ripening. The highest content of CLA was found in all cheeses after 3 months of ripening. The content of this isomer was 3.76 mg g⁻¹ fat in Cheddar cheese, 3.44 mg g⁻¹ fat in Cougar Gold cheese and 3.47 mg g⁻¹ fat in Viking cheese. All cheeses analyzed after 6 months of ripening were characterized by a lower content of CLA than those analyzed after 3 months of ripening. According to KUMAR et al. (2006), the content of *cis9,trans11* CLA in buffalo Cheddar cheeses analyzed after 3 and 6 months of ripening was lower than in the fresh cheeses.

The total content of *trans* C18:1 isomers in milk was 17.13 mg g⁻¹ fat. The total content of these isomers in fresh cheeses was significantly higher than in the milk they were produced from ($P < 0.05$). Only slight changes were observed in total content of C18:1 acid *trans* isomers during 6 weeks of ripening (Table 1). Conducted studies have shown that the content of each marked positional *trans* isomers of C18:1 acid in milk, freshly produced cheeses and cheeses analysed during 6 weeks of ripening have changed. The content of *trans* 6–9 isomers as well as *trans* 12 isomer was significantly higher in freshly produced cheeses than in milk prepared for its production. During ripening period the content of those isomers did not change significantly (Table 1). Fluctuations in the content of 10+11 *trans* isomers and *trans* 16 isomer were observed during ripening period.

The total content of *trans* C18:2 isomers in fresh cheeses was 5.14 mg g⁻¹ fat and was significantly higher than the total content of those isomers in milk they were produced from ($P < 0.05$; Table 1). The total content of *trans* isomers of C18:2 acid in cheeses analyzed during the six weeks of ripening differed significantly, except for cheeses after 2 and 4 weeks (Table 1). The lowest total content of C18:2 acid *trans* isomers was after 1 week of ripening (4.25 mg/g fat) and the highest content was after 6 weeks of ripening (5.65 mg g⁻¹ fat). Results presented in Table 1 indicate that the contents of each marked positional *trans* isomers of C18:2 acid in freshly produced cheeses did not differ significantly from the content of those isomers in milk used for production. During ripening period, the content of those isomers did change. Significantly lower ($P < 0.05$) contents of marked *trans* isomers of C18:2 acid were found in cheeses analysed after 1 week of ripening than in freshly produced cheeses. In cheeses analysed after 2, 4 and 6 weeks of ripening the content of *cis9trans11* and *cis9trans12* isomers fluctuated slightly. The content of *trans11cis15* was

significantly higher ($P < 0.05$) in cheeses analysed after 6 weeks of ripening than in cheeses analysed after 2 and 4 weeks of ripening (Table 1).

The calculated linear Pearson's correlation coefficients indicate that the content of CLA in fat of cheeses was strongly, positively correlated with the content of *trans* C18:1 isomers (Table 2). These results are consistent with the findings of FRITSCHÉ and STEINHART (1998) who obtained a high, positive correlation ($r = 0.806$) between the content of CLA and the sum of *trans* C18:1 isomers in the fat of milk and dairy products. ŽEGARSKA et al. (2008) demonstrated that the CLA content in the fat from hard cheeses was highly positively correlated with the content of *trans* C18:1 isomers ($r = 0.828$, $P \leq 0.01$).

Table 2

The correlation coefficients between the content of *cis9,trans11* CLA, *trans* C18:1 and *trans* C18:2 isomers

Specification	Σ <i>trans</i> C18:1	Σ <i>trans</i> C18:2
<i>cis9,trans11</i> CLA	0.836 *	0.345
Σ <i>trans</i> C18:2	0.074	–

* – values are significantly different at $P \leq 0.001$

Conclusions

The study demonstrated that the time of ripening had a significant impact on the content of *cis9,trans11* CLA as well as on the content of *trans* C18:2 isomers in the cheese. Only a slight change was observed in the content of *trans* C18:1 isomers during 6 weeks of ripening. In the analyzed Kormoran cheese, the content of *cis9,trans11* CLA was significantly higher after 6 weeks of ripening compared to the content of this isomer in fresh cheese and in the cheese after 1, 2 and 4 weeks of ripening.

Comparing obtained results of the content of CLA in the analysed cheese with data obtained by other authors on the contents of this acid in a variety of cheeses, it can be concluded that Kormoran cheese is a good source of CLA in our diet.

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THE FATTY ACIDS CONTENT IN POPULAR BARS

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Key words: bars, fatty acids, MUFA, PUFA, VLCFA.

Abstract

In view of the significance of qualitative fatty acid profile, the aim of this research was to determine and compare the content of fatty acids in two groups popular snacks which were classified into two product groups: healthy or unhealthy. Gas chromatograph was used to analyze the fatty acid profile. The study showed that the products classified as 'unhealthy' generally contained more fatty acids than 'dietary' products. They also had a higher content of fatty acids with atherogenic properties. Approx. 66% of fatty acids in both groups were long-chain fatty acids (LCFAs), followed by *cis* monounsaturated fatty acids (MUFAs) which accounted for 25% of all FAs. In contrast, very long chain fatty acids (VLCFAs) were present in small quantities. We also found quite large amounts of linoleic acid (PUFA), which accounted for 2.6% to 18.5% of the total composition of the snacks and appeared in all samples.

ZAWARTOŚĆ KWASÓW TŁUSZCZOWYCH W POPULARNYCH BATONIKACH

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Słowa kluczowe: batony, kwasy tłuszczowe, MUFA, PUFA, VLCFA.

Abstrakt

Celem pracy była analiza i porównanie zawartości kwasów tłuszczowych w popularnych batonikach, które podzielono na dwie grupy – batoniki „zdrowe” i „niezdrowe”. Analizę przeprowadzono metodą chromatografii gazowej. Wykazano, że produkty oznaczone jako „niezdrowe” ogólnie zawierają znacznie większą ilość kwasów tłuszczowych w porównaniu z produktami oznaczonymi jako „zdrowe”. Dodatkowo zawierają znacznie więcej kwasów tłuszczowych wykazujących właściwości aterogenne. Przeważającą część (ok. 66%) wszystkich kwasów tłuszczowych w obu badanych grupach stanowiły kwasy nasycone długłańcuchowe (LCFA), podczas gdy *cis* jednonienasycone kwasy (MUFA) stanowiły ok. 25% całej puli kwasów. Z kolei długłańcuchowe kwasy tłuszczowe (VLCFA) były obecne w badanych produktach w niewielkich ilościach. W badaniach wykazano, że analizowane produkty zawierały także dość duże ilości kwasu linolowego (PUFA), który stanowił od 2,6% do 18,5% całkowitego składu przekąsek i występował we wszystkich próbkach.

Introduction

The accelerating pace of modern life results in the tendency to maximally shorten the time of food preparation. Yet paradoxically, at the same time dietary preferences of modern consumers reveal a growing interest in healthy lifestyle. Products are now expected to be not only tasty and convenient, but also easy to digest and low in calories. This trend can be seen in the growing popularity of cereal bars, advertized as ideal and dietary snacks whose contents (grains, nuts and fruit) are suggested to be able to replace a complete meal. They are also generally considered a healthier alternative to various chocolate bars and wafers, perceived as unhealthy due to their high sugar and fat content and high calorific value.

Although fats are essential for correct nutrition, they may adversely influence the human body not only through excessive intake in diet, but also through their incorrect profile (WOLAŃSKA and KŁOSIEWICZ-LATOSZEK 2012). For example, the excessive consumption of saturated fatty acids,

especially long chain fatty acids (LCFAs), contributes to an increased plasma cholesterol levels and promotes atherosclerosis (FERNANDEZ and WEST 2005). Artificially derived *trans* fatty acids (TFA) also have a negative impact on health, deteriorating lipid profile parameters and increasing the risk of metabolic syndrome and obesity (KOCHAN et al. 2010). In turn, unsaturated fatty acids (MUFAs and PUFAs) have a generally positive effect on the plasma lipid profile and cardiovascular system, through their anti-atherosclerotic, anti-inflammatory and anti-aggregation actions (HABÁN et al. 2000, KOLANOWSKI 2007).

In view of the significance of qualitative fatty acid profile, the aim of this research was to determine and compare the content of fatty acids in two groups popular snacks: 1) cereal bars popularly regarded as healthy and 2) wafers and bars perceived by most consumers as high-calorie and containing harmful ingredients.

Material and Methods

Material to the study

The study included 15 popular products (bars) containing fruits, grains, nuts, chocolate and caramel, available in various discount stores in Poland. The products were classified into two product groups: healthy (dietary) or unhealthy (non-dietary), according to the results of the online survey, in which respondents ($n = 73$) indicated their opinions on the bars. In the survey, respondents could see the photography of the product and the list of its ingredients. They classified bars by answering the question: 'Into which group would you classify snack bar presented below?' The possible answers were: a) healthy, b) unhealthy. The survey revealed a group of 8 supposedly healthy products and 7 products classified as unhealthy.

Preparation of samples

Products were homogenized, and then 1 g of each product was collected as a representative sample. Fatty acids were extracted with the Folch mixture (2:1; chloroform:methanol, v:v), saponified with 2 M potassium base (KOH) and methylated with the solution of BF_3 in methanol by incubation at 70°C. The resulting methyl esters were extracted with hexane. For separation of the phases, saturated solution of NaCl was added. The upper layer of the solution was taken and transferred to glass vials. The obtained fatty acid esters were subjected to qualitative and quantitative analysis by gas chromatography.

Chromatographic analysis of samples

Gas chromatograph (Agilent Technologies 7890 GC System) was used to analyze the fatty acid profile. The study used a capillary column with dimensions of 15 m x 0.10 mm, 0.10 μm (SUPELCOWAX™ 10 Capillary GC Column, Supelco, Bellefonte, PA, USA). Chromatographic conditions were as follows: the initial temperature was 60°C for 0 min, increased at a rate of 40°C/min to 160°C (0 min), increased at a rate of 30°C/min to 190°C (0.5 min) and then increased at a rate of 30°C/min to 230°C for 2.6 min, where it was maintained for 4.9 min. The total analysis was approximately 8 min and the gas flow rate was 0.8 ml/min with hydrogen as the carrier gas. Qualitative analysis was performed by comparing the peak retention time of the identified a substance with the standard peak retention time. The quantitative analysis included a comparison of surface area of the peaks with the standard peak surface area for heneicosanoic acid (C 21:0).

Statistical analysis

Statistical analysis were performed using Stat Soft Statistica 10.0 and Microsoft Excel 2007. The arithmetic means (AM) and standard deviations of the AM (SD) were calculated for each studied group. The distribution of results for individual variables was obtained with the Shapiro-Wilk test. As most of the distributions deviated from the normal Gaussian distribution, non-parametric tests were used for further analyses. To assess the differences between the studied groups, the non-parametric Mann-Whitney test was used. The level of significance was $p \leq 0.05$.

Results

The study showed that both 'healthy' (group I) and 'unhealthy' (group II) contained fats which varied primarily in terms of composition and quality of the fatty acids (Table 1).

Approx. 67% of fatty acids in both groups were long-chain fatty acids (LCFAs), followed by *cis* monounsaturated fatty acids (MUFAs) which accounted for 17% of all FAs. In contrast, very long chain fatty acids (VLCFAs) in the tested products were present in small quantities.

Moreover, for all analyzed products the atherogenic index (AI) and thrombogenic index (TI) has been calculated by using equations gave by Ulbricht and Southgate (ULBRICHT and SOUTHGATE, 1991). The groups showed no statistically significant differences for AI nor TI. The highest values of both indexes were found for product 5 belonging to the 'healthy' group (Table 1).

Table 1
The percentage content of each fatty acid groups, atherogenic index (AI) and thrombogenic index (TI) of tested products

"Healthy" products									
Sum of %	1	2	3	4	5	6	7	8	mean±SD
MCFA	0.22	10.13	0.16	0.20	40.36	7.21	0.18	0.24	7.34±13.00
LCFA	55.32	42.27	72.11	69.55	52.94	59.96	63.14	85.20	62.52±12.33
VLCFA	0.00	5.91	0.46	0.62	0.19	0.71	1.16	1.56	1.33±1.80
MUFA	11.36	32.67	14.17	16.56	3.71	15.69	27.16	1.71	15.38±9.87
PUFA	33.09	9.02	13.10	13.07	2.80	16.43	8.37	11.29	13.40±8.36
Index AI	0.89	3.39	2.50	2.24	19.15	2.19	1.41	5.75	4.69±5.64
Index TI	0.76	4.00	3.14	4.39	15.06	3.21	3.54	12.96	4.88±4.83
"Unhealthy" products									
Sum of %	9	10	11	12	13	14	15		mean±SD
MCFA	0.20	0.21	0.49	3.11	2.46	0.29	3.08		1.41±1.30
LCFA	46.20	81.72	75.94	78.61	75.28	60.66	78.43		70.98±11.91
VLCFA	1.59	0.74	0.66	0.95	1.32	8.71	1.90		2.27±2.66
MUFA	28.73	12.76	19.36	12.60	18.10	25.17	11.94		18.38±6.10
PUFA	23.28	4.56	3.54	4.73	2.85	5.17	4.64		6.97±6.70
Index AI	0.96	3.53	3.10	3.09	1.95	1.14	3.96		2.53±1.10
Index TI	1.23	9.20	6.75	7.71	7.14	3.91	8.91		6.41±2.66

The table includes data on the content of following fatty acids:

MCFA: caprylic acid C8:0, lauric acid C12:0

LCFA: myristic acid C14:0, pentadecanoic acid C15:0, palmitic acid C16:0, heptadecanoic acid C17:0, stearic acid C18:0

VLCFA: behenic acid C22:0, cerotic acid C26:0

MUFAs: myristoleic acid C14:1, palmitoleic acid C16:1, oleic acid C18:1 n9

PUFAs: linoleic acid C18:2 n6, γ -linolenic acid C18:3n6, α -linolenic acid C18:3n3

Saturated Fatty Acids (SFAs)

Among long chain saturated fatty acids, the groups showed statistically significant differences in the levels of palmitic and stearic acids (Figure 1). Their content in 'unhealthy' bars usually exceeded 10 000 $\mu\text{g g}^{-1}$, although similar values were also found in the healthy bars, in particular in samples 2 and 3, especially regarding palmitic acid. The product 1 contained negligible amounts of both acids. Statistically significant difference was also demonstrated for pentadecanoic acid and heptadecanoic acids (Figure 1), present in greater amounts in 'unhealthy' snacks, especially in

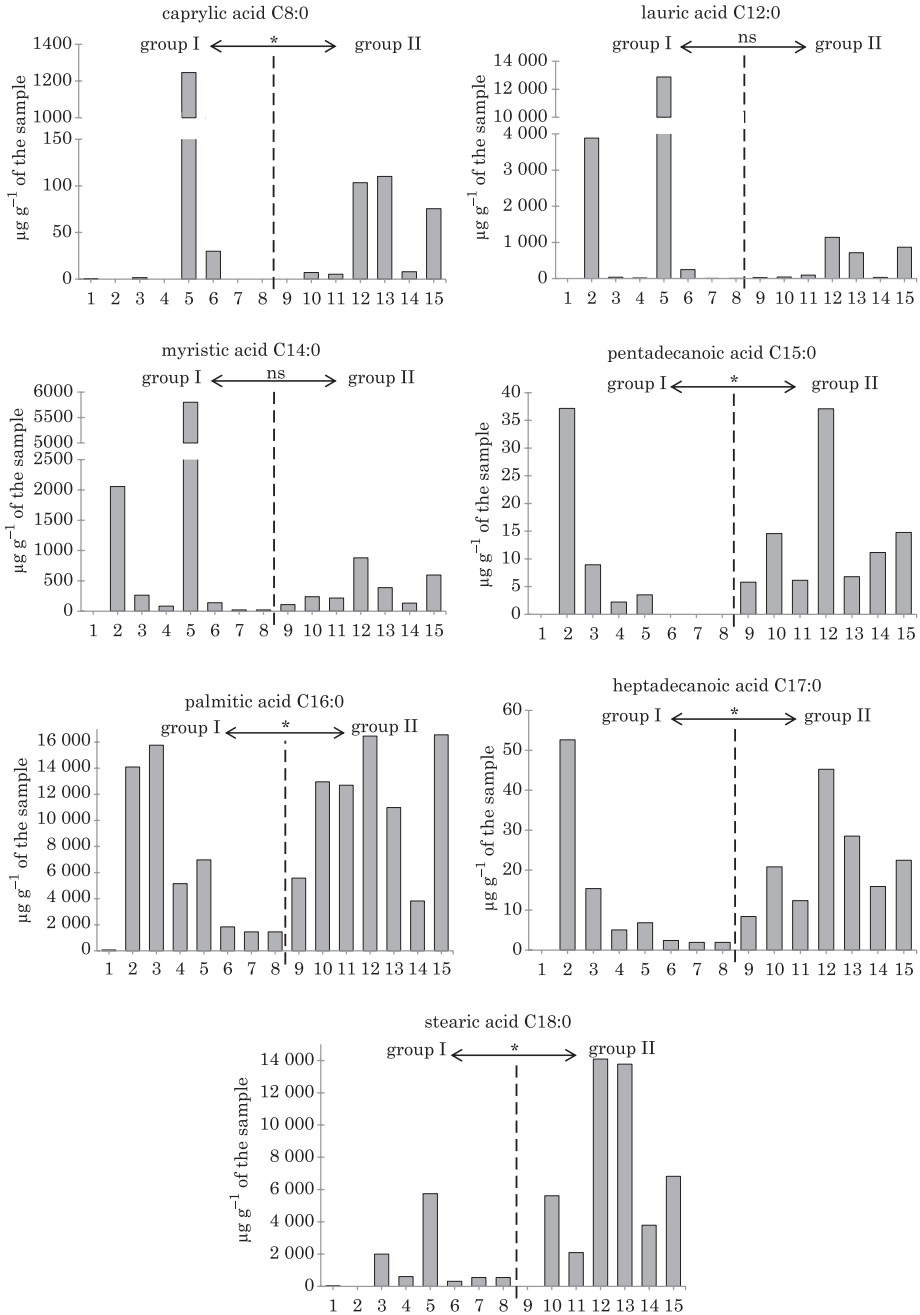


Fig. 1. The contents of medium and long chain saturated fatty acids (MCFA: caprylic and lauric acid; LCFA: pentadecanoic, palmitic, heptadecanoic and stearic acid) in various bars in group I („healthy, dietary”) and group II („unhealthy, nondietary”). * – statistical significance between the two groups, $p \leq 0.05$

the sample 12. However, their levels were the highest in the sample 2 from the 'healthy' group. In contrast, product no. 1 did not contain any of these acids.

Among very long chain fatty acids (VLCFAs) special attention should be paid to cerotic and behenic acids (Figure 2). Statistically significant difference was observed for both of these acids and they were the only ones that were found in each 'unhealthy' product, while in the 'healthy' group their large amounts were found only in sample no. 2, where the level of behenic acid was the highest (2224 $\mu\text{g g}^{-1}$) and much higher than in the other 'healthy' products.

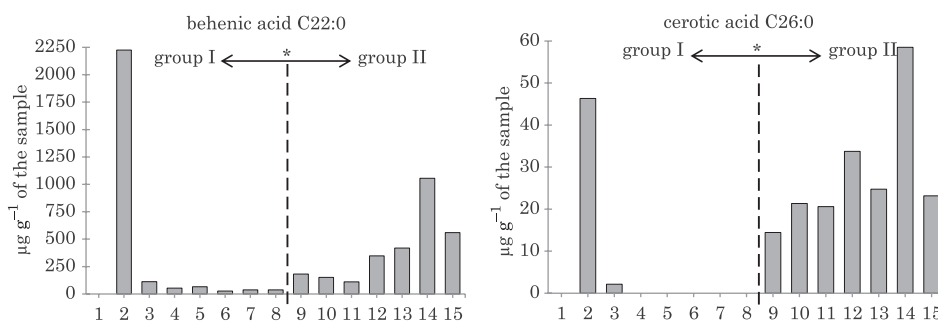


Fig. 2. The content of very long-chain saturated fatty acids (VLCFA: behenic and cerotic acid) in various bars in group I („healthy, dietary”) and group II („unhealthy, nondietary”). * – statistical significance between the two groups, $p \leq 0.05$

Statistically significant differences in saturated fatty acids between the 'healthy' and 'unhealthy' products were also found for caprylic acid belonging to the medium chain fatty acids (MCFAs), but its amounts in the majority of the samples were very low or undetectable. The only exception was the product 5 where its level was above 1200 $\mu\text{g g}^{-1}$ (Figure 1).

The analyzed products also contained SFAs which showed no difference between the groups I and II. These were lauric and myristic acids. It should be noted, however, that in individual samples they were present at levels greater than 1000 $\mu\text{g g}^{-1}$ (Figure 1).

Monounsaturated Fatty Acids (MUFAs)

Analysis of monounsaturated fatty acids, i.e. MUFAs, showed oleic acid was the most plentiful among *cis* acids; it was noted in all products except 1 and 8. In the 'unhealthy' group, its level was more or less stable, exceeding 2000 $\mu\text{g g}^{-1}$, while in the 'healthy' group differences were much higher – ranging from 4100 $\mu\text{g g}^{-1}$ in the product 2 to 570 $\mu\text{g g}^{-1}$ in the

product 6 (Figure 3). The significance of the difference in the oleic acid level between the groups was 0.009. Significant differences between the study groups were also observed for palmitoleic acid, but it was presented only in very small quantities. Its highest values were found in samples 2 and 12. MUFAs also include myristoleic acid, but its selective occurrence in the investigated samples showed no statistically significant differences between the groups.

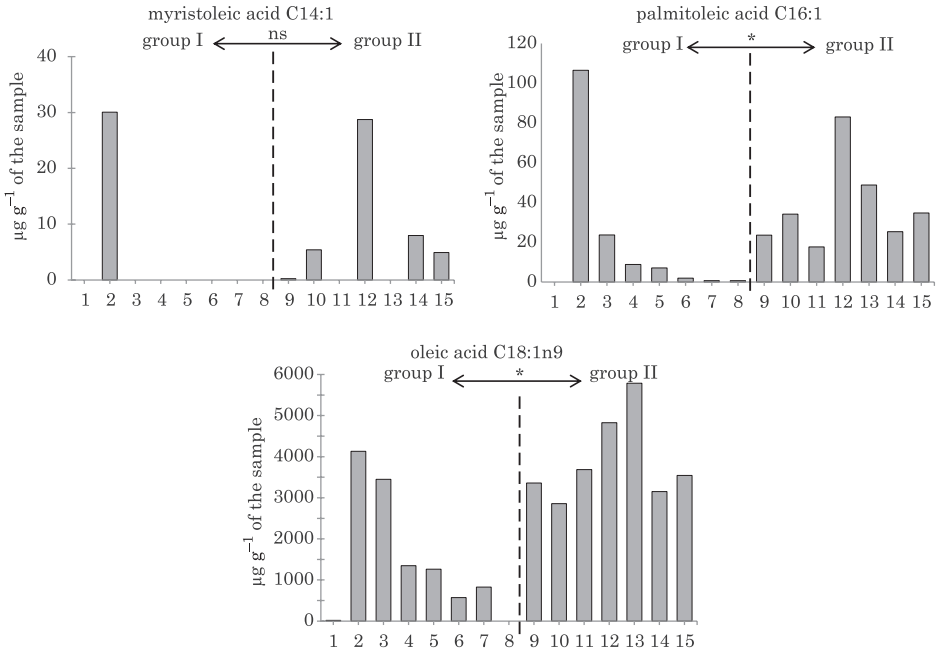


Fig. 3. The content of *cis* monounsaturated fatty acids (*cis*-MUFA: myristoleic, palmitoleic and oleic acid) in various bars in group I („healthy, dietary”) and group II („unhealthy, nondietary”).

* – statistical significance between the two groups, $p \leq 0.05$

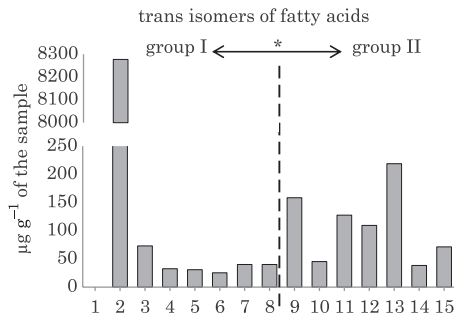


Fig. 4. The content of *trans* monounsaturated fatty acids (*trans*-MUFA) in various bars in group I („healthy, dietary”) and group II („unhealthy, nondietary”). * – statistical significance between the two groups, $p \leq 0.05$

Trans MUFAs were equally important in our analysis. These acids were present in both groups of products, but not exceeding $200 \mu\text{g g}^{-1}$ with the exception of the ‘dietary’ bar 2 which once again revealed extremely high level in comparison to other products ($8277 \mu\text{g g}^{-1}$) sample. In terms of TFA, only product 1 proved to be safe – it contained no such fatty acids. At the same, much larger amounts of TFAs were found in ‘unhealthy’ (Figure 4).

Polyunsaturated Fatty Acids (PUFAs)

When it comes to polyunsaturated fatty acids (PUFAs), the tested products showed the presence of linoleic acid and α -linolenic acid (Figure 5). Although there were no statistically significant differences in the content of these acids between the two groups, it should be noted linoleic acid levels in most products were as high as about $1000 \mu\text{g g}^{-1}$ sample or more.

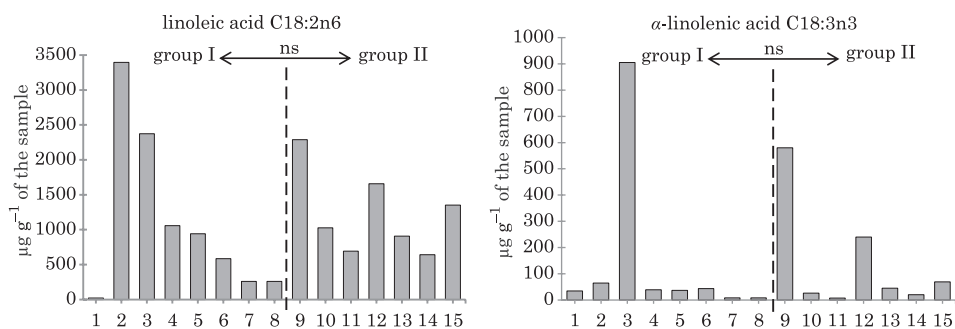


Fig. 5. The content of polyunsaturated fatty acids (PUFA: linoleic and α -linolenic acid) in various bars in group I („healthy, dietary”) and group II („unhealthy, nondietary”).

* – statistical significance between the two groups, $p \leq 0.05$

Discussion

Many studies confirm that the excessive consumption of saturated fatty acids, especially long chain fatty acids (LCFAs), contributes to the incidence of many metabolic disorders resulting from an increase in plasma cholesterol and promotes atherosclerotic lesions. At the same time, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), may reduce the level of cholesterol, although not proportionately. According to WHO recommendations for healthy people, 25 to 30% of energy the diet should come from fat. Saturated fatty acid should not provide more than 10% of energy per day, while omega-6 polyunsaturated

fatty acids from 4 to 8% of energy. Omega-3 fatty acids should be consumed at an amount of 2 g per day (α -linolenic acid) and 200 mg per day for omega-3 long chain eicosapentaenoic and docosahexaenoic acids. The remaining portion of energy should come from monounsaturated fatty acids (CONNOR 1999, ACHREMOWICZ and SZARY-SWORST 2005).

Among saturated fatty acids, most atherogenic properties are shown by lauric C 12:0, myristic C 14:0 and palmitic C 16:0 acids, able to regulate the expression of the LDL receptor gene and inhibit its activity. This results in increased LDL cholesterol and total cholesterol (FERNANDEZ and WEST 2005). The study by WOLAŃSKA et al. (2012) shows a significantly higher consumption of these atherogenic acids in men, who also had a higher total cholesterol than women. Tests carried out by WOLLETT et al. (1992) indicate that a diet rich in palmitic, myristic and lauric acids increases LDL-C by lowering LDL apoB/E receptor activity. This results in an increased production of this cholesterol fraction. Stearic acid has a different effect; although both palmitic and stearic acids belong to the group of saturated fatty acids, stearic acid C 18:0 shows no effect on total cholesterol and LDL-C (EMKEN, 1994).

Our results showed that palmitic acid dominated in the candy bars belonging to the group perceived as 'unhealthy' or 'non-dietary'. Unfortunately, it was also present in bars considered healthy. Particularly worrying is the fact that most of 'healthy' products had high levels of this atherogenic acid. Moreover, two products belonging to the mentioned group had the highest values of atherogenic index and thrombogenic index among all analyzed bars. Therefore, it is very disquieting that respondents basing on the product ingredients could qualified them as 'healthy'. On the other hand, lauric and myristic acids occurred only in some samples, with a statistically insignificantly higher content in 'healthy' snacks.

Very long chain fatty acids (VLCFA) are saturated, unbranched fatty acids whose chains are composed of at least 24 carbon atoms. Many studies show that VLCFAs accumulate in the human body, mainly in the white matter of the central nervous system, adrenal cortex and male gonads (ZGORZALEWICZ-STACHOWIAK et al. 2006).

It is likely that the excessive accumulation of VLCFAs in the nervous system gives rise to an immune reaction involving macrophages and astrocytes, triggered by the impairment of the acylation of gangliosides and phospholipids. The result is the development of inflammation and progressive demyelination of white matter of the brain and – less frequently – in the peripheral nervous system (ZGORZALEWICZ-STACHOWIAK et al. 2006). The excessive accumulation of VLCFAs in the adrenal cortex leads to the disruption of ACTH-induced cortisol secretion. This is due to the formation

of structures in the cytoplasm of the cells in the zona fasciculata and reticularis of the adrenal cortex that include esterified cholesterol of VLCFAs which cannot be used for the synthesis of steroid hormones. Moreover, the high concentrations of VLCFAs increase the viscosity of cell membranes and impede access to ACTH receptors (FICHNA et al. 2004). Our study showed that the analyzed VLCFAs (cerotic acid C 26, behenic acid C 22, tricosanoic acid C 24) can be found in very large quantities in the groups of 'unhealthy' products. However, the presence of these acids at similar levels were also recorded in 'healthy' products, advertised as wholesome snacks.

Monounsaturated fatty acids (MUFAs) are acids which contain one double bond, e.g. oleic acid (C 18:1) commonly found in olive oil. Various studies have shown that MUFAs reduce blood triglycerides and also lower the level of total cholesterol and LDL-cholesterol, while increase HDL-cholesterol (ABIA et al. 2003, HABÁN et al. 2000, WOLAŃSKA and KŁOSIEWICZ-LATOSZEK 2012). Furthermore, LDL enriched with oleic acid reduces the concentration of polyunsaturated fatty acids and their pro-inflammatory properties. It was also found that the content of oleic acid in the LDL inversely correlates with monocyte chemotaxis. CARLUCCIO et al. (1999) found that the incorporation of oleic acid into cultured endothelial cells stimulated with cytokines results in a reduction of leukocyte adhesion to endothelial cells (CARLUCCIO et al. 1999). The activation of factor VII and factor VII antigen concentrations were significantly lower as a result of a diet rich in monounsaturated fatty acids (HABÁN et al. 2000). Enriching a low-calorie diet with MUFAs enhances the beneficial effect of weight loss on the improvement of parameters which constitute risk factors for cardiovascular diseases in obese patients with type 2 diabetes (HABÁN et al. 2000).

Almost all of the naturally occurring unsaturated fatty acids have double *cis* bonds. Some unsaturated fatty acids have a double trans bond and acids containing at least one such bond are known as trans fatty acids (TFAs) (KOCHAN et al. 2010). These acids may occur as solids, which is of great biological importance because it is the type of fatty acids in the phospholipids of cell membranes influences the flexibility of these membranes. TFAs oxidize slower and can be used for repeated frying (CRAIG-SCHMIDT 2006); when incorporated into cell membranes, they alter their permeability and activity, and the number of receptors and enzymes associated with these membranes. This results in a deterioration of vital functions of cells. TFAs are produced by the hydrogenation of saturated fats and the human body they are metabolized similar to saturated fats, causing adverse effects, especially atherosclerotic lesions in blood vessels (ACHREMOWICZ and SZARY-SWORST 2005, KOZŁOWSKA-WOJCIECHOWSKA

2003). MOJSKA (2006) reports that TFA consumption in Poland ranges from 2.8 to 6.9 g/day, which greatly exceeds the nutritional recommendations for the maximum content of *trans* fatty acids in the daily ration. TFA intake should not exceed 1% of energy per day, which is approx. 2 g in the diet of 2000 kcal (ACHREMOWICZ and SZARY-SWORST 2005, MOJSKA 2006). PASZCZYK et al. (2007) analyzed the content of *trans* fatty acids in products bought in Polish stores (biscuits, biscuits and wafers). Of the 32 different cakes, 17 contained TFA at more than 2 g/100 g of the product, and in one case the TFA content was as high as 12 g/100 g. In some popular products in this category the amount of TFAs exceeds the recommended daily ration (PASZCZYK et al. 2007). Our study also showed the presence of *trans* fatty acids in both 'dietary' and 'non-dietary' products. An alarmingly high content of TFAs was observed in one of the dietary products, where it considerably exceeded the levels found in the 'unhealthy' group. Numerous studies have shown that artificially produced *trans* fatty acids impair the blood lipid profile, lowering HDL cholesterol (HDL-C) and increasing the levels of LDL cholesterol (LDL-C) compared to a diet with the predominance of oleic acid (*cis* fatty acid) (ACHREMOWICZ and SZARY-SWORST 2005, MENSINK and KATAN 1990). In addition, TFAs increase the ratio of total cholesterol to HDL (TC/HDL-C), a lipid predictor of coronary artery disease (ACHREMOWICZ and SZARY-SWORST 2005). They increase the levels of atherogenic lipoprotein, decrease plasma apoAI levels and increase the concentration of apoB in the blood, especially compared to MUFAs and PUFAs. TFAs also contribute to an increase in the concentration of triacylglycerols in the blood (ACHREMOWICZ and SZARY-SWORST 2005, LICHTENSTEIN et al. 1999). *Trans* fatty acids increase the risk of coronary heart disease, arrhythmias and sudden cardiac arrest. This is confirmed by numerous studies which show that even small amounts of TFA consumed in the diet (1.6% of energy) have a negative effect on the cardiovascular system. In addition, they contribute to increased weight and body fat, which contributes to the formation of abdominal obesity. This results in a weakening of the sensitivity of cells to insulin, resulting in changes in the structure and function of cell membranes, which can be a risk factor for type 2 diabetes (ACHREMOWICZ and SZARY-SWORST 2005). At the same time, as shown by HU et al. (1999), the replacement of a diet with 2% TFA-derived energy by a diet rich in unsaturated fatty acids (MUFA and PUFA) reduces the risk of cardiovascular disease by 53%.

However, *trans* fatty acids also occur naturally in the milk and meat of ruminants and as such may have a health-promoting effect. They are formed in the stomach of ruminants from α -linolenic acid and linoleic acid by the enzymes of anaerobic bacteria found in the rumen. Intermediate

metabolites are vaccenic acid (C 18:1) and CLA (*cis*-9, *trans*-11 C18:2). It is believed that the CLAs prevent atherosclerotic changes resulting from improper diet, and have antimutagenic and anticancerogenic effects probably due to their antioxidant properties (STACHOWSKA et al. 2004). The results of BARTNIKOWSKA (2000) indicate that CLAs protect cell membranes against free radicals, while their anticancerogenic abilities may consist in the inhibition of the production of eicosanoids, stimulators of cell growth (BARTNIKOWSKA, 2000). Research carried out in 2003 by IP et al. (2003) demonstrated that conjugated linoleic acids (CLAs) are incorporated into lipids in fat cells (where they are accumulated) and indirectly may influence the development of carcinogenesis by inhibiting cell differentiation (BIAŁEK and TOKARZ 2013, IP et al. 2003). In recent years, many publications have shown the contribution of CLAs in inhibiting tumors of the colon and breast. It has been shown that under this acid inhibits the proliferation of colon cancer cells dependent on the activation of PPAR γ receptor. In addition, *trans*-10, *cis*-12 CLA is most likely to inhibit the expression of HER2 protein by interfering with the NF- κ B signaling pathway (BIAŁEK and TOKARZ 2013).

Conclusions

A comparison of the two groups of products was difficult because of the very large differences in the quantity of individual fatty acids between individual products. Nevertheless, the products classified as 'unhealthy' generally contained more fatty acids than 'dietary' products. They also had a higher content of fatty acids with atherogenic properties. Although, there were no statistically significant differences between the groups for atherogenic and thrombogenic indexes, it is worth to mention that several bars had very high levels of AI and TI.

The majority of the total fatty acids in all the products were long-chain saturated C12–18 fatty acids (LCFAs), constituting 41.7–85.1% of the total fatty acids. These were mostly palmitic acid and stearic acid, showing very large differences in their contents between individual products, regardless of the group to which the products were assigned ('healthy' vs 'unhealthy'). Among VLCFAs, statistically significant differences between the groups were found in the levels of behenic and cerotic acids, but their quantities were usually low. In general, VLCFAs usually represented less than 2% of the total fatty acid content in individual samples (with the exception of the product 2–5.9% and product 14–8.7%).

Declaration of interest statement

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**THE LENGTH-WEIGHT RELATIONSHIP
AND CONDITION FACTOR OF THE THREATENED
SNAKEHEAD (*CHANNA STRIATA*) FROM SUNGAI
BATANG RIVER, INDONESIA**

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Key words: Allometric, condition factor, snakehead, Sungai Batang, weight-length.

Abstract

The snakehead (*Channa striata*) in Sungai Batang River, Indonesia has high commercial value and high pressure on population caused by fishing. Meanwhile, scientific information on morphometric characteristic of snakehead is still lacking. Samples of 330 fishes having size range of 75–335 mm (202.68 ± 46.56 mm) total length (TL) and 9–288 g (81.23 ± 53.95 g) weight were taken to estimate length-weight relationship and condition factors. The snakehead grew allometrically ($b = 2.6927-2.9125$), indicating that fish becomes slender as the length increases. Total length and weight of female were significantly higher than male ($P < 0.001$). The highest number of catch was found between 160 and 179 mm TL (24.44%) for male and between 260 and 279 mm TL (16.00%) for female. The heaviest catches of male (33.89%) and female (14.00%) were noted between 30 and 49 g weight class. The mean condition factor (K) value of female was considerably higher than that of male ($P < 0.001$). The mean K values obtained for male and female were 0.83 ± 0.09 and 0.86 ± 0.08 , indicating that the fish in the river was unfavorable condition.

To the best our knowledge, this study is the first reference on length-weight and condition factor for snakehead from this river.

ZWIĄZEK DŁUGOŚCI I MASY CIAŁA ZAGROŻONEGO GATUNKU ŻMIJOGŁOWA (*CHANNA STRIATA*) Z RZEKI SUNGAI BATANG W INDONEZJI

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Słowa kluczowe: wzrost allometryczny, współczynnik kondycji, żmijogłów, Sungai Batang.

Abstrakt

Żmijogłów (*Channa striata*) z rzeki Sungai Batang w Indonezji ma wysoką wartość handlową i dlatego populacja tego gatunku poddana jest silnej presji połowowej. Tymczasem wciąż brakuje naukowych informacji na temat cech morfometrycznych badanego gatunku. W celu oszacowania stosunku długości ciała do jego masy oraz wskazania czynników warunkujących tę cechę, zbadano 330 ryb o całkowitej długości (TL) 75–335 mm (średnio $202,68 \pm 46,56$ mm) i masie 9–288 g ($81,23 \pm 53,95$ g).

Wyniki wskazują na allometryczny wzrost ryb ($b = 2,6927-2,9125$). Całkowita długość i masa samic były znacznie wyższe niż u samców ($P < 0,001$). Największy udział w połowach miały osobniki o długości 160–179 mm TL (24,44%) u samców i 260–279 mm TL (16,00%) u samic. Z kolei pod względem masy największy udział u samców (33,89%) stanowiły ryby o masie 30 g, a u samic (14,00%) – 49 g. Średnia wartość współczynnika kondycji (K) u samic była znacznie wyższa niż u samców ($P < 0,001$). Średnie wartości K uzyskane dla samców i samic wynosiły odpowiednio $0,83 \pm 0,09$ i $0,86 \pm 0,08$, wskazując, że ryby nie miały korzystnych warunków w rzece.

Opracowanie stanowi pierwsze doniesienie na temat związku długości i masy żmijogłowa żyjącego w wodach rzeki Sungai Batang w Indonezji.

Introduction

The snakehead of the family Channidae is economically important freshwater species in Southeast Asia and other countries e.g. Indonesia (MUTHMAINNAH 2013, IRHAMSYAH et al. 2017), Singapore (NG and LIM 1990), Thailand (KHOMSAB and WANNASRI 2017), Malaysia (SONG et al. 2013), the Philippines (BERNAL et al. 2015, JUMAWAN and SERONAY 2017), Viet Nam (QUYEN et al. 2016), Sri Lanka (WIJEYARATNE 1994), Nigeria (AMA-ABASI and OGAR 2013, BOLAJI et al. 2011), Bangladesh (ISLAM et al. 2013), India (DATTA et al. 2013, KASHYAP et al. 2014), Pakistan (NAJERO et al. 2015) and China (WANG et al. 2012, GU et al. 2015), due to delicious, high quality meat fish, and availability throughout the year. The snakehead is air-breathing fish, adaptable to low oxygen, and tolerant to wide temperature range (XIE et al. 2017). It can survive up to four days on land as long as their skin remains moist and survive at colder temperatures by

burrowing into the mud (COURTENAY and WILLIAMS 2004). In nature, snakehead inhabit all freshwater bodies such as rivers, canals, lakes, swamps, marshes and paddy fields (AMILHAT and LORENZEN 2005, DAYAL et al. 2012, ALI et al. 2013), and can be commercially cultured in fish farm, earthen ponds, or hapa system (KUMAR et al. 2011, XIE et al. 2002, HE et al. 2015, QUYEN et al. 2016). Meanwhile, in Europe and North America, snakehead is considered as an invasive species with high risks to aquatic ecosystems (COURTENAY and WILLIAMS 2004, LAPOINTE et al. 2013).

Several works on biological characteristics of snakehead have been reported in recent years (MEHRAJUDDIN et al. 2011, WIDODO 2013, SAKHARE 2015). Each species of snakehead has own its characteristic and they may have different performance and behavior even in the same habitat. To manage the snakehead fishery resource rationally, it is therefore needed in-depth knowledge of its biology, feeding habit and ecology (GREINER and GREGG 2010, SAIKIA et al. 2012, ISTIYANTO and DIANA 2016). The length-weight relationship is the most common scientific approach that used for analyzing growth or morphometric for an individual species of snakehead (HANIFFA et al. 2006, DUA and KUMAR 2006, OLURIN and SAVAGE 2011, RATHOD et al. 2011, ALI et al. 2013), as well as for understanding survival, maturity and reproduction (BOLAJI et al. 2011, ISLAM et al. 2013, ANWAR et al. 2018) of various species from different geographical regions. It is also useful in local and interregional, morphological and life historical comparisons in species and populations (GOSWAMI et al. 2006, KHAN et al. 2012, SONG et al. 2013). GHORBANI et al. (2012) stated that the fish length is the best indicator of production efficiency, while LAWSON (2011) reported that fecundity may increase with increased body size in fish.

Fishing activity for snakehead (*Channa striata*) in Sungai Batang River is open throughout the year regardless of seasonal periods, which is done by both villagers and beyond. Although it is prohibited by the law, in particular circumstance (e.g. low tide), some people still use electricity to collect fish from rivers or swamps because this fishing method is considered as a fast and easy way to earn money. The difficulties maintaining catches using traditional fishing methods to meet the quota are also stirring people to do this. If such condition is not well-managed, it will affect to ecosystem and socio-economic as the whole. On the other side of it, scientific information on morphometric characteristic of snakehead captured is still lack. To get more knowledge in area of study, we carried out the field survey by collecting snakehead samples from local fishermen and investigating the length-weight relationship and condition factor of fish to provide some fundamental suggestions for better fisheries management and conservation for this species in this region.

Materials and Methods

The research was carried out in Sungai Batang River, Martapura of South Kalimantan Province (Figure 1), located on $03^{\circ}22'36''$ S and $114^{\circ}49'29''$ E, determined by GPS-60 Garmin, Taiwan. The village consists mostly of wetland area with water level fluctuation between 0.5 m and 2 m. The wetland is regulated mainly by the rainfall resulting in two contrasts environmental conditions. During rainy season (October–April), the wetland is entirely flooded by water and the fishes are difficult to be caught. Inversely, during the dry season (May–September) the wetland is covered by very dense vegetation and the fish are concentrated on the sludge holes or backwater and allow for people to catch them. This regular changing from water environment to high plant biomass is an important factor in regulating high production of freshwater fishes in the wetland.

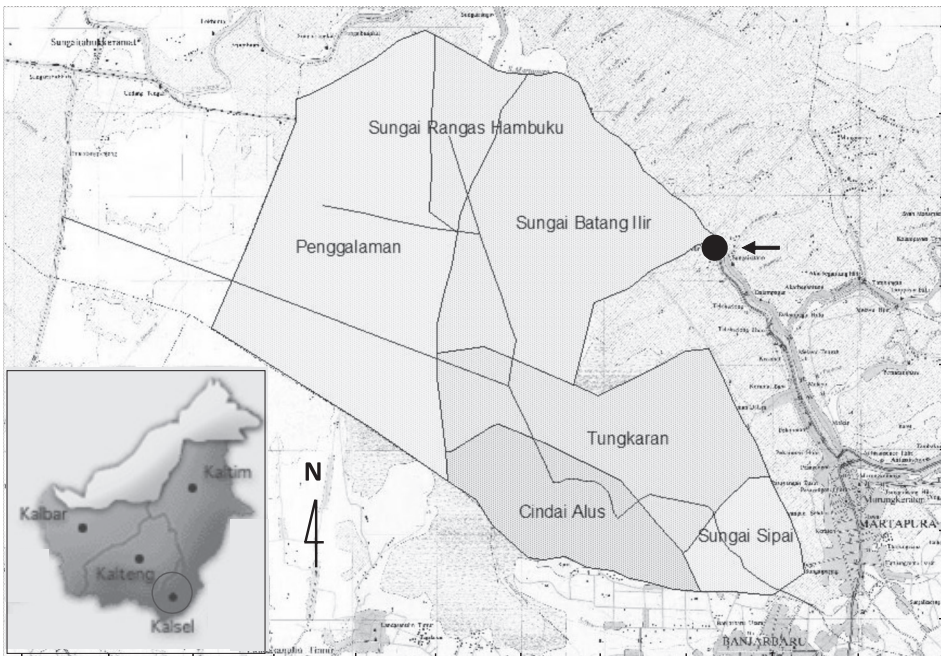


Fig. 1. Map showing the location of sampling site in Sungai Batang River

A total of 330 individuals of snakehead comprising 180 males and 150 females were obtained from local fishermen during May 2017 and February 2018. Fish samples were collected by using different fishing gears such as *banjur* or stage-line ($n = 274$), *lukah* or fish trap ($n = 34$) and also electrofishing ($n = 22$). *Banjur* is consisted of a bamboo stick: 100 cm long,

hook: #10 and a line: 100–160 cm long nylon monofilament. The live frog (*Rana cancarivora*) is used as bait. The fishermen put *banjur* along the riverbank or around the swamp for targeting snakehead. When the fish is being caught, fishermen usually cut the line and leave a hook embedded in a fish's mouth or throat. *Lukah* is an elongated tube-shaped made of bamboo (115–158 cm) diameter of 16–18 cm containing one entry funnel mounted on the inside of conical-shape and tapering inside to about 2.5 cm, called *hinjap* (one-way valve, made of elastic rattan; about 35–40 cm one to other), and containing one exclusion funnel at the opposite side. Thus, fish can enter easily but difficult to escape from the trap. The traps are deployed in the river or swamp under highly vegetated habitats with slow or no current at morning and retrieved at afternoon. The body trap is partly submerged with an oblique angle of approximately 15° on the water surface, which allow for snakehead taking air-breath. We are not able to describe the detailed electrofishing devices used in this study due to a technical barrier (unwillingness of fishermen). Snakehead prefers standing-water habitats with a lot of aquatic vegetation (e.g. *Hydrilla verticillata*, *Eichornia crassipes*, *Ipomea aquatic*, *Pistia stratiotes*, and *Salvinia molesta*). The snakehead catches were identified by sexes, and measured for total length and weight. The total length (TL) and body width (W_d) were measured with a ruler to the nearest mm, while body weight (W) was determined with a digital balance to an accuracy of 0.01 g (Dretec KS-233, Japan). The size distribution of fish sampled was set at 20-mm amplitude and 20-g amplitude. The length-weight relationship of fish can be expressed in either the allometric form:

$$W = aL^b \quad (1)$$

or in the linear form:

$$\text{Log } W = \text{Log } a + b \text{ Log } L \quad (2)$$

where:

- W – the total weight [g];
- L – the total length [mm];
- a – the constant showing the initial growth index;
- b – is the slope showing growth coefficient.

The b exponent with a value between 2.5 and 3.5 is used to describe typical growth dimensions of relative wellbeing (BAGENAL 1978). In other word, this b value has an important biological meaning; if fish retains the same shape and grows increase isometrically ($b = 3$). When weight increases more than length ($b > 3$), it shows positively allometric. When the length increases more than weight ($b < 3$), it indicates negatively allo-

metric (SENGUTTUVAN and SHIVAKUMAR 2012). When scaling is isometric, fish weight (equal to the volume if constant density is assumed) will vary with the length cubed (i.e. $b = 3$), while the standard length will show linear correlation with total length i.e. $b = 1$ (FROESE 2006). If the observed value of b differs from these expectations the relationship is allometric and growth is non-isometric, and an examination of the contributing factors can shed light on species biology and wellbeing. A difference in the b exponent between smaller and larger length classes was also observed. The coefficient of determination (R^2) and the coefficient of correlation (r) of morphological variables between male and female were also computed. The data used for length weight relationship were also utilized for calculating condition factor of male and female by mean of formula (JIN et al. 2015):

$$K = 100 (W/L^3) \quad (3)$$

where:

- K – the Fulton’s condition factor;
- L – total length [cm];
- W – is weight [g].

The factor of 100 is used to bring K close to a value of one. The K value is used in assessing the health condition of fish of different sex and in different seasons. In addition, the Mann-Whitney test was employed to verify if there are no differences between sexes for lengths and weights and for the condition factor. All tests were analysed at the 0.05 level of significance. SPSS for windows version 16.0 statistical software was used for all data analysis.

Results and Discussion

All estimated length-weight relationship of *C. striata* is presented in Table 1. The male size ($n = 180$) ranged from 75 to 280 mm (184.92 ± 39.98 mm) total length and from 9 to 163 g (59.47 ± 36.91 g) weight; while the female ($n = 150$) ranged from 120 to 335 mm (223.99 ± 45.03 mm) total length and from 14 to 288 g (107.33 ± 59.45 g) weight. The pooled ($n = 330$) ranged from 75 to 335 mm (202.68 ± 46.56 mm) total length and from 9 to 288 g (81.23 ± 53.95 g) weight.

Table 1
Total length, weight and condition factor of *C. striata* taken from Sungai Batang River

Sex	n	Total length [mm]			Weight [g]			a	b	R ²	Allometric pattern	K mean ± SD
		min	max	mean ± SD	min	max	mean ± SD					
Males	180	75	280	184.92 ± 39.98	9	163	59.47 ± 36.91	0.00004	2.6927	0.9149	A-	0.83±0.09
Females	150	120	335	223.99 ± 45.03	14	288	107.33 ± 59.45	0.00001	2.9125	0.9662	A-	0.86±0.08
Pooled	330	75	335	202.68 ± 46.56	9	288	81.23 ± 53.95	0.00001	2.9223	0.9725	A-	0.84±0.09

Explanation: n – number of fish samples; SD – standard deviation; a – constant; b – exponent; R² – coefficient of determination; A- – negative allometric; K – condition factor

Significant differences were observed at length-weight relationship of male and female (Figure 2), while b values implied that the body shape displays a negative allometric growth pattern (b < 3), which means that the length increases more than weight. The estimated b values in the WLR

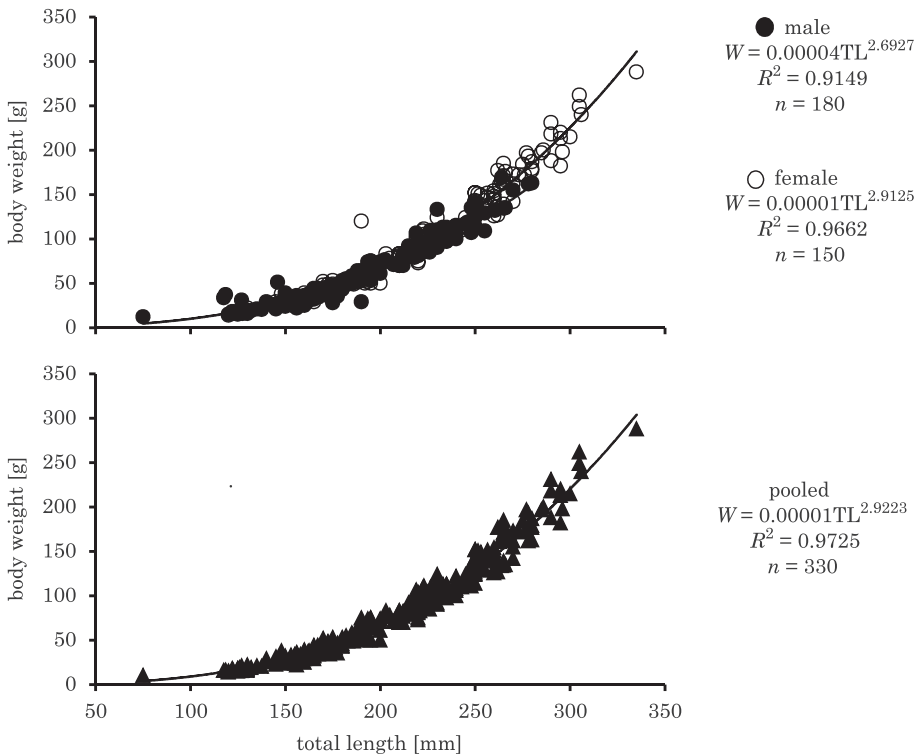


Fig. 2. The relative growth curves for snakehead sampled from Sungai Batang River, displaying a negative allometric growth pattern. The b exponent values obtained were lower than the cubic value (b < 3) across the sampling periods

equations are within the range of 2.6927 for male and 2.9125 for female, with the coefficient of determination (R^2) values ranged from 0.9149 to 0.9662, indicating that > 90% of variability of the weight is explained by the length. The index of correlation (r) of male and female were 0.9565 and 0.9829, found to be higher than 0.5, showing the length weight relationship is positively correlated. Regardless the sex, the pooled b value obtained was 2.9223 with $R^2 = 0.9725$ confirming fish grew allometrically. There were significant differences in total length and total weight between male and female. The body sizes of female were longer and heavier than male ($P < 0.001$). Figure 3 shows the mean ratio of body weight to total length of female was also considerably higher than that of male ($P < 0.001$), but no statistical significant difference in the mean ratio of body width to total length between them was observed ($P > 0.05$), as shown in Figure 4 .

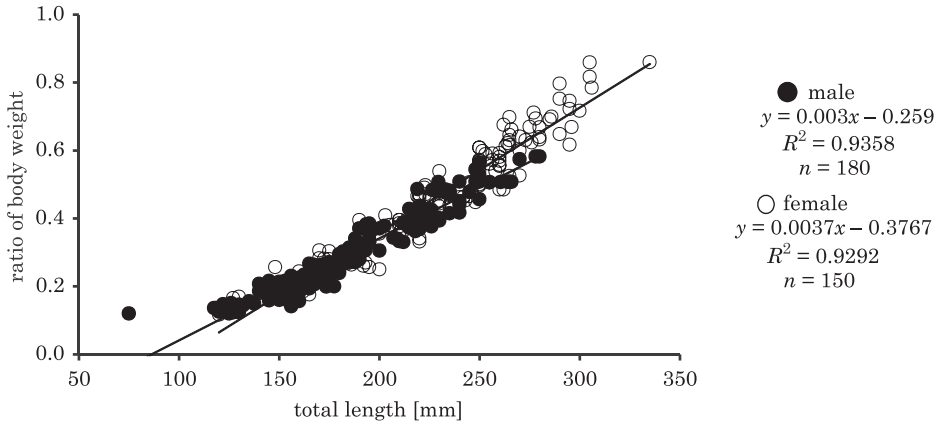


Fig. 3. The mean ratio of body weight to total length of snakehead from Sungai Batang River

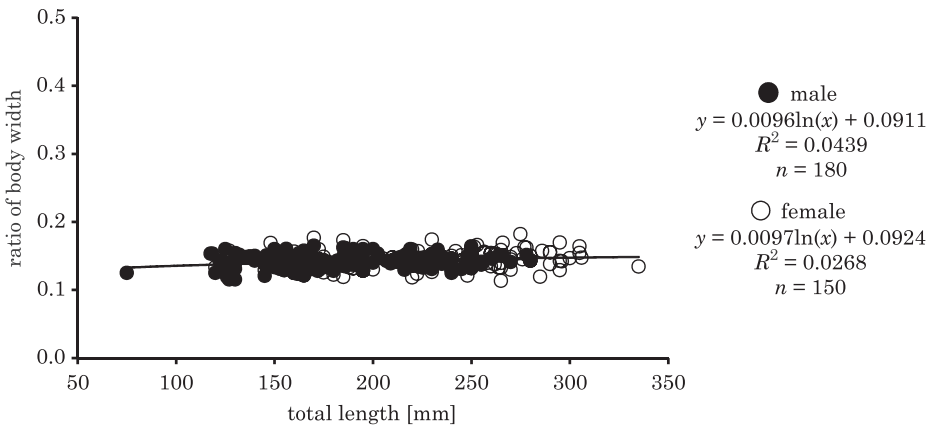


Fig. 4. The mean ratio of body width to total length of snakehead from Sungai Batang River

Table 2
Comparative length-weight relationships and factor conditions of family Channidae from different geographical areas

Species	<i>n</i>	Ratio of W/TL	<i>a</i>	<i>b</i>	<i>R</i> ²	Allometric pattern	Average <i>K</i>	Locations	Country	References
<i>Chanua striata</i>	330	0.859	0.0000	2.922	0.972	A-	0.842	Sungai Batang	Indonesia	Present study
<i>C. striata</i>	144	0.372	0.0140	2.812	0.952	A-	0.839	Sekayu swamp	Indonesia	MUTHAMINAH (2013)
<i>C. striata</i>	140	0.605	0.0020	2.929	0.970	A-	-	River Ganga	India	KHAN et al. (2011)
<i>C. striata</i>	60	0.353	-	1.612	-	A-	0.906	River Siang	India	DAS et al. (2015)
<i>C. striata</i>	1108	1.340	0.0170	2.890	0.972	A-	-	Agusan Marsh	Philippines	JUMAWAN and SERONAY (2017)
<i>C. striatus</i>	451	-	0.0110	2.944	-	A-	-	Chi River	Thailand	SATRAWAHA and PILASAMORN (2009)
<i>C. punctata</i>	1940	-	-1.7140	2.861	0.796	A-	-	Western Ghats River	India	HANIFFA et al. (2006)
<i>C. punctatus</i>	140	-	-2.0000	3.010	0.952	I	-	Gomti River	India	KASHYAP et al. (2014)
<i>C. marulius</i>	100	4.092	0.0210	2.747	0.866	A-	-	Harike Wetland	India	DUA and KUMAR (2006)
<i>C. marulius</i>	32	1.159	1.1670	1.450	0.935	A-	-	Godavari River	India	RATHOD et al. (2011)
<i>C. diplogramma</i>	67	-	1.0330	1.284	0.873	A-	-	Lake Vembanad	India	ALI et al. (2013)
<i>C. limbata</i>	346	0.145	0.2060	1.850	0.900	A-	1.510	Ta Bo - Huai Yai Wildlife Sanctuary	Thailand	KHOMSAB and WAN-NASRI (2017)
<i>C. obscura</i>	49	0.559	0.0160	2.663	0.706	A-	5.230	Ologe Lagoon	Nigeria	KUMOLU-JOHNSON and NDIMELLE (2010)
<i>Parachanna obscura</i>	408	0.143	0.0186	2.697	0.755	A-	-	Enyong Creek	Nigeria	BOLAJI et al. (2011)
<i>P. obscura</i>	85	1.198	0.0040	3.208	0.917	A+	-	Buyo reservoir	West Africa	TAH et al. (2012)
<i>C. striatus</i>	89	0.720	3.2800	3.685	0.933	A+	-	Uttar Pradesh	India	DAYAL et al. (2012)
<i>C. punctata</i>	150	0.103	0.0060	3.579	0.810	A+	1.193	Fish farm	India	DATTA et al. (2013)
<i>C. punctatus</i>	127	0.249	0.006	3.156	0.953	A+	1.722	Gomti River	India	SINGH and SERAJUDDIN (2017)

Explanation: *n* – number of fish samples; *W* – weight [g]; *TL* – total length [mm]; *a* – constant; *b* – exponent; *R*² – coefficient of determination; A- – negative allometric; A+ – positive allometric; I – isometric, and *K* – condition factor

The length-weight relationship and its parameters (a and b) have a wide application in fish biology and fisheries management. In fish, the weight is considered to be a function of length (WEATHERLEY and GILL 1987), while the fish length, according to GHORBANI et al. (2012), is the best indicator of production efficiency. A negative allometric growth pattern in the present study has also been reported for other family Channidae including *C. striata*, *C. diplogramma*, *C. punctata* and *C. marulius* from different geographical areas (Table 2). According to VICENTIN et al. (2012), fish with b value less than 3 consumed more of its energy in axial growth rather than weight. Our finding was contrary to *C. striatus* from Uttar Pradesh, India (DAYAL et al. 2012); *C. punctata* from fish farm and Gomti River, India (DATTA et al. 2013, SINGH and SERAJUDDIN 2017), and *Parachanna obscura* from Buyo reservoir, West Africa (TAH et al., 2012), in which exhibited positive allometric growth pattern ($b > 3$). Meanwhile, *C. punctatus* collected from Gomti River, India was reported to have isometric growth pattern (KASHYAP et al. 2014). Considering the value of the

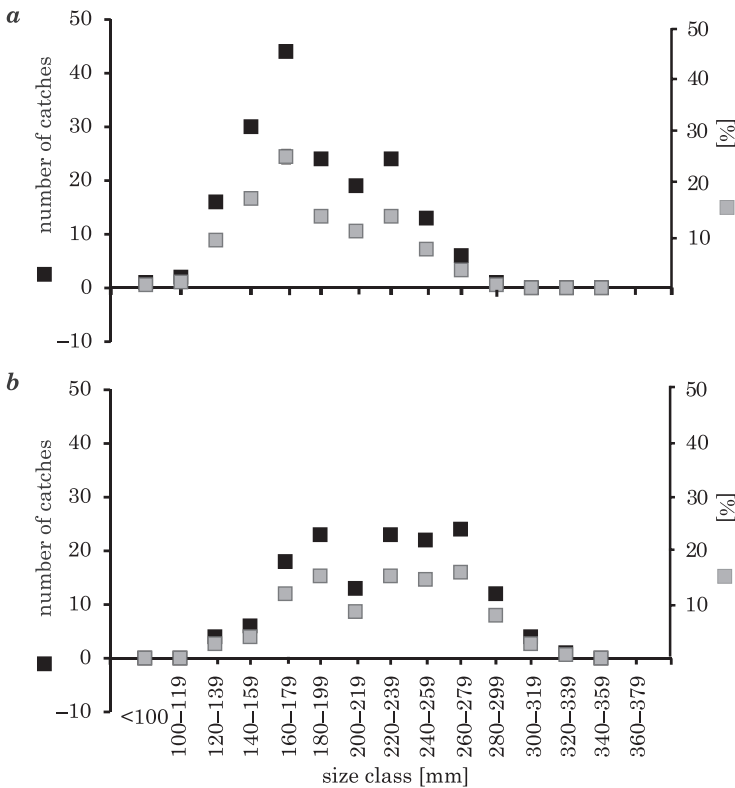


Fig. 5. Total length size distribution of snakehead (20-mm amplitude): a – male; b – female

b coefficient and its significant differences for males and females in the future, one should think about examining the differences in the condition coefficient separately, dividing the fish not only in terms of sex but also in terms of size (sexual maturity), because with the increase in length, differences in the coefficient of fitness between the sexes should vary. Variation in slope may also be attributed to life stages and environmental factors such as food and space (KLEANTHIDS et al. 1999, ABOWEI et al. 2009).

Figure 5 shows the total length size distribution of *C. striata* samples. The highest number of catch was found between 160 and 179 mm TL (24.44%) for male and between 260 and 279 mm TL (16.00%) for female. Meanwhile, the heaviest catches of male (33.89%) and female (14.00%) were recorded between 30 and 49 g (Figure 6). Regardless the sex, we observed that there was a difference in the exponent for smaller length class as compared to larger ones indicating that the species has two growth levels. The smaller individuals (75–140 mm TL) grew with the exponent significantly smaller than the cubic value ($W = 0.2641 \text{ TL}^{0.8916}$,

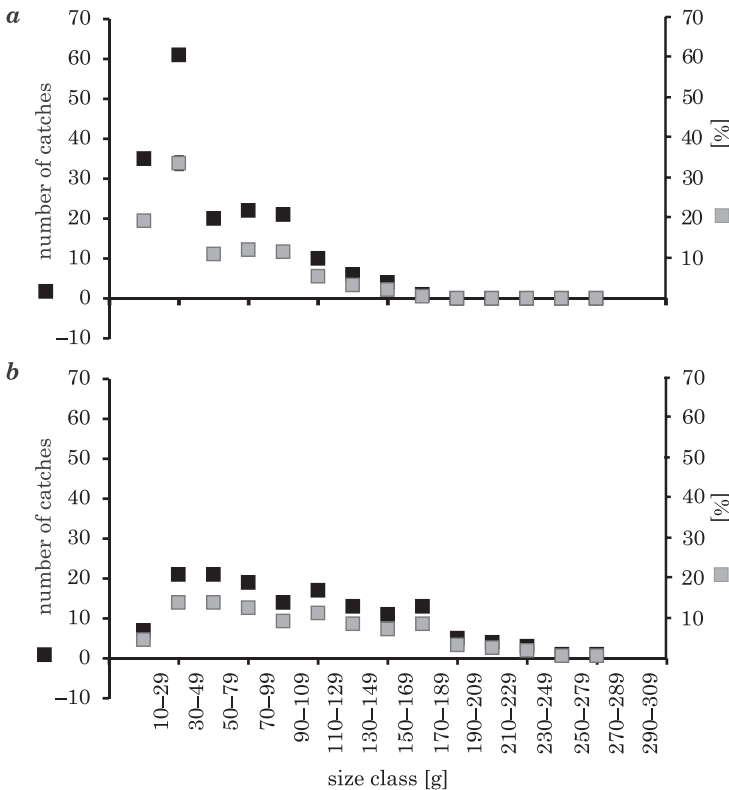


Fig. 6. The weight size distribution snakehead (20-g amplitude): a – male; b – female

$R^2 = 0.1391$). When the length class was extended to be 145 mm TL, we found an exponent of 1.0782, which was slightly higher than the cubic value. Meanwhile, the larger individuals (> 145 mm TL) grew with exponents significantly higher than the cubic value ($W = 8 \cdot 10^{-6} \text{ TL}^{3.0092}$, $R^2 = 0.9583$). RATHOD et al. (2011) observed *C. marulius* from Godavari River, India at the higher length class of 220–380 mm, and found the exponent of 1.45. It meant that such ontogenic variations in the cubic law differ from other fish species of the family Channidae; however, a more detailed analysis regarding the reasons contributing to such variations is necessary. The ratio of body weight to total length of *C. striata* in the present study was more or less similar to those of other snakehead species including *C. striata*, *C. obscura*, *C. punctatus* and *C. limbata* from some other countries (Table 2).

Figure 7 clearly demonstrates that the mean condition factor (K) value of female was significantly higher than that of male ($P < 0.001$). The mean K values obtained were 0.83 ± 0.09 (0.461–1.057) for male and 0.86 ± 0.08 (0.625–1.095) for female. *C. striata* in the present study are also commonly found in the fish species of family Channidae from different geographical areas (see Table 2). BARNHAM and BAXTER (1998) suggested that if the K value is 1.00, the condition of the fish is poor, long and thin. A 1.20 value of K indicates that the fish is of moderate condition and acceptable to many anglers. A good and well-proportioned fish would have a K value that is approximately 1.40. The present K value was found to be less than unity, according to NASH et al. (2006), *C. striata* in this river was unfavorable condition. Variation in the value of the mean K may be attributed to biological interaction involving intraspecific competition for food and space (ARIMORO and MEYE 2007) between species including sex, stages of

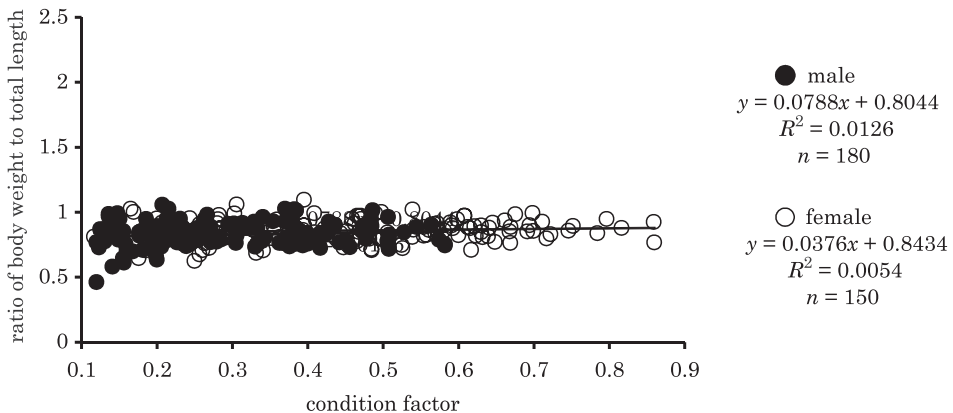


Fig. 7. The relationship between the mean ratio of body weight to total length and condition factor of snakehead from Sungai Batang River

maturity, state of stomach contents and availability of food (SAIKIA et al. 2012, WIDODO et al. 2013, ISTIYANTO and DIANA 2016). Information on condition factor of fish is considerably needed for aquaculture system management particularly to understand specific condition and healthy of fish being cultured. When the fish becomes leaner as the length increases, the manager or fish farmer should take management strategies, for example, by improving the quality of feed contents and its feeding ratio, and rearranging fish density to reduce competition for food and space.

In the investigated area, snakehead has high commercial value and high pressure on population caused by fishing. Local fishermen mostly used stage-line (*banjur*), fish trap (*lukah*) and also electrofishing to collect them and this is on-going throughout the year regardless of seasonal periods. The acquisition of fish from fishermen is associated with fishing selectivity and the preferences of the fishermen themselves. Often, larger individuals do not reach scientists because of their market value. The use of *banjur* is much better than *lukah* or electrofishing in term of gear selectivity because it only captures the larger fish, but the catch is usually no longer survive due to the remain of hook inside their mouths. *Lukah* is considered less selective gear because of having narrow bamboo-lath in its gear construction, resulted in small fish difficult to escape from the trap. In the future, it would be worth considering whether to use, for example, fishing with the use of electricity. Such catches are, firstly, non-selective and, secondly, they allow carrying out this kind of research in an experiential way. In wild sources, snakehead species are being caught by using different fishing gears, for instance, hooks and gillnet in Harike wetlands, India (DUA and KUMAR 2014), cast net and drag nets in Gomti River and Ganga Basin, India (KASHYAP et al. 2014, DAS et al. 2015), fish pot and cast net in Danau Bangkai (IRHAMSYAH et al. 2017). In the Philippines, *C. striata* caught by using improvised purse seine (*lambat*), cast net (*pukot*), gill net (*laya*), improvised fyke net trap (*bantak*), and electrofishing (JUMAWAN and SERONAY 2017). All these studies outlined above describing only on the length-weight relationships of snakehead species that are collected using various fishing gears without considering the detailed composition of fish lengths by each typical gear used. Therefore, it is necessary to use different fishing gears to determine the composition of fish lengths that will be analyzed and compare the results of work with other work done on the species, particularly on the basis of catch selectivity and typical growth dimensions of relative well-being. In addition, we observed in the field that there was no records for snakehead catch in quantity (e.g. number, length and weight) because fishermen or their family members directly sold fish to trader or consumer in some places,

resulted in the ratio of fish exploitation rate to fish growth rate in this river is unpredictable. It is a great challenge for Fisheries Services of Banjar District to improve the quality of inland fishery statistic data for some species of commercial importance including snakehead fishery, and our results provide the first reference on the length-weight relationship of this species. The present length-weight key for snakehead could be used as a useful tool for more effective management of this species since catch tends to decline. This key can facilitate fisheries biologist or researcher to derive weight estimates for un-weighted but measured fish, and help them in assessing the biomass of fish captured in this river.

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**PARALLEL DEVELOPMENT OF GREEN
INFRASTRUCTURE AND SUSTAINABLE TOURISM –
CASE STUDIES FROM HUNGARY**

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Key words: green infrastructure, sustainable tourism, micro-region of Balatonfüred, Csorna and Gönc.

Abstract

In our research we explored the relation of green infrastructure and sustainable tourism development. We hypothesized that the concept of green infrastructure (GI) could be a catalyst for the development of different types of tourism products such as ecotourism and active tourism. Our objective were to analyse the green infrastructure, tourism attractions, and tourism infrastructure; to define the relation between green infrastructure and tourism; to explore the common development possibilities.

In the frames of our project we analysed three pilot areas of different tourist characters. We defined those GI elements which have the most significant development potential for tourism products (eg. hiking trails, open spaces, green spaces of public institutions, lakesides, river banks). Based on the comparison analysis of attraction types and GI assets we defined those elements which can be the base of complex development. These can be public parks, alleys which can increase the value of tourism attractions such as castles, museums or programs. Our results highlighted a specific group of GI elements, the linear GI elements. So the development of linear GI elements is important from touristic, aesthetic and ecologic point of view as well.

RÓWNOLEGLY ROZWÓJ ZIELONEJ INFRASTRUKTURY I ZRÓWNOWAŻONEJ TURYSTYKI NA WĘGRZECH – STUDIA PRZYPADKÓW

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Słowa kluczowe: zielona infrastruktura, turystyka zrównoważona, mikro-region Balatonfüred, Csorna i Gönc.

Abstrakt

Badania dotyczyły związku zielonej infrastruktury i zrównoważonego rozwoju turystyki. Postawiono hipotezę, że koncepcja zielonej infrastruktury (*green infrastructure* GI) może być katalizatorem rozwoju różnego rodzaju produktów turystycznych związanych z ekoturystyką i turystyką aktywną. Celem badań była analiza zielonej infrastruktury, atrakcji turystycznych i infrastruktury turystycznej; określenie związku między zieloną infrastrukturą a turystyką oraz zbadanie możliwości ich wspólnego rozwoju.

W ramach przedstawionego projektu przeanalizowano trzy obszary pilotażowe o różnych uwarunkowaniach dotyczących rozwoju turystyki. Zdefiniowano te elementy GI, które mają najbardziej znaczący potencjał dla rozwoju produktów turystycznych (np. szlaki turystyczne, przestrzenie otwarte, tereny zielone instytucji publicznych, jeziora, brzegi rzek). Na podstawie analizy porównawczej typów atrakcji i zasobów zielonej infrastruktury zdefiniowano te elementy, które mogą być podstawą kompleksowego rozwoju. Mogą to być parki publiczne i aleje, które mogą podnieść wartość atrakcji turystycznych takich jak zamki, muzea lub imprezy. Wyniki badań podkreśliły znaczenie linearnych elementów GI. Rozwój linearnych elementów GI jest ważny zarówno z punktu widzenia turystycznego, estetycznego, jak i ekologicznego.

Introduction

Since the 1980's tourism experts has recognized the importance of environmental issues in order to realize successful tourism development projects (DOWLING and FENNELL 2003). Based on the term of sustainable development (1984 Brundtland report) the concept of sustainable tourism was evolved (*Közös Jövőnk* 1988), and was mentioned firstly in "The Holiday Makers" written by Jost Krippnerdorf in 1987 (PUCZKÓ et al. 1997). Later several similar definitions were elaborated but the concept of The United Nations World Tourism Organization in 1998 can be considered the most significant: "Sustainable tourism development meets the needs of present tourists and host regions while protecting and enhancing opportu-

nity for the future. Sustainable tourism should make optimal use of environmental resources while meeting the economic, social and aesthetic needs of society and maintaining essential ecological processes and helping to conserve natural heritage and biodiversity furthermore respecting the socio-cultural authenticity of communities” (*Saturation of tourist...* 1998).

There have been several attempts to define the major factors of sustainability in tourism development (LEE and JAN 2019, HIGGINS-DESBIOLLES 2018, LANE 2018). A survey from 2005 revealed (VARGÁNÉ CSOBÁN 2010) that according to the majority of experts sustainable tourism should preserve environmental-ecologic, social-economic resources, requires long term approach and regulation and monitoring during realization. Above all these experts highlighted the fact that local (host) population shall recognize the benefits of sustainable tourism (PUCZKÓ et al. 1997, KIPER 2013). Parallel to the term of sustainable tourism the concept of eco-tourism was elaborated even though nature-based tourism existed in ancient times as well (KELEMEN 2006, CHEIA 2013). Just in the 1980’s became the term widespread, which combines tourism forms based on natural and cultural attractions and principles of alternative tourism (LENGYEL 1997). In recent decades, the content of ecotourism has been changed (BRANDFUL COBBINAH et al. 2015, DAS and CHATTERJEE 2015). This process was highly influenced by the World Ecotourism Summit in Canada, 2002 where the “Quebec declaration” was adapted about responsibilities, tasks, methods related to ecotourism development for the state, NGO’s, private sector, local communities and other stakeholders (*World ecotourism...* 2002). Since the beginning of 1980’s several terms have been evolved, however there is still no generally accepted definition (FENNELL 1999, DOMBAY et al. 2008, CHEIA 2013). The World Conservation Union (IUCN) elaborated the most commonly used interpretation which was also applied in the Hungarian Ecotourism Development Strategy 2008 (*Országos ökoturizmus...* 2008). According to the IUCN definition ecotourism is “environmentally responsible travel to natural areas, in order to enjoy and appreciate nature (and accompanying cultural features, both past and present) that promote conservation, have a low visitor impact and provide for beneficially active socio-economic involvement of local peoples” (CEBALLOS-LASCURIÁN 1996).

VARGÁNÉ CSOBÁN (2010) collected a wide range of the existing tourism types, products related to natural environment: green tourism, soft tourism, alternative tourism, responsible tourism. In all these tourism products the responsible, sustainable use and preservation of natural assets are highly important (VASVARI et al. 2015). That’s why our research

is focusing on a recent concept of green infrastructure which could serve long term sustainability. The term of green infrastructure (GI) became widespread all over the world especially in relation to urban planning (initiative of green cities). Because of the complexity and multifunctionality of GI it can be applied in regional and rural development as well. While usually the traditional, grey infrastructure has one single function, the green infrastructure systems can meet many different needs (ELY and PITMAN 2014). That is why GI can serve objectives of nature protection, rural development as well as sustainable tourism development. Several terms and definitions exist for green infrastructure but usually the most widespread is the term elaborated by BENEDICT and MCMAHON (2001) in their book *Green Infrastructure* as “a strategically planned and managed network of wilderness, parks, greenways, conservation easements, and working lands with conservation value that supports native species, maintains natural ecological processes, sustains air and water resources, and contributes to the health and quality of life for America’s communities and people”. There are many ways to group GI elements (CIVIC and SIUTA 2014).

The main objectives of GI development are: development of the network, improving the carrying capacity of the landscape, elaboration of multifunctional zones. The spatial connections, networks can be strengthened by elaboration of hedges, preservation of natural field margins. The carrying capacity of the landscape can be improved by wildlife-friendly land uses and application of agri-environmental farming methods. Multifunctional zones support multiple land uses or activities such as agricultural production, forestry, recreation, nature protection (EC 2010). The European Union intends to integrate the concept of green infrastructure into several policy fields, strategies such as *Biodiversity Strategy 2020* (COM (2011) 244 final), *Roadmap to a Resource Efficient Europe* (COM (2011) 571), *Proposal on specific provisions concerning the European Regional Development Fund and the Investment for growth and jobs goal* (COM (2011) 612 final/2), *the CAP towards 2020: Meeting the food, natural resources and territorial challenges of the future* (COM (2010) 672 final), *new Forest Strategy* (COM (2013) 659 final) (this is extremely important as several GI elements relate to forest patches, alleys, forest belts). *The Biodiversity Strategy* sets the following targets among others by 2020: ecosystems and their services are maintained and enhanced by establishing green infrastructure and restoring at least 15% of degraded ecosystems.

The above described researches, papers, strategies highlight the role of GI in environmental, nature, landscape protection, and recreational possibilities furthermore healthcare but according to our opinion the

tourism related use connected to recreation is also highly important and relevant. So in our research project we integrated the GI concept into tourism and analyzed the role of the GI assets in tourism development. Our research objectives were:

- define the GI development potential and needs of the study areas,
- explore the tourism potential of the study areas,
- define the matching points of GI planning and tourism,
- highlight those GI assets which contribute to tourism development in the study areas,
- define general regularities between green infrastructure and tourism in study areas with different potentials.

Materials and Methods

We have chosen study areas of different conditions: micro-region of Gönc with partly well-developed tourism infrastructure, micro-region of Csorna, the mostly agricultural landscape with eco- and rural tourism potential and the highly popular micro-region of Balatonfüred. The pilot

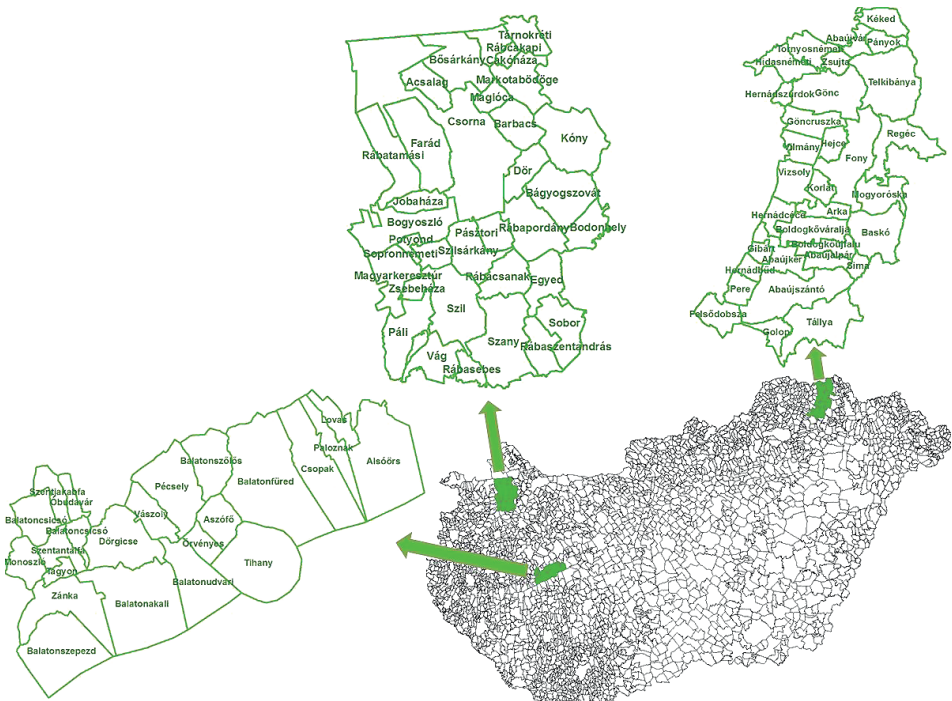


Fig. 1. Study areas: micro-region of Balatonfüred, Csorna and Gönc

regions have different natural and social conditions but all are rural areas and consists of mostly villages (settlements with less than 1000 inhabitants) – Figure 1.

Micro-region of Balatonfüred is located in county Veszprém, and consists of the most popular settlements of Lake Balaton Recreational Area – such as Tihany – totally 22 settlements with city of Balatonfüred (Figure 2). It is a highly diverse, mosaic-like landscape with valleys, diverse settlement system located at the merger line of Transdanubian Mountain Range and Transdanubian Hills. The settlements on the shoreline of Balaton often referred as Balaton-Riviera are especially tourism oriented, but the economic base of villages in the neighbouring hilly landscape is agriculture especially vine and fruit production. The proportion of nature protection areas is really high: the Balaton Uplands National Park, several Natura2000 areas, European Diploma area are located here. The majority of the region is also protected as landscape scenery protection zone in the National Land Use Framework Plan.

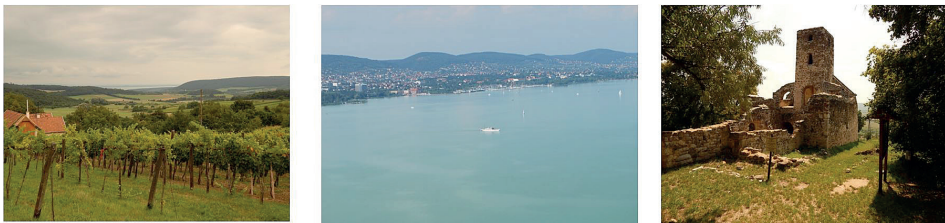


Fig. 2. Micro-region of Balatonfüred

Micro-region Csorna is situated in Western-Hungarian Region, between Győr and Sopron, the two economic centers of county Győr–Moson–Sopron (Figure 3). In spite of the fact that it is situated in one of the most developed regions of Hungary, our study area is considered as an inner periphery.



Fig. 3. Micro-region of Csorna

The micro-region consists of three landscape units: Rábaköz, Hanság and Tóköz. The centre of the micro-region, Csorna is located at the crossing of two important arteries of the region, M85 and M86. The micro-region is

a rural landscape, dominated by small villages (30% of settlements have less than 1000 inhabitants, and 50% have less than 500 inhabitants). The remnants of the former vast wetlands and marshes, meadows and alluvial forests of Hanság have high ecologic value, protected by National Park Fertő-Hanság and Natura 2000 areas. Unfortunately due to the intensive drainage works just small proportion of the former wetlands remained, but thanks to the recent landscape rehabilitation projects there are two major wetlands with large open water surfaces (Nyirkai-Hany, Oslí-Hany). Tóköz is the continuation of the marshlands and waters of Hanság wedged into the cultivated landscape of Rábaköz. Since centuries Rábaköz has been an intensively cultivated agricultural landscape with maximum 9% forest cover, where mostly the vegetation along the watercourses, riparian forests of river Rába and the meadows are of high ecologic value.

Micro-region of Gönc is one of the most disadvantaged areas of Hungary located in North-Hungarian Region, in county (Figure 4) Borsod-Abaúj-Zemplén. From its 32 settlements two are towns (Gönc és Abaújszántó), 19 settlements have less than 1000 inhabitants. All the most important social and economic indicators show the unfavourable situation of the micro-region: in 2012 the unemployment rate is 21,8% (national average 9%), total income per capita 480 502 Ft (national average 810 000 Ft).



Fig. 4. Micro-region of Gönc

In contrast to the social-economic situation Gönc is rich in natural, cultural heritage. The study area lies along the Hungarian-Slovakian border. The Eastern-Southern Slovakian regional centre, Košice with its 300 000 population has a remarkable influence on the neighbouring Hungarian areas. The recent connections have traditions since previously the region was called as “pantry of Košice”. The main transportation corridor of the region is stretching parallel to river Hernád (road Nr 3.). The majority of the smaller settlements are dead end villages.

Our research project consisted of four phases:

1. We elaborated databases of natural and cultural heritage, tourism infrastructure and elements of green infrastructure in all pilot regions.

2. Based on the collected data we carried out assessments in order to compare the pilot regions.

3. We carried out comparison analysis to reveal the relation between GI elements and tourism product types.

4. Finally the development potential of green infrastructure and tourism were analysed.

We listed and categorized the GI elements of the study areas. Various definitions and groupings of GI elements exist. A common approach is that the GI network consists of natural and semi-natural areas designed and managed to deliver a wide range of ecosystem services. It includes green spaces in rural and urban settings and it can be considered in local and regional scale as well. From the point of view of tourism the accessibility is highly important so unlike the majority of theories we distinguished major groups of GI elements according to their availability for the public. We considered freely available GI elements all kinds of natural, semi-natural areas in the surrounding of built environment: forests, grassland, agricultural areas including nature protection areas furthermore the open spaces, public parks, gardens of open access in cities, towns, and villages. GI elements of restricted access are mostly the gardens of institutional facilities such as resorts, health centers, ecclesiastical or other institutions. We considered GI elements not available for the public those strictly protected areas where because of the endangered habitats or species national authorities declared nature reserves.

We used various data sources, maps for our research (Hungarian Central Statistical Office, TÉKA database, Hungarian Database of Spatial Planning, Hungarian Nature Protection Database) furthermore we integrated our experiences of field trips, interviews.

Results and Discussion

Green infrastructure of the pilot regions

We grouped the green infrastructure assets according to the following categories: GI elements of open access, restricted access, private (no public access). We assessed quality/state, size/extent, distribution/location, connection/network in case of all GI elements in the three study areas. The results of the assessment is shown in Table 1, the short summary of the results about the other micro-regions is included in the study.

Table 1
Assessment of GI network of micro-region of Gönc

Types of GI (according to availability)	Quality/ state	Size/ extension	Distribution/ location	Connection/ network
Freely available	The GI elements of the region generally are in good shape (Zemplén Landscape Protection Area is outstanding). In case of natural values of smaller extent illegal waste disposal (streets and valleys) and gravel mining (riparian forests) present a risk. In several settlements (Gönc, Abaujvári), the quality of open space is not appropriate. Meanwhile many settlement centres were recently reconstructed with EU co-financing (Pányok, Golop). The water quality of Hernád is good, the factories in Könc pose the most important risk.	The forests of Zemplén-mountains are of largest extent. There are fruit gardens and meadows also of remarkable extent. Mostly other GI elements are of linear character.	The distribution of GI elements in the region is of dual character. The GI elements of the largest extension and significance are situated in the Eastern part of the region (Forests of Zemplén), and along river Hernád (riparian forests). And in-between there are just GI elements in small patches (lakes, grass fields).	The problems of the network erect from the location of the main GI elements, between the core areas of ecologic network (Zemplén Landscape Protection Area and river Hernád) just smaller valleys provide connection. In this area arable land dominates the landscape. In the small settlements this doesn't cause problems as the GI elements directly frame them.
Restricted availability	Several mansion gardens serve as institutional open spaces (Abaujkér, Kékéd). These gardens have not just environmental but dendro-logic and historic values as well. The state of gardens in private property are of appropriate (Melzer Palace Hotel Kékéd), while the gardens of public institutions need to be reconstructed (Abaujkér). The environment of hotels, camps is usually in good conditions.	these GI assets are usually point-like situated in the built areas	the GI elements of restricted availability are situated usually in the built areas of the settlements distributed evenly in the region	these GI elements are point-like so they do not create a network but many of them (caste gardens) are located on the peripheries of the built up areas so they are connected to the freely available GI assets (forests)
Not available for the public	The strictly protected areas which belong to the most pristine areas of the country are in outstanding condition in the core of Zemplén Landscape Protection Area	the most strictly protected areas cover approx. 10% of Zemplén Landscape Protection Area (more than 2000 ha), partly located in the study region	the strictly protected areas are located in the inner parts of the mountain in larger blocks	the strictly protected areas not available for the public are located in larger blocks but directly connected with other GI elements (e.g. forests of Zemplén Landscape Protection Area)

The majority of open access GI elements are forest patches in micro-region of Balatonfüred, which are mostly in good state, but the ratio of adventive species is high. Forests of Csicsó and Balatonfüred are ecologically valuable with old, matured trees. The traditional land use forms of Balaton Uplands are represented by vineyards and fruitgardens. There are many new plantations at the foothills, but we can see many abandoned vineyards on the steep slopes. There is a strong process of afforestation parallel to disappearing the traditional meadows. There are valuable wetlands close to the lake and on the top of the former volcanos and along the shoreline vast contiguous reedbeds (*Natura* 2000) provide habitat for rich wildlife. The majority of watercourses are temporary without natural vegetation. The GI elements in the settlements are well maintained and diverse. Several settlements lie on foothills with wonderful panorama on the well kept cemeteries, calvaries and chapels. The shoreline settlements are oversized, overgrown urban areas, suited for the crowded summer season while the hillside villages have preserved their medieval structure. The study area has really varied geographic conditions with forested hills, vineyards, fruitgardens on the slopes of the mountains and larger residential and recreational areas stretching on the plain landscape along the lake.

The GI elements of restricted access: the gardens of resorts, health centers, ecclesiastical or other institutional facilities are well kept. The open spaces of the former abandoned summer camps are deteriorated. The sportfields are well kept but their environment is usually untidy without ecologic values. On Tihany-peninsula there are strictly protected natural areas.

The open access GI elements in micro-region of Csorna are usually well kept but sometimes reconstruction is necessary, and the majority of the small villages do not have any public open spaces. The illegal waste disposal is a major threat on open spaces. Gravel mining is a common phenomenon in the region, unfortunately the abandoned mines (mostly lakes) are not suitable for recreational use. Recently many village centers have been reconstructed co-funded by the European Union. The water quality of river Rába is good, but the riverside is very often not suitable for recreational use. The riparian forests of Rába are of great ecologic and aesthetic value. The distribution of GI elements is characterized by a spectacular duality in the pilot region. The most valuable natural areas are located in the North (Hanság) which is protected under National Park Fertő-Hanság and the Southern line of riparian forests of river Rába. In-between there is the intensively cultivated landscape of Rábaköz with really small patches of meadows, forests of high ecologic value. Highly important corridors are between the core areas the small watercourses.

The gardens of restricted access of different institutions are situated mostly in the centre of the micro-region, in Csorna and a few larger settlements. There are several valuable castles in the small villages, but these are very often abandoned with deteriorated gardens. The church gardens could be important element of the open space system of small villages but they are very often closed for the public. The institutional facilities of the centre of the micro-region are usually well kept. Vast strictly protected areas of National Park Fertő-Hanság (mostly in Tóköz) are not accessible for the public. The strictly protected lake Barbacs need reconstruction. The recently created vast wetlands by the landscape rehabilitation projects (Osli-Hany, Nyirkai-Hany) could be important attractions for eco-tourism.

Tourism attractions of pilot regions

There are many possibilities for grouping tourism attractions. We distinguished three major categories: built or artificial values, natural values, and cultural programs. In all cases we assessed: quality/state, size/extension, distribution/location, connection/network, and significance/recognition. We carried out the assessment in all study areas and summarized the results in tables (see the results of micro-region of Balatonfüred in Table 2, the short summaries of the other pilot-regions are included in the study).

There are remarkable differences in the state of built heritage in micro-region of Csorna. A lot of values are in really deteriorated state (especially the smaller castles, mansions, and some traditional farmhouses). The most significant attraction of the region, the Norbertine Abbey of Csorna is in a really good state but it has lower tourism potential. Almost all settlements can be characterized by less significant cultural values as rural churches, mansions. The most significant castle is situated in Rábabes.

The most important natural attractions are parts of Fertő-Hanság National Park open for the public as Nyirkai Hany, Exhibition of Eszterházy Madárvárta and study trail of Hany Istók, Fehértó, ornithological camp, and recreational potential of river Rába. The protected areas of Fertő-Hanság National Park represent the largest natural value of the region. It's parts are situated in several separated blocks in the Western-Southern part of the region. The valuable areas along river Rába represent smaller areas of linear character. The natural values are concentrated and there is no proper connection between them, a matured network is missing. Considering tourism potential, the problem is, that the most attractive, popular parts of Fertő-Hanság National Park are situated around Lake Fertő.

Table 2

Assessment of tourism attractions in micro-region Balatonfüred

Attractions	Quality/state	Size/extension	Distribution/location	Connection/network	Significance
Built environment, architectural values	Balaton Uplands has been settled since pre-historic times (ancient paint mine in Lovas) and there are wide range of historic values in the region from ancient times such as religious (churches from the Arpadian ages, calvarias, stone crosses), military (castle Zádor), residential (Török-house), industrial (mill), agricultural (Granary), transportation (stone bridge) facilities. In almost all villages we can find values in good state. Many values (cellars, church ruins from Arpadian age) on private property are deteriorating. There are significant folk architecture, these traditional houses generally are in good shape. A lot of attractions are related to active tourism: modern golf course, thematic park, marina, beaches, and amusement parks.	There are several point-like attractions and others of larger extent such as castle gardens (castle Sebestyén Balatonszepezd), calvarias, public parks (Szentjakabfa-Kutaszó) amusement parks. Interesting areas are the environment of 'walkers' related to traditional lifestyles (Óbudavár). There are entire settlements or city districts representing value in themselves (Paloznak, centre of Balatonakali or Balatonfüred downtown).	In all of the settlements national or cultural heritage elements can be found. Attractions of modern-day are concentrated mostly in shoreline settlements. Wine cellars serve guests in shoreline and background settlements as well.	The wine routes have been developed into networks connecting mostly 2-5 settlements but there are only one settlement wine routes as well. Marinas provide connection with all marinas of the lake providing an inner hierarchy for the sailor society different from administrative units. The micro-region is part of Balaton Uplands influencing the settlements in focus.	The attractions of Tihany and Balatonfüred are of international significance and relevance. With the growing importance of gastro and wine tourism the attractiveness of the background settlements, the small villages of the hillside landscapes is growing as well.

<p>Natural heritage</p>	<p>Divers, hilly landscape, lake, climate are attractive for tourism. On the hillsides and hilltops there is always a great panorama, there are lot lookout towers which are mostly in good shape. Unfortunately we can see untidy areas with garbage and invasive species. One of the most important factor of the wonderful landscape are wine production, wine yards and cellars. Wine production developed remarkably recently. In all settlements there are always a few abandoned wine yards.</p>	<p>The National Park covers the entire hilly landscape, the shoreline reed beds are Natura 2000 areas, the wine yards are mostly landscape scenery areas and even in the settlements there are protected values (springs, gardens, and plants).</p>	<p>the natural values are distributed evenly in the region</p>	<p>The geographic conditions influence the relations, the valleys and basins represent organic units for the society and visual connections as well. The Northern forests connect several settlements.</p>	<p>The cultural and natural values of the Balaton Uplands are intertwined, the region is internationally known and popular. The landscape represents the most important attraction for tourists.</p>
<p>Programs</p>	<p>In all settlements different cultural programs, festivals (wine, fishing festival, fairs, Balaton cross swimming) different competitions such as swimming, sailing and fishing competitions are organized. Several guided tours on study trails and thematic routes await guests. Horse riding has a growing popularity. The cultural centres offer wide range of events as well (dance, arts and crafts). Museums, libraries, open workshops (even in beaches) have divers programs.</p>	<p>the festivals which last several days attract a lot of people so the guests have the occasion to visit the region as well</p>	<p>the festivals are organized mostly in the shoreline settlements</p>	<p>several programs connect the shoreline and background settlements</p>	<p>The festivals are mostly of regional and national significance. The other programs mostly try to balance the effects of the changes of the weather and keeps guests occupied or busy in bad weather. The cultural events of small villages mostly serve local needs.</p>

The cultural programs of the smaller settlements have mostly local significance, the Fertő-Hanság National Park organizes programs as camps, guided tours, but the majority of them around Lake Fertő.

The state and significance of built values of micro-region of Gönc show great differences. Several values are in really poor condition (especially the smaller castles, mansions, traditional farmhouses) in some cases they are threatened by total destruction. Meanwhile the most significant values are in proper condition, several were recently reconstructed (castel of Regéc, Chapel of Saint Chaterine and Medieval Hospital). The state of cultural values often correlate with the geographic situation: the built values in the South, in the Tokaj Wine region are in good condition. The most significant built values are concentrated in the Southern part of the region or in some larger settlements (Gönc, Boldogkőváralja). There are values of national importance as Church of Vizsoly, Bible Museum of Gönc, castles of Boldogkővár and Regéc, built heritage related to wine production in Tállya and Abaújszántó). Built values of less significance as rural churches, mansions can be found in all settlements.

The natural values are in good conditions and well-kept. The largest nature protection area is Zempléni Landscape Protection Area situated in the Eastern part of the region covering approximately 30% of it's territory. The nature protection areas along river Hernád are of smaller extent. The natural values of the micro-region are concentrated (Zemplén Landscape Protection Area and river Hernád) and only smaller watercourses offer connection between them.

There are several cultural programs of national significance (Apricot Festival of Gönc, Medieval Tournaments of Castle Boldogkő and Country Fair of Tállya).

Tourism infrastructure of study areas

We grouped tourism infrastructure according to the following: accomodation, catering, tourist trails and cycling routes, tourism information system, accessibility and other facilities. We assessed quality/state, quantity, and distribution/location. The results of the assessment were presented in table form (as example see the results of pilot region Csorna in Table 3. and short summaries in the following about the other micro-regions).

In micro-region Balatonfüred all kinds of accommodations can be found. The former camps are of lower quality but there are apartmants, guest houses and luxury hotels meeting various needs. There are always more apartmants and hostels providing quality for reasonable price. Accommodations for larger number of guest are located in the shoreline settlements and rural tourism is characteristic for the background settlements.

Table 3
Assessment of tourism infrastructure of micro-region Csorna

Types of tourism infrastructure	Quality/state	Quantity	Distribution/location
Accommodation	there are just a few rural accommodation in the region mostly in Hanság and Tóköz; the state or quality of accommodations is appropriate, with reasonable prices; in Csorna there is a camping as well	low number of accommodations, in the majority of settlements there is no accommodation available at all	uneven distribution of available accommodations, especially just in settlements of Hanság, Tóköz
Catering	catering available mostly in settlements along roads Nr 85 and 86 (Bósárkány, Kóny) because of high transit traffic and in Csorna; in the small villages there are only pubs mostly not appropriate for tourists	low number of catering facilities	catering facilities are concentrated in major settlements and settlements along roads Nr 85, 86 similar to accommodations; there are no catering facilities in the majority of small villages
Hiking trails, cycling routes	there are just a few hiking trails in the region, in Tóköz and South Rábaköz, a hiking trail connects Lake Fertő with Western-Hanság; marked cycling routes lead mostly on roads not separated from traffic or on dirt roads	just a few hiking trails and cycling routes	there are just a few hiking trails; Recommended cycling routes are distributed evenly in the region
Tourism information system	marking of attractions is not sufficient; mostly settlements have homepage but not for tourists; there is no common information system	not relevant	not relevant
Accessibility	roads Nr 85 and 86 cross the micro-region and the newly constructed M85-M86; the minor roads are often of low quality; busy and modern rail lines also cross the region	generally good accessibility (roads, rail lines); the small villages have low public transport access	the transport infrastructure network is uneven; outstanding accessibility along roads Nr 85-86, low accessibility in the small villages
Other tourism facilities	there is no other relevant tourism facility in the region	not relevant	not relevant

Wine tourism is very popular in the region. In the shoreline settlements there are plenty of old canteens, the majority of them are reconstructed and offer modern food. Seasonality is very strong, many caterings close for the winter. In several background settlements there are no caterings at all but in many vineyards the hosts offer excellent hospitality for pre-booking guests.

There is the Balaton cycling ring road along the shoreline with trunk roads leading to the uplands as well. Dense network of hiking trails help to explore the region and there are thematic and study trails, and wine routes as well. Accessibility is good, the train connects the shoreline settlements although the trains are slow, the road network is dense. Public bus transportation is quite rare and mostly offers Northern-Southern connections. During summer season there are often traffic jams along the shoreline roads. Ships connect the shoreline settlements. Usually there are plenty of information, signs etc about the attractions and there are tourism offices especially in the shoreline settlements. There are other tourism facilities as well: yacht marinas, golf course at Balatonudvari, go-kart courses, riding schools, and numerous lookout towers. It is worth to mention the heart hospital and sanatorium in Balatonfüred.

In micro-region of Gönc we can find mostly rural accommodation of adequate quality and medium price level. In several settlements there are campsites or children's camps with lower comfort levels. In contrast, there are mainly higher quality and therefore more expensive accommodation in the southern settlements. The distribution of accommodation is uneven, they are concentrated especially in Tokaj Wine Region. In several small villages the only catering facilities are pubs which are not really suitable for tourists. Meanwhile there are several really unique restaurants or catering facilities as well such as mediaval restaurant in Boldokgővára, Bestillo Pálinka House, cellars of Tállya. These are also concentrated in the Southern settlements (eg. Telkibánya, Boldogkővára).

The micro-region has a dense, well marked hiking trail network, even the national Blue Trail, which connects the most beautiful landscapes of Hungary, crosses the region. The marked cycling routes are dangerous sometimes because they are not separated from the road traffic. The hiking trails mostly concentrated in the Eastern, Zemplén region meanwhile in Hernád-valley there are just really a few tourist trails. The distribution of marked cycling routes is also uneven. There are plenty of information about the settlements (websites), attractions but there is no common information system, the co-operation is missing. The road Nr 3. borders the region in the West offering good connection to other parts of the country, but the quality of minor roads is usually not satisfactory, some sections are really neglected, even dangerous. The railway line between Miskolc and Kosice is electrified, while the average age of trains and, buses is high.

Table 4

Relation between green infrastructure types and potential tourism products

Types of Tourism Products/ Main Types of GI	Active tourism	Rural and eco-tourism	Gastro- and wine tourism	Health tourism	Cultural tourism	Total
Public Parks	0	1	1	1	1	4
Sport fields	2	0	0	2	0	4
Alley	1	0	1	2	1	5
Green buffer along roads	2	2	0	0	1	5
Cycling routes and their environment	2	2	0	2	0	6
Study trails	2	2	0	0	1	5
Hiking trails	2	2	0	2	0	6
Institutional open spaces, gardens	0	1	2	2	2	7
Beaches	2	0	1	2	0	5
Lakes	2	2	0	2	0	6
Rivers, riversides	2	2	0	2	0	6
Stream valleys	2	2	0	1	0	5
Row of trees, forest belts	1	2	0	0	1	4
Forest patches	2	1	0	1	0	4
Forests	2	2	0	1	0	5
Grass fields (meadow and pastures)	2	2	0	0	0	4
Wetlands (swamp, moor, reed)	1	1	0	0	0	2
Boundaries (strip of grass, stone wall, retaining wall etc.)	1	2	0	0	0	3
Gardens, fruit gardens, vineyards of high ecologic value	1	2	2	0	1	6
Total	29	28	7	20	8	-

0: not relevant; 1: partly relevant, 2 dominant

Comparison of main green infrastructure types and potential tourism products

We distinguished 19 types among the GI elements of the three pilot regions. We analysed which GI types in what extent can positively influence certain tourism products. In our pilot regions the following tourism types can be distinguished: active tourism, rural and eco-tourism, gastronomy. Considering the potential use of GI assets in tourism types we distinguished not relevant, partially relevant and dominant categories.

According to Table 4 the most significant role of certain GI elements can have in active, rural and eco-tourism furthermore health and well-being tourism. The development of GI network will influence mostly these tourism types. The table also highlights those GI types which have the most significant positive effect on the majority of tourism types: cycling routes, hiking trails, gardens of institutions, lakes, riverbanks, valuable fruitgardens, and vineyards.

Defining complex development possibilities

There have been 19 types of tourism attractions distinguished. In the last phase of our research we analysed the connection and synergies of the tourism attractions and different GI elements, namely what kind of complex tourism and green infrastructure development projects could be possible. We distinguished neutral pairs/categories, pairs which may be combined directly and indirectly.

Our results highlight the fact that several green infrastructure elements can be combined with tourism attractions, the common, complex development projects have wide range of positive synergies (Table 5). The development of public spaces such as parks, alleys are highly important from the point of view of tourism as well. It can improve the attractiveness of castles, folk architecture, museums and even cultural programs (festivals, trade fairs). Our results highlight the importance of a special groups of GI assets: the linear green infrastructure elements (study trails, hiking routes, buffer zones along cycling routes, greenways). This proves the fact that preservation, development of linear GI elements are highly important and useful next to nature protection aspects for tourism as well by strengthening the connections and network of attractions.

Recommendations for the study areas

We formulated recommendations and trends for complex green infrastructure and tourism development in the study area. In all regions one of the most important group of recommendations relate to the development of GI network, creating, improving connections through GI elements. Greenways are really good example for common management of tourism and green infrastructure, greenways are a characteristic phenomenon of positive synergies of GI and tourism development. Greenways are interpreted as linear open spaces offering non motorised active recreational opportunities meanwhile protecting the environment even improving the ecologic value of the landscape. Recognizing the benefits of greenways several researches, plans, projects focused on greenway development in Hungary.

In micro-region of Balatonfüred the most important objective is to connect the open spaces of downtown with the surrounding GI elements in the landscape. This is especially important in the shoreline settlements with significant tourism load. In the background settlement there is an opposite objective of development, GI development shall connect the less significant attractions increasing tourist volume. By diversifying the active recreational opportunities such as horse riding, cycling, it is also possible to widen the tourist competitiveness and attractiveness not just spatially but seasonally as well. Furthermore it is highly important to maintain the permeability between the valleys by ecologic corridors, greenways, thematic routes.

In micro-region of Csorna the local rural development strategy defines remarkable eco-touristic development objectives. Partly thanks to these initiatives several good examples were realized in the last decade. The green infrastructure in Hanság and along river Rába has great touristic potential but the improvement of the tourist infrastructure is necessary. In the intensive cultivated landscape of Rábaköz complex green infrastructure development is inevitable to develop and connect the less significant attractions into complex tourist product.

We consider highly important the development of green infrastructure network in order to connect the less significant tourist attractions in micro-region of Gönc also. These initiatives shall focus on the Western part of the study area in order to improve the relations with the most popular tourist destinations (Boldogkőváralja, Tállya, Regéc, Telkibánya). The landscaping of the surrounding of the major built heritage (Bible Museum in Vizsoly) can improve their attractiveness.

Conclusions

In our research project we defined and analyzed the green infrastructure system, touristic attractions and tourist infrastructure of three Hungarian micro-regions. We concluded that the complex tourism and GI development is significant in the field of active, rural, eco-tourism, and furthermore health and medical tourism. It is highly important to connect the ecologically core areas of Hanság and Rába by complex development including restoration of watercourses, habitat development of the Rábaköz in order to improve the life quality of local people and ecological value of the micro-regions and rising tourism potential. In case of Gönc micro-region green infrastructure development can enhance the complex tourism potential by connecting smaller scale attractions.

We defined those GI assets which have the most significant positive influence on tourism: surrounding of cycling routes, hiking trails, green spaces of public institutions, lakesides and riverbanks, traditional fruit-gardens and vineyards. The recreational development of the beautiful, varied and traditional landscape of Balatonfüred micro-region can enhance the tourism potential of background settlements. The connection of open spaces of downtown with the surrounding GI elements in the landscape can enhance the aesthetic, ecological value so the tourism potential of the tourism destinations in micro-regions of Balatonfüred.

Based on the comparison analysis of attraction types and GI assets we defined those elements which can be the base of complex development (they have synergies). These can be public parks, alleys which can increase the value of tourism attractions such as castles, museums or programs. Especially in micro-region of Gönc and Csorna the development of the surrounding of the major built heritage can improve their tourism potential. Our results highlighted a specific group of GI elements, the linear GI elements. So the development of linear GI elements is important from touristic, aesthetic and ecologic point of view as well. Among these assets we see great potential in development of greenways. Greenway development could be a good common development and management program with positive synergies for tourism and green infrastructure as well in all study areas but especially in micro-region of Csorna.

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