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A COMPARISON OF THE NUMBER OF SELECTED GROUPS OF EPIPHYTIC MICROORGANISMS IN MEADOW SWARD FERTILISED WITH VARIOUS DOSES OF NATURAL FERTILISERS AND NPK

Summary

Increasing interest in organic farming and consequently in the application of natural fertilisers poses a risk of modifying qualitative and quantitative composition of microorganisms' populations living on plants fertilised with these fertilisers. Composition of epiphytic microflora on plant material intended for ensilage affects fermentation processes and stability and hygienic quality of obtained silage. Studies carried out in the years 2009-2011 were aimed at assessing the effect of fertilisation of permanent meadow with natural fertilisers (manure and liquid manure) and mineral fertilisers (NPK) on the number of selected groups of epiphytic microorganisms living on meadow sward. Plant samples were taken three times in vegetation seasons (before the 1st, 2nd and 3rd cut). In total, 207 samples of vegetation were analysed for: total number of aerobic bacteria, bacteria of the genus Enterobacteriaceae, yeasts and mould fungi. No differences in the number of studied groups of microorganisms living on meadow plants fertilised with different fertilisers applied at different doses were found. However, the number of particular groups of microorganisms depended mainly on the time of sampling.
***Key words**: epiphytic microflora, aerobic bacteria, yeasts, mould fungi, enterobacteria*

PORÓWNANIE LICZEBNOŚCI WYBRANYCH GRUP EPIFITYCZNYCH MIKROORGANIZMÓW RUNI ŁĄKOWEJ NAWOŻONEJ RÓŻNYMI DAWKAMI NAWOZÓW NATURALNYCH I NPK

Streszczenie

Rosnące zainteresowanie rolnictwem ekologicznym oraz wynikające z jego zasad stosowanie nawozów naturalnych niesie ze sobą niebezpieczeństwo modyfikacji składu jakościowego i ilościowego populacji mikroorganizmów naturalnie bytujących na roślinności nawożonej tymi nawozami. Skład mikroflory epifitycznej znajdującej się w materiale roślinnym przeznaczonym do zakiszania wpływa na przebieg procesów fermentacyjnych, stabilność i jakość higieniczną uzyskanej kiszonki. Celem badań przeprowadzonych w latach 2009-2011 była ocena wpływu nawożenia łąki trwałej nawozami naturalnymi (obornikiem i gnojówką) i nawozami mineralnymi NPK na liczebność wybranych grup epifitycznych mikroorganizmów bytujących na runi łąkowej. Próbki roślin do badań pobierano trzykrotnie w trakcie sezonu wegetacyjnego (przed I, II i III pokosem). Łącznie przebadano 207 próbek zielonki. W ramach badań oceniano: ogólną liczebność bakterii tlenowych, bakterii z rodzaju Enterobacteriaceae oraz drożdży i grzybów pleśniowych. W wyniku przeprowadzonych badań nie wykazano różnic w liczebności badanych grup drobnoustrojów bytujących na roślinności łąkowej nawożonej różnymi nawozami i ich dawkami. Stwierdzono jednak, że liczebność poszczególnych grup mikroorganizmów zależała przede wszystkim od terminu poboru próby.
***Słowa kluczowe**: mikroflora epifityczna, bakterie tlenowe, drożdże, grzyby pleśniowe, enterobakterie*

1. Introduction

Plant surface is a complex system which enables development of a large community of mutually interacting epiphytic microorganisms. Epiphytes occupy a narrow ecological niche; their functioning depends on plant properties and habitat conditions like moisture, temperature, wind speed, solar radiation and land use [1, 2]. Soil and rain are the sources of microorganisms on plants. Microorganisms are also spread by insects and wind [1, 3]. Their numbers are strongly affected by anthropogenic factors including farming [4]. Epiphytic microorganisms exert strong and variable effects on plants they inhabit [1]. Bacteria and fungi may stimulate plant growth and repel herbivores, protect from pathogens and increase plants' tolerance to drought which translates into plant condition and the amount and quality of yield. On the other hand, microorganisms may infect plant tissues [5].

Ensilage is more and more common method of meadow sward conservation. Composition of epiphytic microflora on plant material intended for ensilage affects fermentation processes and stability and quality of obtained silage [1, 6]. Lactic acid bacteria are among epiphytic microflora of meadow sward. These microorganisms favourably affect ensilage and constitute about 1% of epiphytes living on meadow plants. Microorganisms undesired by silage producers stem from bacilli of the genera Clostridium and Bacillus, rod-shaped bacteria of the family Enterobacteriaceae, moulds and yeasts. Contamination of meadow sward by pathogens may result in contamination of silages [7].

Increasing interest in organic farming and associated application of natural fertilisers poses a risk of modifying the composition (both quantitative and qualitative) of microbial communities living on plants fertilised with these fertilisers [8]. Natural fertilisers are often habitats for vari-

ous pathogens, parasites and microorganisms that are harmful for silage fermentation. For example, bacteria of the genera Clostridium and Bacillus negatively affect milk quality and its usefulness for further processing [9]. Fodder contaminated by pathogenic microorganisms may pose a risk for human health due to a possibility of pathogen transfer through animals to animal products [9, 10].

This study was aimed at assessing the effect of fertilisation of permanent meadow with natural fertilisers (manure and liquid manure) and with mineral NPK fertilisers on the number of selected groups of epiphytic microorganisms on meadow sward.

2. Materials and methods

Studies were carried out in the years 2009-2011 at the Institute of Technology and Life Sciences (ITLS) in Falenty. Study material consisted of samples of meadow sward from an experiment set up on permanent meadow of the Experimental Farm of the ITLS.

2.1. Methods of field experiment

Experiment was set up on meadow situated in Laszczki on mineral, degraded black earth of grain size of light silty loam. Nine experimental plots were selected of an area of 0.3 ha each and fertilised with cattle liquid manure, solid manure or mineral (NPK) fertilizers. Fertilisers were applied in three different doses corresponding to three levels of nitrogen fertilisation. At the first level of fertilisation (N-60) annual doses of nutrients were: 60 kg N, 30 kg P and 60 kg K per ha, at the second level (N-90) - 90 kg N, 45 kg P and 90 kg K per ha and at the third level (N-120) - 120 kg N, 60 kg P and 120 kg K per ha. The amount of applied natural fertilisers differed from year to year depending on nitrogen content in fertilisers. At the first level (N-60), from 24.0 to 30.0 t ha^{-1} of manure and from 24.0 to 28.0 m^3 ha^{-1} of liquid manure were applied in subsequent years. At higher levels of fertilisation (N-90 and N-120) doses of natural fertilisers were increased by 50% and 100%, respectively. Solid manure after preliminary fermentation (20% dry mass) was applied once in autumn or spring with the use of manure spreader. Liquid manure was applied directly to soil with special injectors in two equal doses – in spring and after the first cut. Phosphorus deficits in liquid manure were supplemented with phosphorite powder. Mineral fertilisers were used in the form of ammonium saltpetre, phosphorite powder and potassium sulphate. They were applied in spring (1/3 of annual dose of N and K and the whole dose of P) and after the first and second cut (the remaining two doses of N and K).

2.2. Methods of microbiological analyses

Samples of meadow sward for microbiological analyses were taken three times in vegetation season, during the first, second and third cut. Three samples of plants were taken from each plot. In fresh plant samples the total number of aerobic bacteria, bacteria of the family *Enterobacteriaceae* and the number of yeasts and mould fungi were determined using cultures on PetrifilmTM 3M plates. All samples were shaken in a buffer. Then 1 ml of subsequent dilutions was taken and inoculated on PetrifilmTM 3M plates. 3M™ Petrifilm™ Aerobic Count Plates were incubated at 28°C for 72 h, 3M™ Petrifilm™ *Enterobacteriaceae* Count Plates at 35°C for 24 h while 3M™ Petrifilm™ Rapid Yeast and Mould Count Plates were incubated at 25 °C for 48 h.

2.3. Statistical analysis of results

Obtained data on the number of selected groups of epiphytic microorganisms were statistically assessed using two-way analysis of variance with type of fertiliser and its dose as factors. The effect of study year and sampling time (cuts) was also analysed. Significance of differences was checked with the Tukey HSD test at α=0.05. Correlations between the number of studied groups of organisms and the sum of precipitation [mm] and mean daily temperature [^oC] were also calculated. All tests were made with the use of Statistica ver. 6 (Statsoft, Poland), module ANOVA for factorial design.

3. Results and discussion

It is commonly known that growing agricultural plants decreases the diversity of epiphytic microorganisms and alters their species composition. This is important since these microorganisms may be beneficial or harmful for plants. For example, it was found that a great number of bacteria on winter wheat increased yielding and limited the intensity of symptoms of Septoria disease [10, 11, 12]. Some sources indicate that contamination of soils of permanent grasslands with faecal bacteria translates into contamination of meadow sward and has in turn negative impact on silage quality [13, 14, 15].

Performed studies did not show significant differences in the number of aerobic bacteria among the types and doses of applied fertiliser (Table 1).

Table 1. The total aerobic bacteria count (log cfu·g^{-1}) in subsequent sampling terms depending on the type and dose of fertilization (means from three years of study)
Tab. 1. Liczebność ogólnej liczby bakterii tlenowych (log jtk·g^{-1}) w kolejnych terminach poboru prób w zależności od rodzaju i dawki nawożenia (średnie z trzech lat badań)

Fertilizer type	Fertiliser dose	Sampling term			Mean from three samplings
		I cut	II cut	III cut	
NPK	N-60	7.50	7.56	8.19	7.75
	N-90	6.95	8.11	8.26	7.77
	N-120	7.51	7.97	8.67	8.05
Manure	N-60	6.45	7.42	7.94	7.27
	N-90	6.74	7.84	8.22	7.60
	N-120	6.83	7.52	8.46	7.60
Liquide manure	N-60	6.60	7.62	8.09	7.44
	N-90	6.41	7.79	7.67	7.29
	N-120	6.38	7.82	7.96	7.39
Mean					
NPK		7,37	7.88	8.37	7.87b
Manure		6,64	7.55	8.18	7.46ab
Liquide manure		6,47	7.73	7.94	7.38a
Mean					
N-60		6,73	7.52	8.05	7.43
N-90		6,70	7.91	8.05	7.55
N-120		6.84	7.74	8.32	7.63

means in columns followed by the same letter are not significantly different at 5% level of probability (Tukey's test, $p<0.05$)

Source: own work / Źródło: opracowanie własne

The number of bacteria varied from 6.38 log cfu·g^{-1} (meadow sward from the first cut fertilised with liquid manure at a dose of 120 kg N/ha) to 8.46 log cfu·g^{-1} (the third cut of sward fertilised with manure at a dose of 120 kg N/ha). Values averaged over three times of sampling showed the greatest number of aerobic bacteria (8.05 log cfu·g^{-1}) on plants grown in soil fertilised with mineral fertiliser at nitrogen dose of 120 kg N/ha and the lowest number (7.27 log cfu·g^{-1}) in that fertilised with manure at a dose equivalent to 60 kg N/ha. Considering the source of nitrogen, aerobic bacteria were, on average, more numerous on plant samples from plots fertilised with mineral fertilisers and with manure (7.87 and 7.46 log cfu·g^{-1}, respectively) than on plants fertilised with liquid manure (7.38 log cfu·g^{-1}). No significant differences in the number of analysed microorganisms were found among the intensities of N fertilisation. Mean number of bacteria on plants from plots fertilised with 120 kg N/ha was 7.63 log cfu·g^{-1} and that from plots fertilised with 60 kg N/ha was 7.43 log cfu·g^{-1}).

The greatest number of bacteria of the family *Enterobacteriaceae* were found in our study in plant samples from the third cut fertilised with manure at a dose corresponding to 90 kg N/ha (6.82 log cfu·g^{-1}), while the lowest number (4.33 log cfu·g^{-1}) - in samples from the first cut fertilised with liquid manure at the same nitrogen dose (Table 2).

Table 2. *Enterobacteriaceae* count (log cfu·g^{-1}) in subsequent sampling terms depending on the type and dose of fertilization (means from three years of study)

Tab. 2. Liczebność bakterii z rodzaju Enterobacteriaceae (log jtk·g^{-1}) w kolejnych terminach poboru prób w zależności od rodzaju i dawki nawożenia (średnie z trzech lat badań)

Fertilizer type	Fertiliser dose	Sampling term			Mean from three samplings
		I cut	II cut	III cut	
NPK	N-60	4.93	6.06	6.29ab	5.76
	N-90	4.98	6.05	6.54ab	5.86
	N-120	5.04	6.25	6.64ab	5.98
Manure	N-60	4.79	5.69	6.26ab	5.58
	N-90	5.97	6.13	6.82b	6.12
	N-120	4.73	5.98	6.28ab	5.66
Liquide manure	N-60	4.88	5.72	5.53a	5.38
	N-90	4.33	5.92	5.88ab	5.38
	N-120	4.56	5.84	6.09ab	5.50
Mean					
NPK		4.98	6.12	6.49b	5.86
Manure		5.03	5.89	6.40b	5.77
Liquide manure		4.62	5.81	5.83a	5.42
Mean					
N-60		4.85	5.78	6.03	5.55
N-90		5.09	6.03	6.41	5.84
N-120		4.74	6.00	6.30	5.68

means in columns followed by the same letter are not significantly different at 5% level of probability (Tukey's test, p<0.05)

Source: own work / Źródło: opracowanie własne

No significant differences were found among the numbers of bacteria in various plots for both the first and second cut. Significant effect of applied fertilisation on the number of microorganisms was noted in sward sampled from the third cut (Table 2). On average, irrespective of the level of fertilisation, significantly higher numbers of *Enterobacteriaceae* were found in samples from plots fertilised with mineral fertilisers and manure (6.49 and 6.40 log cfu·g^{-1}, respectively) compared with the sward fertilised with liquid manure (5.83 log cfu·g^{-1}). When averaged across all cuts, the greatest number of *Enterobacteriaceae* was also found on plants from plots fertilised with mineral fertilisers (5.86 log cfu·g^{-1}). The level of nitrogen fertilisation did not markedly affect the number of analysed microorganisms. It varied from 5.84 log cfu·g^{-1} on sward fertilised with manure to 5.55 log cfu·g^{-1} for sward fertilised with mineral fertilisers on average.

The effect of epiphytic yeasts on plants may vary. Some species of the genus *Spetoria* [4, 11] cause plant diseases, other may inhibit the development of pathogens by competing for habitat (for example fungi of the genus *Sporobolomyces*) or by inducing protection mechanisms in plant tissues (for example *Cryptococcus* and *Rhodotorula*) [11]. For the production of silage, yeasts are probably the most important aerobic microorganisms that determine its quality. Development of yeasts is not inhibited by pH since they are able to grow and reproduce at pH from 3 to 8 [4, 16]. Some species may develop in anaerobic conditions and perform fermentation of sugars to ethanol. Silages with a high content of ethanol may be quite variable with respect to oxygen stability [1, 17].

The number of yeasts in our study was variable. No significant effect of analysed factors was, however, found on the number of these microorganisms (Table 3).

Table 3. Yeasts count (log cfu·g^{-1}) in subsequent sampling terms depending on the type and dose of fertilization (means from three years of study)

Tab. 3. Liczebność drożdży (log jtk·g^{-1}) w kolejnych terminach poboru prób w zależności od rodzaju i dawki nawożenia (średnie z trzech lat badań)

Fertilizser type	Fertilizer dose	Sampling term			Mean from three samplings
		I cut	II cut	III cut	
NPK	N-60	0.80	1.72	2.05	1.52
	N-90	0.67	1.04	0.93	0.88
	N-120	0.48	1.83	1.68	1.33
Manure	N-60	0.54	1.78	1.43	1.25
	N-90	0.00	3.51	0.94	1.48
	N-120	0.26	2.17	1.15	1.19
Liquide manure	N-60	0.11	1.13	1.42	0.89
	N-90	0.38	0.98	1.02	0.79
	N-120	0.61	1.35	1.77	1.24
Mean					
NPK		0.65	1.53	1.55	1.24
Manure		0.32	2.29	1.23	1.28
Liquide manure		0.37	1.17	1.45	1.00
Mean					
N-60		0.45	1.55	1.57	1.19
N-90		0.35	1.84	0.96	1.05
N-120		0.45	1.77	1.52	1.25

means in columns followed by the same letter are not significantly different at 5% level of probability (Tukey's test, p<0.05)

Source: own work / Źródło: opracowanie własne

In samples taken during the first cut from plot fertilised with manure at a dose corresponding to 90 kg N/ha these organisms were not found at all and their highest number (3.51 log cfu·g^{-1}) was noted on plants from the same plot during the second cut. On average, yeasts were most numerous on plants from plot fertilised with manure at a dose corresponding to 90 kg N/ha (1.48 log cfu·g^{-1}) and least numerous in meadow sward fertilised with liquid manure at the same dose (0.79 log cfu·g^{-1}). When compared among the

types of fertilisers, the number of yeasts was slightly higher for variants with manure (1.28 log cfu·g^{-1}) and mineral fertilisation (1.24 log cfu·g^{-1}) than for those fertilised with liquid manure (1.00 log cfu·g^{-1}). With respect to fertilisation level, the number of yeasts varied from 0.35 log cfu·g^{-1} in samples from the first cut at N fertilisation level of 90 kg/ha to 1.84 log cfu·g^{-1} in meadow sward of the second cut on the same plot. On average, yeasts were most numerous on meadow sward fertilised with nitrogen at a dose of 120 kg/ha (1.25 log cfu·g^{-1}) and least numerous on sward fertilised with N dose of 90 kg N/ha (1.05 log cfu·g^{-1}).

Moulds are filamentous fungi common on plants. Their populations, although able to grow on various substrata, are seldom numerous enough to affect parameters of silage produced and stored in proper conditions [4, 6]. Moulds develop when silage quality get worse as a result of yeasts and other aerobic bacteria activity. Moulds may develop exclusively in aerobic conditions; hence their visible presence in silage is an evidence of excess oxygen in a silo [1] and bad quality of silage [4]. Moreover, moulds produce mycotoxins, which are harmful for the health of animals and humans. Mycotoxins are usually produced under stress conditions for moulds and environmental stressors initiating their production vary largely depending on species. At appropriate ensilage, concentrations of mycotoxins in silage are similar to those in green plant biomass.

The number of moulds in analysed samples of meadow sward was quite variable and ranged from 3.99 log cfu·g^{-1} on plants from the first cut on plot fertilised with manure at a dose corresponding to 90 kg N/ha to 5.90 log cfu·g^{-1} on plants of the first cut fertilised with mineral fertilisers at a dose of 90 kg N kg/ha (Table 4).

Table 4. Moulds count (log cfu·g^{-1}) in subsequent sampling terms depending on the type and dose of fertilization (means from three years of study)
Tab. 4. Liczebność grzybów pleśniowych (log jtk·g^{-1}) w kolejnych terminach poboru prób w zależności od rodzaju i dawki nawożenia (średnie z trzech lat badań)

Fertilizer type	Fertilizer dose	Sampling term			Mean from three samplings
		I cut	II cut	III cut	
NPK	N-60	4.54	5.90	5.80	5.41
	N-90	4.13	5.43	5.14	4.90
	N-120	5.37	5.84	5.22	5.48
Manure	N-60	4.40	5.44	5.50	5.11
	N-90	3.99	5.26	5.17	4.81
	N-120	4.67	5.43	5.61	5.24
Liquide manure	N-60	5.09	5.71	5.61	5.47
	N-90	4.16	5.27	5.10	4.84
	N-120	4.30	5.51	5.19	5.00
Mean					
NPK		4.68	5.72	5.38	5.26
Manure		4.40	5.39	5.46	5.08
Liquide manure		4.56	5.53	5.33	5.14
Mean					
N-60		4.66	5.63	5.60b	5.30b
N-90		4.09	5.32	5.14a	4.85a
N-120		4.71	5.56	5.35ab	5.21b

means in columns followed by the same letter are not significantly different at 5% level of probability (Tukey's test, p<0.05)
Source: own work / Źródło: opracowanie własne

No significant effect of analysed factors on the numbers of this group of microorganisms was, however, found. On average, the number of moulds varied from 4.81 log cfu·g^{-1} on sward fertilised with manure at a dose equivalent to 90 kg N/ha to 5.41 log cfu·g^{-1} on plots with mineral fertilisation at a dose of 60 kg N/ha. The type of fertilisation had no significant effect on the number of moulds, which ranged from 4.40 log cfu·g^{-1} (third cut of plots fertilised with manure) to 5.72 log cfu·g^{-1} (second cut of plots fertilised with NPK). The only significant effect was found of the level of nitrogen fertilisation (Table 4). The number of moulds was significantly higher at N doses of 60 and 120 kg/ha (5.30 and 5.21 log cfu·g^{-1}, respectively) than at a dose of 90 kg/ha (4.85 log cfu·g^{-1}).

There were statistically significant positive correlations between the numbers of particular groups of microorganisms (Table 5). The numbers of aerobic bacteria strongly correlated with those of *Enterobacteriaceae* (r=0.71) and moulds (r=0.54). Moreover, there was significant relationship between the numbers of enterobacteria and yeasts (r=0.47).

Table 5. Spearman's Rank Correlation Coefficient
Tab. 5. Korelacja porządku rang Spearmana

	Aerobic bacteria	*Enterobacteriaceae*	Yeasts	Moulds
Aerobic bacteria	1.00	0.71**	0.43**	0.54**
Enterobacteriaceae	0.71**	1.00	0.47**	0.35**
Yeasts	0.43**	0.47**	1.00	0.30**
Moulds	0.54**	0.35**	0.30**	1.00

** correlations significant at p <0,01
Source: own work / Źródło: opracowanie własne

As in other studies [4, 18, 19], the numbers of all studied groups of microorganisms depended significantly on the time of sampling (cuts) and year (Table 6). On average, the highest numbers of aerobic bacteria (8.65 log cfu·g^{-1}) were noted in plant samples from the third cut in 2010 and the lowest (5.56 log cfu·g^{-1}) in plants from the first cut of 2009. *Enterobacteriaceae* achieved the highest numbers in the third cut of 2010 and the lowest number of these microorganisms was noted in sward from the first cut of 2011. Both yeasts and moulds were most numerous on plants from the second cut of 2010 (3.44 and 5.88 log cfu·g^{-1}, respectively) and least numerous in samples from the first cut of 2009.

Performed comparisons showed that the study year had significant effect on the numbers of studied microorganisms. From the analysis of meteorological conditions in growing season in the study period (Fig. 1, 2) it appeared that the most favourable conditions for microorganisms were those in 2010, when the vegetation season was characterised by the highest rainfalls and mean temperature (15.5°C). Moreover, there were significant positive correlations between the sum of precipitation in the vegetation season and the total number of aerobic bacteria (r=0.26), *Enterobacteriaceae* (r=0.30) and yeasts (r=0.27) and between mean air temperature in the vegetation season and the number of *Enterobacteriaceae* (r=0.15) and yeasts (r=0.23). The strongest correlation was found for the total number of bacteria, whose average numbers were 8.10 log cfu·g^{-1} in 2010 and 6.88 log cfu·g^{-1} and 7.5 log cfu·g^{-1} in 2009 and 2011, respectively. Equally distinct effect of the study year was noted for yeasts. The number of these microorganisms in 2010 was 1.98 log cfu·g^{-1} while in 2009 and 2011 the numbers were 0.41 log cfu·g^{-1} and 0.97 log cfu·g^{-1}, respectively. All studied epiphytic microorganisms

were least numerous in the year 2009, which was characterised by the lowest precipitations and mean air temperatures during the vegetation season.

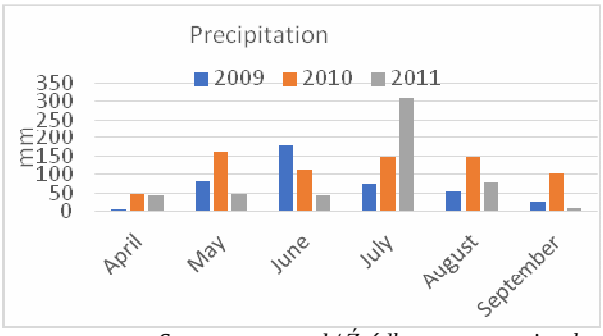
Table 6. The count of selected epiphytic groups of microorganisms (log cfu·g^{-1}) depending on the year and term of sampling

Tab. 6. Liczebność wybranych grup epifitycznych mikroorganizmów runi łąkowej (log jtk·g^{-1}) w zależności od roku badań i terminu poboru próbek

Year	Cut	Aerobic bacteria	*Enterobacteriaceae*	Yeasts	Moulds
2009	I	5.56a	4.19a	0.04a	3.97a
	II	7.47b	5.40b	0.87ab	5.05b
	III	7.61bc	5.84b	0.33a	5.04b
2010	I	7.59bc	6.08bc	0.80ab	4.32a
	II	8.05c	6.54c	3.44d	5.88d
	III	8.65d	6.61c	1.71bc	5.78cd
2011	I	7.12b	4.00a	0.36a	5.59cd
	II	7.47bc	5.69b	0.21a	5.63cd
	III	8.10cd	6.16bc	2.33cd	5.29c
Mean					
2009		6,88a	5.14a	0.41a	4.69a
2010		8,10c	6.41b	1.98c	5.32b
2011		7,56b	5.29a	0.97b	5.51b
Mean					
I cut		6,76a	4.88a	0.42a	4.53a
II cut		7,70b	5.92b	1.70b	5.53b
III cut		8.14c	6.22b	1.39b	5.40b

means in columns followed by the same letter are not significantly different at 5% level of probability (Tukey's test, $p<0.05$)

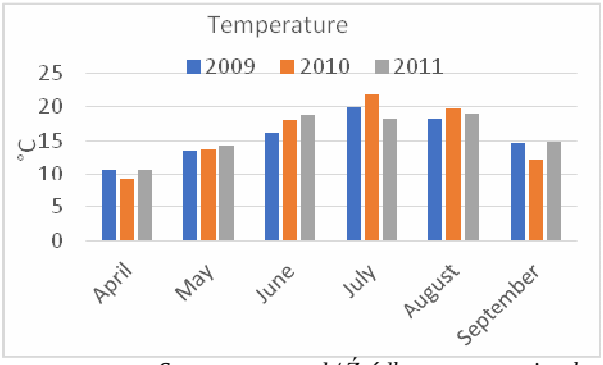
Source: own work / Źródło: opracowanie własne



Source: own work/ Źródło: opracowanie własne

Fig. 1. Precipitation (mm) in growing season in 2009, 2010 and 2011

Rys. 1. Suma opadów (mm) w okresie wegetacyjnym w latach 2009-2011



Source: own work/ Źródło: opracowanie własne

Fig. 2. Monthly mean air temperature in growing season in 2009, 2010 and 2011

Rys. 2. Średnia miesięczna temperatura powietrza (°C) w sezonie wegetacyjnym w latach 2009-2011

Apart from the study year, the time of sampling was also an important factor. The numbers of all microorganisms on meadow sward were significantly lower in the first cut and the highest in the third cut. The number of yeasts most numerous on plants from the second cut was the exception.

4. Conclusions

Natural fertilisation applied in the study did not affect the numbers of aerobic bacteria, *Enterobacteriaceae*, yeasts and moulds present on meadow sward compared with the numbers of these microorganisms on plants fertilised with mineral fertilisers.

Nitrogen doses applied in fertilisers (60, 90 and 120 kg/ha) did not differentiate the numbers of aerobic bacteria, *Enterobacteriaceae* and yeasts but had a significant effect on the number of moulds.

The strongest effect on the numbers of studied microorganisms was exerted by the time of plant sampling. All studied epiphytes were most numerous during the third cut.

As expected, increased numbers of studied microorganisms were noted in the year of higher daily mean temperature and higher sum of precipitation in the vegetation season.

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