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CORRELATIONS BETWEEN SOIL ORGANIC CARBON PROPERTIES AND SOIL MICROORGANISM INDICES

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Abstract

The connection between soil organic matter (SOM) and microorganisms has been investigated for a long time. On a global scale the microbial biomass (MB) in the soil is mainly influenced by the SOM content. Among other activities, microorganisms take part in the decomposition and formation of SOM. In the present research, soils from a long-term field trial involving organic and inorganic N fertilization (IOSDV, Keszthely, Hungary) were used to investigate the relationship between the amount and quality of SOM and the amount and activity of MB. The short-term (MB) and long-term (SOM) effects of fertilization on soils were also investigated. The quantity of microorganisms (microbial biomass carbon – MBC or C_{mic}) was determined using the chloroform-fumigation extraction method, while their activity was measured by means of fluorescein-diacetate (FDA) hydrolysis. The quality of SOM was measured using the E4/E6 method.

The determination of MBC and soil organic carbon (SOC or C_{org}) revealed that the C_{mic}/C_{org} ratio differed in the various treatments and was affected by organic amendments but not by the inorganic N fertilization dose. The C_{mic}/C_{org} ratio was the highest in treatments involving crop residue incorporation and the lowest in the unamended treatments. Similarly, the amount and activity of MB did not depend on the dose of inorganic N fertilization but was influenced by organic amendments. The results between the C_{mic}/C_{org} ratio and the amount and activity of microorganisms showed a correlation.

Keywords: soil organic carbon (SOC), E4/E6 method, microbial biomass carbon (MBC), fluorescein diacetate (FDA) hydrolysing activity, long-term fertilization experiment

Összefoglalás

A talajok szervesanyag-tartalma és a talajban élő mikroorganizmusok közötti kapcsolatok hosszú ideje kutatások tárgya. Globálisan a talajban élő mikrobák mennyiségét alapvetően meghatározza a szervesanyag-tartalom. A mikroorganizmusok, számos más aktivitásuk mellett, a szervesanyagok lebontásában és létrehozásában egyaránt részt vesznek. Kutatásainkban a Keszthely melletti IOSDV szerves és szervetlen N trágyázási tartamkísérlet talajait felhasználva vizsgáltuk a szervesanyag minősége, illetve mennyisége és a mikroorganizmusok mennyisége, illetve aktivitása közti összefüggéseket. A trágyázás talajra gyakorolt rövid- (mikrobiális biomassza) és hosszútávú (szervesanyag tartalom) hatásait szintén vizsgáltuk. A mikroorganizmusok mennyiségét szerves széntartalmuk alapján, kloroform fumigációs-extrakciós módszerrel, aktivitásukat fluoreszcein-diacetát (FDA) hidrolízis mérésével határoztuk meg. A szervesanyagok minőségét a humuszminőség vizsgálatára kidolgozott E4/E6 módszer felhasználásával jellemeztük.

A mikrobiális szén és a talaj összes szerves szén arányát kifejező C_{mic}/C_{org} hányadost vizsgálva megállapítottuk, hogy a különböző kezelésekben értéke nem állandó, változását nem a N-műtrágyázás adagja, hanem a szervesanyag kiegészítés befolyásolja. A C_{mic}/C_{org}

hányadost a növényi maradvány alászántásos kezelésekben volt a legmagasabb és a szerves kiegészítést nem kapott kezelésekben a legalacsonyabb. Hasonlóképpen a mikrobiális biomassza és aktivitása nem függött az N-műtrágyakezelés dózisától, de a szervesanyag kiegészítéstől igen. A C_{mic}/C_{org} arány korrelált a mikroorganizmusok mennyiségével és aktivitásával.

Introduction

Soil organic matter (SOM) sustains many key soil functions by providing energy, substrates and biological diversity to support biological activity, which affects soil aggregation, water infiltration and nutrient availability (Reeves, 1997). Recently, SOM has received growing attention from a new aspect. The global soil organic carbon (SOC or C_{org}) pool is approximately twice the size of the atmospheric pool (Lal, 2004); hence, a small change in the C loss from soils exerts a significant influence on the carbon dioxide concentration of the atmosphere (Smith et al., 2008). Soil C sequestration plays an important role in mitigating climate change (Lal, 2004; Smith et al., 2008); therefore, it is essential to understand the mechanisms underlying SOC turnover and dynamics (Tan et al., 2014).

Besides the amount of SOM, its quality is also important. Various physical and chemical methods exist for the determination of SOM or humus quality. Physical methods include ultraviolet visible (UV VIS) spectroscopy, Fourier transformed infrared (FT IR) spectroscopy, “steady-state” fluorescence spectroscopy and ^{13}C nuclear magnetic resonance (NMR) (Enev et al., 2014, Filep et al. 2016). Chemical methods are usually based on the use of various extractants, such as dilute NaOH solution or hot water. The E4/E6 method which determines the ratio of optical densities of dilute, aqueous humic and fulvic acid solutions measured at

465 and 665 nm, is frequently used to characterize humus quality. A lower E4/E6 ratio means a larger proportion of higher molecular weight humic substances (HS) containing more condensed aromatic rings (Kononova, 1966). Recently, Nadi (2012) published E4/E6 ratios for different soils in Hungary. This method was also used to characterize humus quality in the present experiment. From the theoretical point of view, more soluble, less complex HS can be more readily utilized for microorganisms as an energy source than less soluble, more complex HS. Therefore, increasing the proportion of less complex HS (higher E4/E6 ratio) can be expected to increase the microbial biomass and activity.

Although soil organisms comprise <1% of the total mass of a soil, they have a vital role in many soil processes: mineralization, recycling nutrients, humus formation, improving soil structure, promoting plant growth, controlling pests and diseases, etc. The amount of soil microorganisms (bacteria plus fungi) is usually measured by some form of chloroform fumigation method, expressed in carbon units and referred to as microbial biomass carbon (C_{mic}). In forest and grassland soils, C_{mic} is estimated to be <5.0% of C_{org}, but in agricultural soils it is usually less than 2.5% (Kallenbach and Grandy, 2011). Despite its small size, the soil MB is known to play a fundamental role in soil organic matter dynamics. Further, MB has a short turnover time and is highly sensitive to soil environmental conditions and disturbances, which makes it a useful index for diagnosing early changes in soil C (Sparling, 1992).

SOM and MB are in complex relation. SOM is mainly plant-derived and microbially processed. There is wide agreement that the amount of total soil organic matter determines the amount of microbial biomass in the soil and that there is a linear correlation between them on a local or global scale (Paul, 2007, Fierer et al., 2009; Kallenbach and Grandy, 2011; Ussiri and Lal, 2013). The data used in these meta-analyses and reviews were collected from experiments in different locations, climate zones and soil types. MB is tightly coupled both to the quantity and quality of C inputs and to SOM concentrations (Kallenbach and Grandy, 2011). Microbial responses to organic C inputs also depend on other biotic or abiotic factors, such as soil texture, crop rotation, management and changes in soil pH, etc., so the simplified notion, hat more organic matter input into soil means greater C_{org} content is misleading. In certain circumstances the input of organic matter increases the decomposition of SOM, which is known as the priming effect (Kuzyakov et al., 2000; Kuzyakov, 2010). The ratio of decomposition and humification depends on the C:N or C:N:P ratios of microbial biomass, SOM and organic matter input (Cleveland and Liptzin, 2007).

In soils, only a small part of the microbial biomass is active, usually about 2–3% or even less (Blagodatsky et al, 2000; Blagodatskaya and Kuzyakov, 2013). The rate of ecologically important processes, such as organic matter decomposition or the transformations of nutrients depends to a large extent on the active fraction of the microbial biomass (Blagodatsky et al., 2000). The activity of soil microorganisms can be determined by measuring soil respiration (carbon dioxide emission) or the activity of any enzyme. The measurement of FDA hydrolysing enzyme activity was suggested by Schnürer and Rosswall (1982) for determining total hydrolytic activity of the soil. This is now one of the most widely accepted methods both in Hungary and internationally (Adam and Duncan, 2001; Villányi et al., 2006; Bíró et al., 2014).

The direct quantification of changes in SOM content is greatly hindered by the high degree of spatial heterogeneity in soils and the relative imprecision of methods for quantifying soil carbon stocks (Paterson et al., 2009). Long-term field trials allow investigations on the effects of fertilizers and different management systems on C_{org}, C_{mic} and their ratio. Many long-term fertilization trials were primarily set up to study the impact of fertilizers on crop production, but researchers are taking advantage of these well-documented experiments to study soil microbial communities. In a meta-analysis, based on the data of 107 datasets from 64 long-term trials, Geisseler and Scow (2014) found that the long-term fertilization of agricultural soils led to increased C_{mic} content, which is to be caused by the associated increases in C_{org} due to the higher crop productivity. Across all the datasets, the C_{mic}/C_{org} ratio was not significantly affected by fertilization. Increased C_{mic} content was observed in fertilized soils compared with that observed in unmanaged ecosystems, where C_{mic} often decreased as a result of N input (Geisseler and Scow, 2014). The C_{mic}/C_{org} ratio has been thoroughly investigated, as it has relevance in many models describing carbon flows in soil. From the eco-physiological point of view, the C_{mic}/C_{org} ratio reflects the carbon available for microbial growth (Anderson, 2003).

In the present research, the amount and activity of soil MB and the amount and quality of SOM were investigated in the IOSDV long-term fertilization experiment (near Keszthely, Hungary) set up to study the effects of organic and inorganic N fertilization. Since IOSDV was set up in 1983 different SOM levels have developed in the different plots, making it possible to study soils of a single type (Eutric Cambisol) with different SOM concentrations, while the climate, cultivation method, the plant species and plant protection methods were the same. The short- and long-term effects of mineral fertilization and organic amendments on the soils were also investigated.

To reveal detailed relationships between SOM and MB, measurements were made on the amount (C_{org}) and quality (E4/E6) of SOM and on the amount (C_{mic}) and FDA hydrolysing activity of MB, after which the C_{mic}/C_{org} ratio was calculated. Answers were sought to the following questions:

1. Do mineral fertilization and organic amendments have any significant effect on the measured (C_{org}, C_{mic}, FDA activity, E4/E6) and calculated (C_{mic}/C_{org}) soil parameters?
2. Is there a close significant correlation between C_{mic} and C_{org}?
3. Which factors effect on C_{mic}/C_{org} ratio if this ratio has changed?
4. Does a higher ratio of more soluble, less complex humic substances (HS) in the soil increase the amount and activity of microorganisms?

Materials and methods

The International Long-term Fertilization Experiment (IOSDV) is located in Keszthely, Hungary (46° 45' N, 17° 14 E) at 115 m above sea level. This area has moderate rainfall and mild temperature conditions. The annual precipitation is 683 mm and the mean temperature over the 1901-2000 100 year long period is 10.51 °C when averaged. The soil type at the experiment location is Ramann’s brown forest soil (Eutric Cambisol – WRB, 2014) with sandy loam texture. At the start of the experiment it was poor in organic carbon (~1.0% C_{org}), with a medium amount of available K (AL-K_2O ~ 135 mg/kg), available P-content was low (AL-P_2O_5 ~ 20 mg/kg). The pH_{KCl} varied between pH 6.8-7.0 (Kismányoky and Balázs, 1996).

The IOSDV is a bifactorial field experiment set up in 1983 with a cereal crop rotation in a strip block design. The crop rotation consists of maize, winter wheat and winter barley, sown in three replications with a plot size of 48 m^2. The factors in the experiment are increasing rates of N fertilizer and three kinds of organic amendments. I: inorganic fertilizer only (control); F: I+35 t ha^{-1} farmyard manure every 3rd year before sowing maize; S: I+stalk, straw and green manure (GM) incorporation. Green manure is applied as oilseed radish (*Raphanus* *sativus* var. Oleiformis) grown only once in the rotation after barley as a second crop before maize. In case of straw incorporation 10 kg N was added per 1 t of straw (Kismányoky and Balázs, 1996).

As basic fertilization every plot (including the N control) was given 100 kg ha^{-1} P_2O_5 and K_2O mineral fertilizer, while N was applied at five rates (N1, N2, N3, N4, N5), with 0-70-140-210-280 kg ha^{-1} for maize, and 0-50-100 (50+50)-150 (50+50-50)-200 (100+50+50) kg ha^{-1} for wheat.

Microbial biomass carbon (C_{mic}) contents were measured using the fumigation extraction method, according to Vance et al. (1987). The soil samples for MB (and for all other) measurements were taken from a depth of 0-20 cm at the following sampling times:

- 23rd May 2014, after maize germinated, when the plants were in phenophases 14-16 on the BBCH scale (Lancashire et al., 1991);
- 9th September 2014, before the maize harvest;
- 30th April 2015, when the wheat was in phenophase on the 37 on the Zadock (1974) scale;
- 27th October 2015, before the sowing of winter barley;
- 13th April 2016, when the barley was in phenophase 47 on the Zadock (1974) scale (head in the boot);
- 29th October 2016 after the barley harvest.

The determination of soil organic carbon content (C_{org}) was carried out according to the Hungarian standard (MSZ 08-0452:1980) in 2013. As C_{org} changes very slowly and no further measurements were made, these C_{org} values were used in all the comparisons. The C_{mic}/C_{org} ratio was calculated by dividing C_{mic} by C_{org}.

Measurements of FDA hydrolysing activity were carried out according to Alef and Nannipieri (1998) in autumn 2015 and spring and autumn 2016 by incubating 0.5 g wet soil was incubated with 20 ml buffered FDA solution for 3 h at 37 °C, under continuous shaking. The enzymatic reaction was stopped by the addition of 20 ml acetone, and the concentration of fluorescein was determined as absorbance at 490 nm (Hitachi U-1100 spectrophotometer). Enzyme activities were expressed as the amount of product released per hour and per gram of dried soil.

In the E4/E6 method for measuring humus quality, 1.25 g soil was dissolved in 50 ml 0.5% NaOH solution and mixed thoroughly. Following decantation, absorbance was measured at 465 and 665 nm (Hitachi U-1100 spectrophotometer). The ratio of the two absorbance values is the E4/E6 ratio. The measurements of humus quality were carried out in autumn 2014, spring 2015 and autumn 2016.

After sampling, the soil samples were stored in open nylon packs in a refrigerator (~4 °C) for at most 6 weeks prior to analysis.

The statistical evaluation of the experimental data involving univariate variance analysis, Duncan test and correlation analysis was done with SPSS Student Version 15.0 and Microsoft Excel.

Results

270 soil samples were analysed during the three years of the experiments. The statistical properties of the measured C_{org}, C_{mic}, E4/E6 and FDA data are shown in Table 1 (range, average, median and standard deviation). Only the C_{mic} values were measured twice a year (spring, autumn).

Table 1. Statistical properties of the measured soil parameters. N: number of data

Soil parameter	Range	Lowest value	Highest value	Mean	Median	Standard deviation	N
C_{org} (%)	0.39	1.03	1.42	1.19	1.15	0.10	45
E4/E6	6.70	2.91	9.61	6.26	6.29	1.44	90
C_{mic} (mg C kg^{-1} soil)	730.3	15.4	754.7	217.7	182.8	121.6	270
FDA (µg fluorescein g^{-1} soil h^{-1})	60.3	0.5	60.8	32.9	34.1	12.9	135

Univariate ANOVA was used to analyse the effects of three different factors (N fertilizer dose, organic amendment, year) on the measured soil parameters (Table 2). Interactions between the factors are also shown in Table 2. The N fertilizer dose had a significant effect on both the C_{org} and the grain yield (data not shown). The type of organic amendment influenced all the measured soil parameters significantly, as well as the grain yield (data not shown). The year also has a significant effect on all soil parameters except FDA hydrolysing activity.

Table 2. Effects of three different factors on the measured soil parameters. The significance levels are the results of univariate ANOVA.

Factors	C_{mic}	FDA	C_{org}	E4/E6	C_{mic}/C_{org}
Dose of N fertilizer (D)	0.594	0.422	0.003	0.220	0.418
Organic amendment (A)	0.000	0.000	0.000	0.001	0.000
Year (Y)	0.000	0.005	-	0.000	0.000
D*A	0.387	0.985	0.155	0.781	0.382
D*Y	0.145	0.975	-	0.635	0.117
A*Y	0.000	0.025	-	0.000	0.000
D*A*Y	0.382	0.999	-	0.786	0.346

Tables 3 and 4 show the mean values recorded in the different N fertilizer and organic amendment treatments and the results of the Duncan tests.

Table 3. Effect of the N fertilizer dose on the measured parameters. Letters after the mean values denote the results of Duncan tests.

Dose of N-fertilizer	C_{mic} (mg C kg^{-1} soil)	FDA (µg fluorescein g^{-1} soil h^{-1})	C_{org} (%)	E4/E6	C_{mic}/C_{org} (%)
N0	222.6 a	29.6 a	1.16 a	6.40 a	1.94 a
N1	228.7 a	31.1 a	1.20 b	6.36 a	1.91 a
N2	210.6 a	33.2 a	1.21 b	6.44 a	1.75 a
N3	217.4 a	36.0 a	1.18 ab	6.23 a	1.84 a
N4	209.0 a	34.4 a	1.19 ab	5.88 a	1.77 a

Table 4. Effect of organic amendments on the measured parameters. Letters after the mean values denote the results of Duncan tests.

Type of organic amendment	C_{mic} (mg C kg^{-1} soil)	FDA (µg fluorescein g^{-1} soil h^{-1})	C_{org} (%)	E4/E6	C_{mic}/C_{org} (%)
None	164.9 a	25.1 a	1.13 a	6.52 a	1.47 a
Farmyard manure	220.3 b	35.5 b	1.29 b	6.44 a	1.71 b
Crop residues incorporation	267.7 c	37.9 b	1.14 a	5.81 b	2.34 c

Correlations between the soil parameters were investigated using Pearson correlation analysis. The correlation coefficients and significance levels between the measured and calculated soil parameters are shown in Table 5. The correlation coefficients were not significant for C_{org} but were usually significant for C_{mic} and the C_{mic}/C_{org} ratio.

The results of correlation analysis showed that an increase in the E4/E6 ratio was correlated with a decrease in both the amount (C_{mic}) and activity (FDA) of soil microbial biomass (Table 5). An increase in the E4/E6 ratio thus led to lower humus quality.

Table 5. Correlation coefficients and significance levels between the measured and calculated soil parameters (C_{mic}/C_{org}, C_{mic}, C_{org}, E4/E6 and FDA).

	C_{mic}/C_{org}	C_{mic}	C_{org}	E4/E6	FDA
C_{mic}/C_{org}	1	0.989**	-0.064	-0.348**	0.263**
C_{mic}		1	0.075	-0.343**	0.268**
C_{org}			1	0.058	0.056
E4/E6				1	0.082
FDA					1

**: correlation significant at the 0.01 level.

Discussion

The results indicated that C_{org} was significantly affected both by the dose of N fertilizer and by organic amendments. These findings are in accordance with the results of other authors (Rees et al., 2001; Geisseler and Scow, 2014). One surprising result of the statistical analyses was that C_{org} did not correlate with any of the investigated soil parameters (humus quality, C_{mic}, C_{mic}/C_{org} and FDA). SOM has a dominant role in almost all soil properties, including the amount and activity of MB, but the present results suggest that the relationship is not linear. The lack of a correlation between C_{org} and C_{mic} could be the combined effect of various factors, such as the narrow range of C_{org}, the quantity and quality of other organic inputs into soil and the year effect.

In the last 32 years of the IOSDV experiment, different organic matter concentrations developed in the variously treated plots, but the range was narrow, with values varying between 1.03 and 1.42% C_{org} (Table 1). The quality of the organic matter showed greater variation, ranging (in a single soil type) from 4.8 to 9.6. E4/E6 ratios recently published by Nadi (2012) ranged from 2.1 to 13.5 for different Hungarian soils. The N fertilizer had no direct effect on the humus quality, but crop residue incorporation increased it (lower E4/E6 value means better humus quality). Unlike crop residue incorporation, the addition of farmyard manure had no significant effect on humus quality compared to the control. This finding emphasizes the importance of crop residue management (Chen et al., 2014, Nicholson et al., 2014). The amount of incorporated crop residue was dependent on the N fertilizer dose, so it had an indirect effect on humus quality.

The total amount of microbial biomass is usually relatively small, 50-2000 mg C kg^{-1} soil The soil microbial parameters, C_{mic} and FDA values were in the range normally found in arable soils (Blagodatskaya and Kuzyakov, 2013). The FDA values (average 32.2 mg fluorescein kg^{-1} soil h^{-1}) were within the range of 21-56 mg fluorescein kg^{-1} soil h^{-1}, reported by Adam and Duncan (2001). The dose of N fertilizer had no significant effect on the microbial parameters. The type of organic amendments influenced the quantity and activity of microbial biomass, which exhibited the highest values in the case of crop residue incorporation. These results were in accordance with those reported by Kautz et al. (2004), based on similar treatments in an IOSDV trial located in Berlin-Dahlem, Germany. This could be due to the fact that crop residue incorporation was carried out every year, while organic manure was only added every third year (in autumn 2013, deep ploughing).

FDA hydrolysing activity and C_{mic} together reflect the ratio of active and dormant microbes in the soil. Only a tiny portion of the total microbial biomass maintains an active state in soil without an input of easily available substrates, while a large proportion of living cells are inactive, dormant (Prosser et al., 2007; Blagodatskaya and Kuzyakov, 2013). In the present experiment these two values correlated with each other, so the treatments did not change the active/dormant microorganism ratio.

The C_{mic}/C_{org} ratio of agricultural and forest soils at neutral pH is very similar, in the range of 2.0 to 4.4% C_{mic} to total C_{org}, depending on the nutrient status and soil management (Anderson, 2003). The values calculated in the present work (1.47-2.34%) were within this range. Anderson and Domsch (2010) analysed over 100 plots with a long-term management history (at least 15 years to approximate a quasi-equilibrium state) from 26 sites at different locations in Europe, and found that the mean C_{mic}/C_{org} for crop rotation soils was 2.9, a value somewhat higher than that found in the present experiment, where the type of organic amendment and the year had a significant effect on C_{mic}/C_{org} ratio. Both farmyard manure and crop residue incorporation increased the C_{mic}/C_{org} ratio compared to the unamended treatments. A high C_{mic}/C_{org} ratio is indicative of the accumulation of labile C in the soil, forming a favourable environment for microbial growth, whereas a low ratio is usually closely linked to organic matter of poor quality (Cheng et al., 2013). It was reported by Anderson and Domsch (2010) that the pH had a pronounced effect on the C_{mic}/C_{org} ratio, so differences in pH between the IOSDV plots might explain the differences in C_{mic}/C_{org} ratio, together with the organic matter input into the soil. In the present experiments the pH values were not measured. However, such measurements are planned in a future step, to provide a better elucidation of the results.

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DECOMPOSITION OF SALIX AND POPULUS LEAVES IN THE AREA OF LAKE BALATON AND KIS-BALATON WETLAND

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Összefoglalás

A vízbe kerülő avar bomlása nagymértékben meghatározza az anyagforgalmi folyamatok alakulását, így annak vizsgálata kiemelt fontosságú. Kísérletünkben célul tűztük ki meghatározni fűz és nyár avar lebontási ütemét egy téli és egy nyári időszakban, makrogerinctelen szervezetek jelenlétében és azok hiányában, a Balaton és a Kis-Balaton területén. Ehhez a szakirodalomban is alkalmazott avarzsákos módszert alkalmaztuk, kis és nagy lyukbőségű zsákokkal. Meghatároztuk a bomlási ütemét, illetve a víz kémiai tulajdonságait (pH, vezetőképesség, NH_4^+, NO_3^-, SO_4^{2-}, PO_4^{3-}, Cl^-). A nyári időszakban az avar bomlása lényegesen gyorsabb ütemben történt, mint télen. A két víztest esetében elmondható, hogy az avar gyorsabban bomlott a Balatonban, mint a Kis-Balatonban. A vízkémiai változók tekintetében megállapítható, hogy nincs szignifikáns különbség sem a víztestek, sem a mintavételi helyek között.

Kulcsszavak: avar lebontás, fűz, nyár

Abstract

Decomposition of plant litter in water influences metabolic processes, so its investigation has a major priority. In our study the aim was to determine *Salix* and *Poplar* leaf mass loss in a winter and in a summer period in presence and deprivation of macroinvertebrates in the area of Lake Balaton and Kis-Balaton Wetland. As a common method in the literature, leaf litter bag technique was used with small and big mesh sizes. Decomposition rates and chemical parameters of the water (pH, conductivity, NH_4^+, NO_3^-, SO_4^{2-}, PO_4^{3-}, Cl^-) were determined. In summer decomposition was much faster, than in winter. In respect of the two study sites, leaves decomposed faster in Lake Balaton, compared to Kis-Balaton Wetland. In respect of the water chemical variables there was no significant difference between the water bodies and the sampling sites, either.

Keywords: leaf litter decomposition, *Salix*, *Willow*

Introduction

Deciduous leaf litter represents a major energy source for the food webs in fresh water habitats (Fisher et al., 1973). The decomposition rate of leaves depends on abiotic factors such as temperature, flow, physical abrasion (Petersen & Cummins, 1974), pH (Faye, 2006), nitrate and phosphate concentrations (Pozo, 1993), and biotic factors such as initial litter quality (Melillo et al., 1984), invertebrates and microbial colonization (Abelho, 2008). During leaf-litter decomposition, a succession of organisms takes place expressing an adjustment of biological communities to the direct environment (Frankland, 1998). Microorganisms colonizing leaves (Graça, 1993; 2001) accelerate leaf litter mineralization and enhance food quality for shredders (Gessner et al., 1999). Shredders comminute leaf litter into smaller parts and may consume fungi directly or compete with them for food resources (Prokhorov et al., 2007; Suberkropp, 1998).

The decomposition process can be affected by changes in biodiversity at various levels including species richness of microbial communities (Duarte et al., 2006), detritivorous invertebrates (Schälder & Brandl, 2005), and plant litter itself (Kominoski et al., 2007). In the last century, forested areas have been destroyed in many parts of Europe, especially lowland areas. The wood itself was used for many industrial purposes; the bare areas were used for pasture (Fujisaka et al., 1993).

An experiment was carried out to study leaf litter decomposition in two Hungarian midland freshwater habitats. Litterbags were filled with leaves of two species and placed into the water. The aim of the study was to determine leaf mass loss in litterbags in a winter and in a summer period that can correlate to bed morphology, chemical parameters or temperature. Other important factor was to investigate the impact of presence and deprivation of macroinvertebrates during the decomposition process. We hypothesized that leaf breakdown is slower in the water period and in the lack of shredding organisms.

Materials and methods

Site description

A lake (Lake Balaton: surface area 596 km^2; average depth 3.3 m) and a wetland (Kis-Balaton Wetland: 68 km^2) were chosen in southwestern Hungary to carry out the research. The study sites were Keszthely Bay (17°14'46.3" E and 46°43'32.1" N) and Kis-Balaton Wetland, Ingói Bay (17°11'46.4" E and 46°38'37.4"N N)(Fig. 1.).

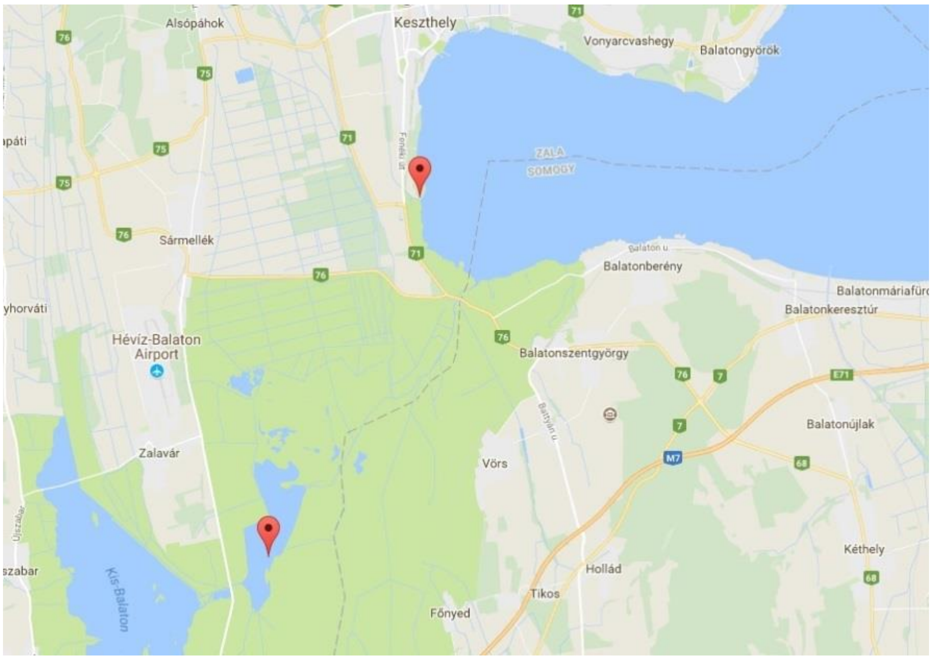

Figure 1: The locations of the two study sites (https://www.google.hu/maps)

At the sampling sites the dominant tree species is *Populus tremula* and *Salix Alba*. The depth of water is 1-2 m. The bed is composed of psammal/psammopelal (*Φ*>6 µm to 2 mm, sand/sand with mud, including organic mud und sludge), and at some points argyllal (*Φ*<6 µm silt, loam, and clay (inorganic).

Leaf-litter bags

Populus tremula and *Salix alba* leaves were collected shortly after the fall (2016 September) put into big paper boxes to avoid breaking and transported to the laboratory. The leaves were dried at 70 °C to constant mass and 10 g were put into two kinds of litter bags (following the method of Gessner, 2005). The size of the bags was the same (10 cm×10 cm), but the mesh size differed: 3 mm (leaf litter bag) and 900 µm (plankton net bag) mesh sizes were used to deprive and let the macroinvertebrates in. The bags contained only one kind of leaf, the leaves were not mixed. The top of the bags was glued carefully to keep the litter inside. Leaf litter bags were fixed to plastic racks and incubated *in situ* at 1 m depth below the surface in the littoral zone. In the field bags were sprinkled to avoid leaf-litter break before they were placed into the water. The experiment was set up twice, in a winter (30 November 2016 - 17 May 2017) and in a summer (25 May 2017 - 3 August 2017) period. At once three litterbags were collected from each site. The oven dry mass of the cleaned leaves were measured in the laboratory, to determine leaf mass loss. Exponential decay coefficients (*k*) for leaves were calculated by using the exponential decay curve below. Regression analysis assuming negative exponential decay was used (Boulton & Boon, 1991): $M_t=M_0e^{-kt}$, where M_t is mass at time *t*, M_0 is mass at time 0, *k* is exponential decay coefficient and *t* is time in days. Based on their daily decay coefficients, leaves have been classified as “fast” ($k > 0.01$), “medium” ($k = 0.005\text{-}0.01$) and “slow” ($k < 0.005$) (Bärlocher et al., 2005; Petersen & Cummins, 1974).

The halving times of the detritus were calculated using the formula proposed: $T_H = \ln2 \cdot k^{-1}$. Only those data were adverted, where the bags were not empty.

Physical and chemical parameters of the water

Conductivity and pH and were measured in the field with a multi-parameter field sensor (Neotek-Ponsel Odeon). Water samples were taken each time, the main chemical variables (NO_3^-, NH_4^+, SO_4^{2-}, PO_4^{3-} and Cl^-) were analyzed in laboratory (Lovibond MultiDirect photometer).

Results and discussion

Leaf mass loss

Decay curves are well characterized by exponential decay models for all leaf species at both sites in both seasons (Fig. 2.). In summer decomposition was much faster, than in winter. In respect of the two study sites, leaves decomposed faster in Lake Balaton, compared to Kis-Balaton Wetland.

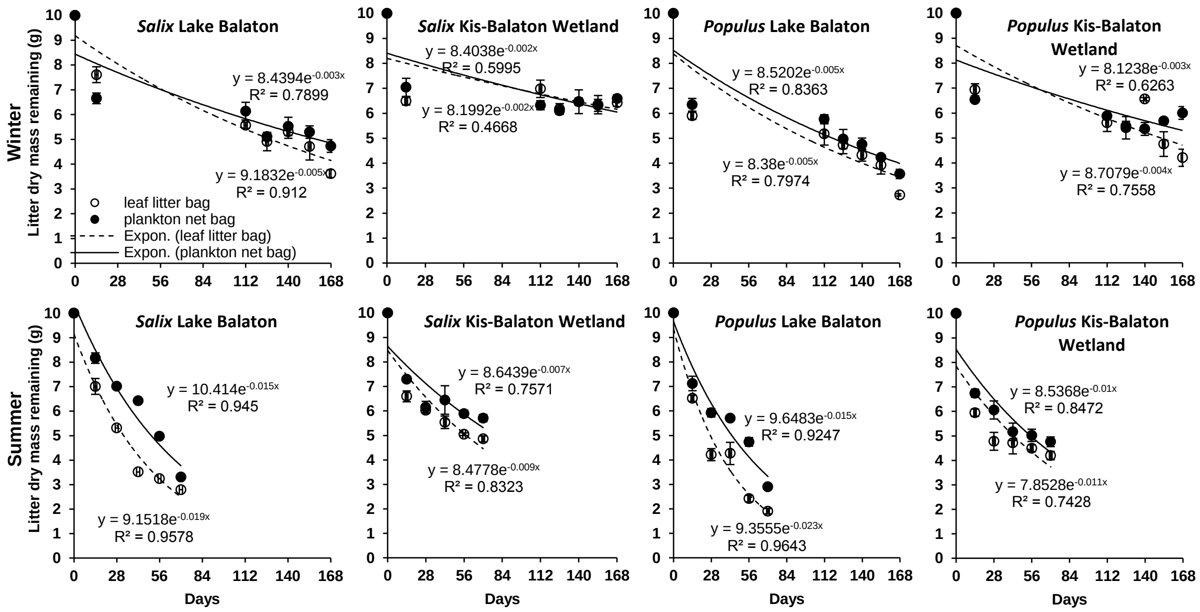

Figure 2: The litter dry mass of Salix and Poplar leaves remaining in the litterbags in Lake Balaton and Kis-Balaton Wetland in the winter and summer period

In Lake Balaton the leaf litter decay in the bags was fast for double reasons. On one hand, there is a shortage of food and on the other, drifting is higher in the open water bed, than at the other location.

Table 1: Exponential decay coefficients (k) and halving times of Salix and Populus in the winter and summer period in Lake Balaton and Kis-Balaton Wetland

Season	Leaf litter	Study site	Type of bag	Exponential decay coefficient±SD	Decomposition category	Halving time (day)
Winter	*Salix*	Lake-Balaton	leaf litter bag	0.0081 ± 0.0051	medium	85.1
			plakton net bag	0.0090 ± 0.0090	medium	76.8
		Kis-Balaton Wetland	leaf litter bag	0.0081 ± 0.0102	medium	85.9
			plakton net bag	0.0073 ± 0.0080	medium	95.4
	Populus	Lake-Balaton	leaf litter bag	0.0121 ± 0.0115	fast	57.1
			plakton net bag	0.0105 ± 0.0098	fast	65.9
		Kis-Balaton Wetland	leaf litter bag	0.0086 ± 0.0079	medium	80.5
			plakton net bag	0.0089 ± 0.0096	medium	77.7
Summer	*Salix*	Lake-Balaton	leaf litter bag	0.0190 ± 0.0068	fast	36.5
			plakton net bag	0.0114 ± 0.0043	fast	61
		Kis-Balaton Wetland	leaf litter bag	0.0143 ± 0.0083	fast	48.5
			plakton net bag	0.0111 ± 0.0060	fast	62.5
	Populus	Lake-Balaton	leaf litter bag	0.0217 ± 0.0073	fast	31.9
			plakton net bag	0.0148 ± 0.0062	fast	46.8
		Kis-Balaton Wetland	leaf litter bag	0.0178 ± 0.0102	fast	38.8
			plakton net bag	0.0163 ± 0.0090	fast	42.6

Exponential decay coefficients (k) and halving times of *Salix* and *Populus* in the winter and summer period in Lake Balaton and Kis-Balaton Wetland are shown in Table 1. Higher decomposition rates were detected in summer, compared to the winter period. The *k*-values of *Populus* leaves were higher than that of for *Salix* in Kis-Balaton Wetland in two mesh size litterbags.

Using two mesh sizes has an effect on macroinvertebrate flow and activity. Bigger mesh size results faster fragmentation, catabolism and leaching (Danell & Sjöberg, 1979; Brock et al., 1985; Neely, 1994). In our study this was detectable only in the summer period. Seasonal investigation causes faster mass loss in the summer period (Wrubleski et al., 1997). The timing of leaf collection, and its water content (dried or fresh) also contributes to the observed fluctuation of leaf litter decay rates (Gessner 1991).

The degree of leaf litter decomposition shows a declining trend, which is confirmed by the experiment of Pérez-Corona et al. (2006) in Spain.

One leaf litterbag contained only one kind of leaf. However, because of natural input and drift, some leaves may have attached to the outer surface of the bags. Recent research shows that mixed leaf species accelerate leaf litter decomposition (Markus & Gessner, 2009).

Physical and chemical parameters of the water

The seasonal average values of the water chemical variables (Tab. 2.) at the sampling locations corresponded, at average, to similar values from streams in the Carpathian Basin (Kovács et al., 2006). The pH values of the study sites were slightly alkaline. Nitrate was under range during the study period. The water chemical results of the surveyed areas are in line with expectations in Hungary. Based on the measured water chemistry parameters, it is concluded that the water chemical conditions of the sites were not extremely good and not extremely poor either. There was no significant difference between the water bodies and the sampling sites, either.

Table 2: Physical and chemical parameters of the water in the winter and summer period in Lake Balaton and Kis-Balaton Wetland

	Winter		**Summer**	
	Lake Balaton	**Kis-Balaton Wetland**	**Lake Balaton**	**Kis-Balaton Wetland**
pH	8.3 ± 0.5	8.0 ± 0.4	8.7 ± 0,6	8.8 ± 0.2
conductivity ($\mu S\ cm^{-1}$)	661 ± 48.9	701 ± 151	823 ± 517	597 ± 120
NH_4^+ ($mg\ l^{-1}$)	0.47 ± 0.13	0.59 ± 0.15	0.53 ± 0.28	1.14 ± 0.19
SO_4^{2-} ($mg\ l^{-1}$)	90.4 ± 16.2	82.1 ± 27.9	108 ± 32.8	72 ± 9.96
PO_4^{3-} ($mg\ l^{-1}$)	0.22 ± 0.06	0.37 ± 0.34	0.21 ± 0.07	0.4 ± 0.24
Cl^- ($mg\ l^{-1}$)	17 ± 6.4	12 ± 3.2	24 ± 3.7	10 ± 4.5

Conclusion

In conclusion, leaf mass loss was faster in summer, compared to the winter period. Due to higher drifting in Lake Balaton, a greater fragmentation of leaves was detectable. Using big mesh size allows macroinvertebrate actions, which makes fragmentation, catabolism and leaching faster. In our study the results show that the activity of macroinvertebrates is not essential to increase the decomposition rates in the winter season.

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DECOMPOSITION OF SALIX AND POPULUS LEAVES IN STANDARD CLASS "A" EVAPORATION PANS

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Összefoglalás

A lebontás és a párolgás a vízi ökoszisztémák alapvető mutatója, ennek ellenére ritkán vizsgálják őket egymással összehasonlítva. Egy kísérletet állítottunk be négy párolgásmérő A-káddal. Az egyiket vízzel töltöttük meg (kontroll kád), míg a többi hármat balatoni üledékkel, amire avarzsákokat helyeztünk. A kádakban mértük a lebontás ütemét és a napi párolgást. Az avar lebontása az exponenciális lebontási görbét követte, a lebontási ráták a gyors kategóriába estek. A kádakban a víz hőmérséklete a léghőmérséklettől függött. A meteorológiai elemek közül a levegő napi középhőmérséklete jelentősen befolyásolja a kádak vizének hőmérsékletét, a párolgás mértéke a hőmérséklettel növekszik.

Kulcsszavak: avar lebontás, fűz, nyár, A-kád

Abstract

Decomposition and evaporation are both basic measures of aquatic ecosystems, nevertheless they are rarely studied compared to each other. A research was carried out with four class “A” evaporation pans. One of them was filled with water (control pan), while the other three with sediment from Lake Balaton, on which leaf litter bags were placed. Decomposition rate and daily evaporation were measured in the pans. Leaf mass losses followed the exponential decay curve, decomposition rates were in the fast category. Water temperature in the pans was driven by air temperature. Among the meteorological elements, the daily average air temperature greatly influences the evaporation values, the rate of evaporation increases with warming.

Keywords: leaf litter decomposition, *Salix*, *Willow*, class “A” pan

Introduction

In aquatic environments leaf litter input from the coastal vegetation represents a major nutrient and plays a basic structural and functional role in several ecosystems (Fisher et al., 1973). Pan evaporation (E_p) is a basic physical measure of atmospheric evaporative demand widely measured using standard containers filled with water. Due to its simplicity and low cost, E_p measurement networks have been established worldwide under an institutional framework of meteorological services over half a century (Lim et al., 2013). Ep measurements have many functions, for example the estimation of a water budget (Kisi, 2015).

Decomposition studies are usually carried out under natural circumstances (*in situ*) or in the laboratory (*in vitro*), but not in class “A” evaporation pans. This new, pioneer method was chosen to investigate decomposition and evaporation together.

Materials and methods

The research was carried out at the Agrometeorological Research Station of Keszthely (latitude: 46° 44′ N, longitude: 17° 14′ E, elevation: 124 m above sea level), which belongs to the observation network of the Hungarian Meteorological Service (Fig 1.).

Figure 1: The locations of the study site (https://www.google.hu/maps)

Four Class A pans (Fig. 2.) were laid out in the meteorological garden from 25 May 2017 till 31 August 2017 (in the summer season). One of them was filled with water (control pan), while the other three with sediment from Lake Balaton, on which leaf litter bags were placed.

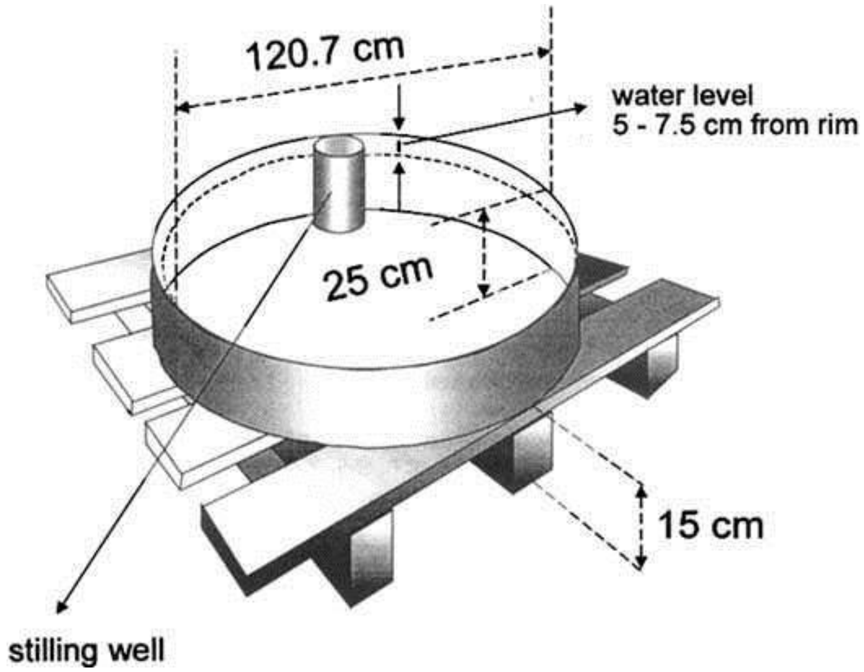

Figure 2: Class A evaporation pan (http://www.fao.org/docrep/X0490E/x0490e08.htm)

The Class A Evaporation pan is circular, 120.7 cm in diameter and 25 cm deep. The pan is mounted on a wooden open frame platform which is 15 cm above ground level. The soil is built up to within 5 cm of the bottom of the pan. It is filled with water to 5 cm below the rim, and the water level should not be allowed to drop to more than 7.5 cm below the rim. The water should be regularly renewed, at least weekly, to eliminate extreme turbidity. Pans should be protected by fences to keep animals from drinking. Pan readings were taken daily in the early morning at the same time that precipitation is measured. Measurements are made in a stilling well that is situated in the pan near one edge. The stilling well is a metal cylinder of about 10 cm in diameter and some 20 cm deep with a small hole at the bottom.

Populus tremula and *Salix alba* leaves were collected shortly after the fall (2016 September). The leaves were dried at 70 °C to constant mass and 10 g were put into two kinds of litter bags (following the method of Gessner, 2005). The size of the bags was the same (10 cm×10 cm), but the mesh size differed: 3 mm (leaf litter bag) and 900 μm (plankton net bag) mesh sizes were used to deprive and let the macroinvertebrates in. The bags contained only one kind of leaf, the leaves were not mixed. Leaf litter bags were fixed to plastic racks and incubated in the pans (Fig. 3.).

Figure 3: Class A evaporation pan with the litter bags fixed to plastic racks

At once one of each type of litterbags was collected from each pan. In the laboratory macroinvertebrates were separated from the samples and stored in 70% alcohol till classification. Sediment was thoroughly washed from the leaves under tap water. The oven dry mass of the cleaned leaves were measured, to determine leaf mass loss. Exponential decay coefficients (*k*) for leaves were calculated by using the exponential decay curve below. Regression analysis assuming negative exponential decay was used (Boulton & Boon, 1991): $M_t=M_0e^{-kt}$, where M_t is mass at time t, M_0 is mass at time 0, k is exponential decay coefficient and t is time in days. Based on their daily decay coefficients, leaves have been classified as "fast" ($k > 0.01$), "medium" ($k = 0.005$-0.01) and "slow" ($k < 0.005$) (Bärlocher et al., 2005; Petersen & Cummins, 1974). The halving times of the detritus were calculated using the formula proposed: $T_H= \ln 2 \cdot k^{-1}$.

Results and discussion

Leaf mass loss of the two species and the two kinds of bags followed the exponential decay curve (Fig. 4.). The curves of leaf litter bags and plankton net bags almost covered each other.

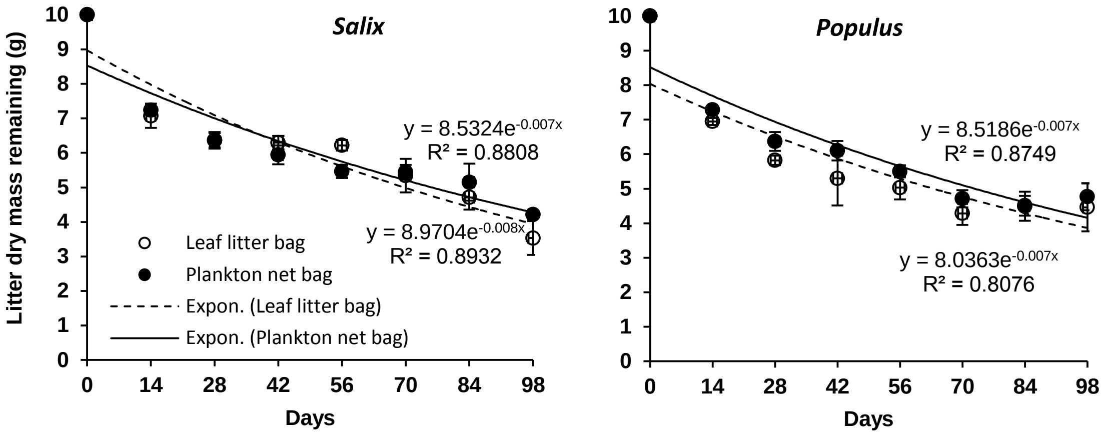

Figure 4: The litter dry mass of Salix and Poplar leaves remaining in the two kinds of bags

Both leaves in both types of bags fell into the fast category. Despite of the lack of drifting, high temperature in the pans caused fast decomposition rates, which matches the findings of Boulton & Boon (1991) under warm circumstances. Decomposition of deciduous leaves are less influenced by temperature, than conifer leaves (Whiles & Wallace, 1997). So the effect of temperature is regionally variable (Tam et al., 1998).

Table 1: Exponential decay coefficients (k) and halving times of Salix and Populus leaves in the pans

Leaf litter	Type of bag	Exponential decay coefficient±SD	Decomposition category	Halving time (day)
Salix	leaf litter bag	0.0127 ± 0.0055	fast	54.6
	plakton net bag	0.0125 ± 0.0050	fast	55.2
Pupulus	leaf litter bag	0.0146 ± 0.0057	fast	47.3
	plakton net bag	0.0127 ± 0.0047	fast	54.5

Water temperate varied from 19.5 to 32.9, while air temperature from 15.5 to 29.2 °C (Fig. 5.). Water temperature in the decomposition pans was continuously about 20% higher, than air temperature. The fluctuation of water temperature in the pans was driven by air temperature.

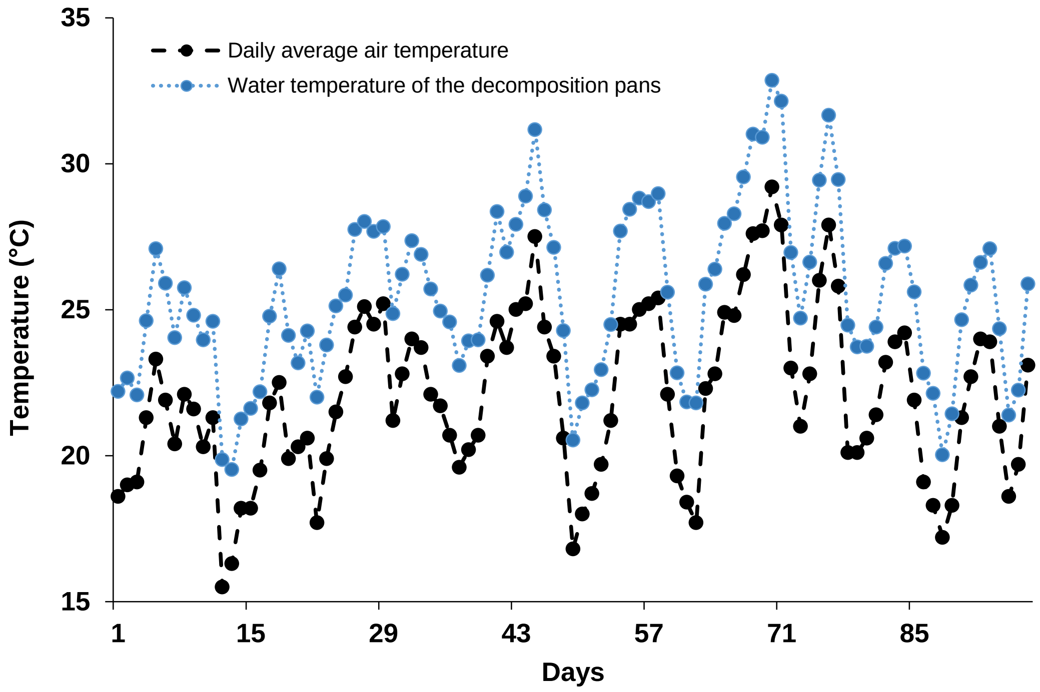

Figure 5: The daily average air temperature and water temperature of the decomposition pans

Among the meteorological elements, the daily average air temperature greatly influences the evaporation values, the rate of evaporation increases with warming (Fig. 6.) Evaporation of the control pan (R^2=0.2222) correlates better with air temperature, than the decomposition pans (R^2=0.2152). The measured evaporation values varied from 3.7 to 7.6 in the control pan, and from 3.3 to 7.5 mm day^{-1} in the decomposition pans.

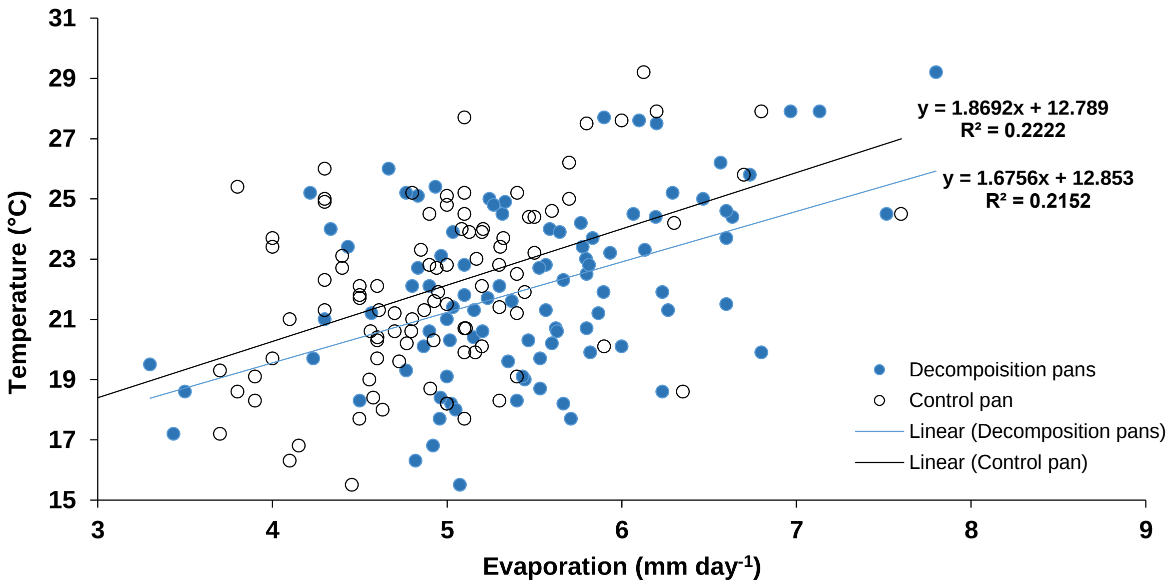

Figure 6: The daily average air temperature and evaporation of the decomposition and control pans

McMahon et al. (2013) proved, that the evaporation of water covered by macrovegetation is higher, than the evaporation of the open water surface. The evaporation of the decomposition pans were higher, than control pans. It can be explained by the dark color of the sludge and leaves, which – additionally with the biological processes of the plants - increased the water temperature and the radiation properties changed.

Conclusion

Leaf mass losses followed the exponential decay curve, decomposition rates were in the fast category. Evaporation has changed due to different circumstances (dark sediment and presence of biomass), which means a better correlation in case of the control pan with air temperature, than the decomposition pans. The experiment should be repeated as far as possible, further expanding of the examined environmental variables is needed.

Acknowledgement

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PRELIMINARY FINDING ABOUT SUGAR COMPOSITION AND ORGANIC ACID STRUCTURE OF WOODLAND GRAPE (*VITIS SYLVESTRIS* GMEL.)

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Abstract

In this study, a total of 6 *Vitis sylvestris* GMEL. genotypes were compared to cultivated grapes of *Vitis vinifera* L. i.e. their sugar composition and acid structure were measured by

HPLC (High Performance Liquid Chromatography) and AAS (Atomic Absorption Spectroscopy) in two different years (2014 and 2015).

The predominant sugars in *Vitis sylvestris* GMEL. and *Vitis vinifera* L. (Welschriesling and Pinot noir) berries were glucose and fructose. Considering the total acid content of woodland grape berries, the amount of tartaric acid was lower than the amount of malic acid, in both years. Succinic acid was found in some *Vitis sylvestris* GMEL. genotypes (in 2014), which was absent from control European grapevine cultivars. Element analysis examinations by AAS indicated a higher concentration of potassium in the total mineral content of woodland grapes, than of other minerals.

The preliminary results of this study pointed out interesting differences in the constitution of organic acids, sugar and mineral element content of the grape-juice of *Vitis sylvestris* GMEL. genotypes and *Vitis vinifera* L. cultivars.

Keywords: minerals, sugars, organic acids, woodland grape

Összefoglalás

Kutatásunkban 6 ligeti szőlő genotípust és termesztett kerti szőlő fajtákat hasonlítottunk össze illetve ezek cukor-, valamint savtartalmát mértük HPLC-vel (High Performance Liquid Chromatography) és AAS-el (Atomic Absorption Spectroscopy) két különböző évben (2014-ben és 2015-ben).

A cukrok közül a glükóz és a fruktóz volt legnagyobb mértékben jelen a *Vitis sylvestris* GMEL.és a *Vitis vinifera* L. fajták (Olasz rizling, Pinot noir) mustjaiban. Mindkét évben a ligeti szőlő mustjának összes savtartalmát nézve a borkősav mennyisége alacsonyabb volt, mint az almasavé. A 2014-es évben a ligeti szőlő beltartalmi értékeit vizsgálva a

borostyánkősav is kimutatható volt, ami a kerti szőlő fajták mustjaiból hiányzott. Az AAS vizsgálatok kimutatták, hogy az összes ásványi anyagot tekintve a kálium mértéke volt a legmagasabb a többi elemhez képest.

Az elsődleges vizsgálatok alapján elmondhatjuk, hogy jelentős különbségek vannak a *Vitis sylvestris* GMEL és a *Vitis vinifera* L. fajták mustjának sav-, cukor- és ásványi elem összetétele között.

Introduction

The evolution of cultivated plants played an important role in the ascent of humanity. Research of their origin and evolution started at the beginning of the 20th century, but till now, a lot of questions have remained open. A large number of theories exist about the evolution of the European grapevine (*Vitis vinifer*a L.).

The *Vitis sylvestris* GMEL. is a protected species in Hungary (Farkas, 1999). The quest and reservation of its populations are significant in terms of nature conservation and reserve of biodiversity as well. Based on theoretical and practical researches, this species is supposed to be, either in itself, or in a crossing with other species the possible progenitor of the European grapevine (*Vitis vinifera* L.) (De Candolle, 1894, Kozma, 1991 and Terpó, 1986).

Vitis sylvestris GMEL. occurs in flood basins, with tendrils for climbing and lobed leaves. Its berries are blue with 1-5 seeds (usually 2-seeded), which are brownish, short-billed. The exocarpium is naturally thin with gelled berry flesh and a low amount of must can be gained from it. The colour is solely provided by anthocyanins containing monoglycoside (Bartha & Kevey, 2010).

HPLC is used to analyse the content and composition of organic acids and sugars. Nowadays, several methods have been developed for identifying and quantifying these organic acids in grape juices and wines (so much) individually, like non-enzymatic spectrophotometric and enzymatic methods or as a group of them simultaneously, like chromatographic and electrophoretic methods (Mato, Suarez-Luque & Huidobro, 2005, Saavedra & Barbas, 2003 and Vereda, Garcia de Torres, Rivero & Cano, 1998).

Most methods for the analysis of sugars and organic acids in grapevine berries and wines that rely on high performance liquid chromatography (HPLC) have commonly used only the grape musts and/or juices with different sample extraction protocols (Castellari, Versari, Spinabelli, Galassi & Amati, 2000, Crippen & Morrison, 1986, Frayne. 1986, Liu, Wu, Fan, Li & Li, 2006, McCord, Trousdale & Ryu, 1984 and Sabir, Kafkas, & Tangolar, 2010). In analytical chemistry, the atomic absorption spectroscopy (AAS) technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed.

Sugars and organic acids are important primary metabolites, which contribute to grapevine growth and berry development. These compounds are also considered key factors in grape and wine quality. The hexoses, glucose and fructose, as well as the organic acids, like malic and tartaric acid are the most abundant compounds contributing to the grape juices' sweetness and acidity, respectively. Their concentrations and/or ratios vary during the berry development and maturation stages. Organic acids are produced in both the grape leaves and berries and start to accumulate in the grapevine berry at early stages of berry development (Conde et al. 2007).

It is very important to know the change in the acidity of the grapes since it affects the composition of the future wine (Kállay, 2010). It is important to determine the organic acids in grape juices and wines, because they have influence on the organoleptic properties (flavour, colour, and aroma) and on the stability and microbiological control of the products. Tartaric and malic acids are the predominant organic acids in grape juices and succinic, as well as citric acids are present in minor proportion. In the case of wines, a common differentiation is made between the acids which come directly from the grape (tartaric, malic and citric acids) and those that are originated, fundamentally, in the fermentation process (succinic, lactic and acetic acids) (Belitz & Grosch, 1992 and Peynaud, 1999).

Wineries need to monitor the concentrations of organic acids during the wine making process in order to ensure the quality of the products.

The low molecular organic acids can dramatically affect the pH values and can also have implications on biological stability, sensory properties (Caccamo, Carfagnini, Di Corcia & Samperi, 1986 and Tusseau & Benoit, 1987), and the colour of the wine (García-Romero, Sánchez-Munoz, Martín-Álvarez & Cabezudo-Ibánez, 1993).

The succinic acid is a two-phase acid with four carbon atoms. Formula: COOH-CH2-CH2-COOH. It has a slightly sour, unpleasant taste and is the secondary product of the alcoholic fermentation. In 1852, Pasteur (1859, 1969) showed that during the alcoholic fermentation, succinic acid always arises, in a concentration of approx. 1 g in 100 g of alcohol. In fact, the amount of generated succinate varies between 0.5 and 1.5 quantities g L^{-1} depending on the conditions of fermentation. This succinate content remains in the wine. With its typical complex salt and bitter - sour taste, it plays a great role in the typical wine taste, as the most flavourful acid in wine. It has an important physiological role, as the anion form of succinate is involved in the citric acid cycle (Kállay, 2010 and Krebs & Lowenstein, 1960).

The French paradox suggests that consuming red wine daily not only helps the cardiovascular system, but it also increases lifespan due to the resveratrol (found in the skins and tannins of red grapes) content in red wine (Catalgol, Batirel, Taga & Ozer, 2012). Resveratrol has been linked to preventing decline in cardiovascular function caused by age (Das, Mukherjee & Ray, 2011). France surpasses many countries in average life expectancy partly due to the common practice of drinking red wine with meals (Brownlee, 2006). However, not all wine is "created equal," with red wine containing eight times as many flavonoids as white wine (Catanese, 2013).

Among polyphenols, trans-resveratrol can be emphasized, about which (some publications mention) is said to have beside its antioxidant quality, a lot of other positive effects, e.g. regulating the cholesterol level, preventing renal insufficiency, inhibiting prostate tumour, as well as having a liver defending function, HIV disablement, retardation of the cerebral aging processes, prevention of gastric ulcer and the diseases of the lungs etc. The presence of minerals is always ambivalent, as some of the physiologically important ions make the wines instable. These are for example the K^{+}, $Fe^{2+/3+}$ and the Ca^{2+}, which showed noticeable differences in these technological experiences (Kállay, Májer, Jahnke & Veress, 2007).

This research is interesting because to our knowledge, different *Vitis sylvestris* GMEL. genotypes have not previously been studied by HPLC and AAS.

Based on elementary studies, the positive physiology effects of woodland grapes can be used in our future breeding program.

Materials and methods

The *Vitis sylvestris* GMEL. genotypes came from the area of Szigetköz and Fertő- Hanság National Park and were ex-situ conserved in the NARIC Research Institute for Viticulture and Enology, Badacsony. Berry samples were collected from the different *Vitis sylvestris* GMEL. genotypes and *Vitis vinifera* L. cultivars at harvest time in 2014 and 2015. Grape juice was pressed out of the berries, and samples for analyses were prepared as described later.

The organic acids and sugars were determined by HPLC (High Performance Liquid Chromatography) and the minerals by AAS (Atomic Absorption Spectroscopy).

For the HPLC analyses, samples were cleaned by active carbon and 0,45 NYL syringe filter. Shimadzu (Japan) system was used with Rezex ROA-Organic Acid H^+ (8%) column. The analysis was processed at a constant temperature of 41°C. 70 µl/sample was injected. For data recording and processing, LC Solutions software provided by the producer was used. (Shimadzu, Japan).

For AAS analyses, samples were prepared as follows: the must was centrifuged, and the clear supernatant was filtered. 1 ml of ionising buffer (36 g L^{-1} strontium-chloride) was added to 1 ml cleared must. For the determination of calcium and magnesium, an additional 8 ml (10x dilution), as for potassium determination 98 ml (100x dilution) of distilled water was added. For the determination of sodium, only 1 ml of ionising buffer was added to 10 ml of original cleared must (1,1x dilution), because of the low Na concentration of the must.

For the analyses, GBC 932 plus system and for the data processing, GBC Avanta Ver 1.33 software (GBC, Australia) were used. For the determination of potassium and sodium content of the samples, the emission at 766,5 nm and at 589 nm wavelength with Curent Lamp were measured respectively. For the determination of calcium and magnesium content of the samples, the absorption at 422,7 nm or 285,2 nm wavelength with Cathode or Hollow Cathode Lamp were measured respectively.

For the statistical analysis, two way ANOVA (Excel 2013 Analysis Tool Pak) was used.

Results

Table 1. shows the meteorological data in both investigated years (2014 and 2015).

Table 1: Meteorological data in the year 2014 and 2015, in Badacsony (Hungary)

Month	Sum of sunny hours			Temperature (°C)			Rainfall (mm)		
	Average of many years	2014	Difference	Average of many years	2014	Difference	Average of many years	2014	Difference
January	63,2	51,3	-11,9	-0,4	2,7	3,1	36,2	24,2	-12,0
February	95,2	72,5	-22,7	1,6	4,1	2,5	36,3	114,4	78,1
March	149,5	174,3	24,8	6,3	10,0	3,7	38,5	18,2	-20,3
April	189,5	207,0	17,5	11,8	13,1	1,3	45,4	34,2	-11,2
May	243,1	259,6	16,5	16,9	15,4	-1,5	57,5	77,3	19,8
June	253,3	298,1	44,8	20,1	20,9	0,8	75,4	58,2	-17,2
July	272,9	257,7	-15,2	22,0	22,6	0,6	71,8	103,8	32,0
August	249,5	252,5	3,0	21,5	20,3	-1,2	71,4	236,9	165,5
September	187,8	153,0	-34,8	17,2	16,5	-0,7	52,3	137,8	85,5
October	145,5	139,0	-6,5	11,9	13,0	1,1	45,6	91,9	46,3
November	67,1	88,0	20,9	5,9	7,8	1,9	62,0	37,3	-24,7
December	45,0	95,0	50,0	1,3	2,5	1,2	47,9	47,7	-0,2
Total:	1961,6	2048,0	86,4	_	_	_	640,3	981,9	341,6
Average:	_	_	_	11,3	12,4	1,1	_	_	_

Continuing Table 1: Meteorological data in the year 2014 and 2015, in Badacsony (Hungary)

Month	Sum of sunny hours			Temperature (°C)			Rainfall (mm)		
	Average of many years	2015	Difference	Average of many years	2015	Difference	Average of many years	2015	Difference
January	62,9	87,0	24,1	-0,3	1,9	2,2	36,0	63,2	27,2
February	94,8	104,0	9,2	1,6	1,9	0,3	37,7	57,4	19,7
March	150,0	176,0	26,0	6,3	6,8	0,5	38,1	16,2	-21,9
April	189,8	266,0	76,2	11,9	11,7	-0,2	45,2	4,0	-41,2
May	243,4	242,0	-1,4	16,9	16,4	-0,5	57,9	104,7	46,8
June	254,2	315,0	60,8	20,1	21,2	1,1	75,1	12,9	-62,2
July	272,6	300,0	27,4	22,0	24,7	2,7	72,3	38,7	-33,6
August	249,6	303,0	53,4	21,4	24,8	3,4	74,3	56,6	-17,7
September	187,1			17,2			53,8		
October	145,4			11,9			46,4		
November	67,5			5,9			61,6		
December	46,0			1,3			47,9		
Total:	1963,3			_	_	_	646,3		
Average:	_	_	_	11,4			_	_	_

Results of the HPLC and AAS analyses are detailed in Table 2.

Table 2. Sugars, organic acid and Na, K, Ca, Mg contents in grape berries (Badacsony, 2014-2015).

Accessions	Year	Glucose (g/l)	Fructose (g/l)	Glucose+ Fructose (g/l)	G/F ratio	Citric acid (g/l)	Tartaric acid (g/l)	Malic acid (g/l)	Succinic acid (g/l)
S-4/1	2014	84,77	84,61	169,38	1,00	0,61	8,43	15,10	4,14
S-4/2	2014	79,62	73,15	152,77	1,09	0,62	8,03	21,17	4,23
S-6/1	2014	83,15	75,64	158,79	1,10	0,44	5,23	24,92	6,22
S-6/2	2014	64,46	65,70	130,16	0,99	1,00	7,94	25,10	6,69
S-B.1	2014	99,56	108,43	207,99	0,92	1,53	8,65	21,52	1,87
S-B.48	2014	90,5	101,60	192,10	0,89	0,48	5,23	27,96	5,04
Vinifera	2014	80,48	61,67	142,14	1,31	0,38	6,29	5,38	0,10
S-4/1	2015	69,89	86,46	156,36	0,81	0,02	5,19	6,705	0,06
S-4/2	2015	70,72	86,32	157,04	0,81	0,03	4,79	7,605	0,12
S-6/1	2015	66,97	81,11	148,08	0,82	0,04	5,45	7,73	0,11
S-6/2	2015	44,90	59,51	104,41	0,75	0,06	8,45	8,05	0,13
S-B.1	2015	53,08	69,26	122,34	0,77	0,12	3,70	9,62	0,08
S-B.48	2015	64,74	84,01	148,75	0,77	0,02	7,74	7,03	0,08
Vinifera	2015	81,81	91,55	173,36	0,89	0,03	4,04	6,15	0,04

Varieties	Year	Na (mg/l)	K (mg/l)	Ca (mg/l)	Mg (mg/l)
S-4/1	2014	0,05	1159,12	187,99	106,00
S-4/2	2014	0,49	1510,95	94,70	57,00
S-6/1	2014	1,04	1943,95	228,85	89,30
S-6/2	2014	4,94	1803,06	280,46	211,80
S-B.1	2014	3,57	1001,81	131,79	136,20
S-B.48	2014	0,87	998,27	125,19	60,16
Vinifera	2014	3,02	1250,67	33,80	60,00
S-4/1	2015	0,585	1237,43	165,53	83,98
S-4/2	2015	0,90	1275,42	119,94	72,59
S-6/1	2015	1,54	1431,715	166,56	94,43
S-6/2	2015	0,85	1135,12	158,15	125,13
S-B.1	2015	1,77	1648,42	94,98	75,75
S-B.48	2015	0,90	1013,26	138,59	63,86
Vinifera	2015	1,36	1548,77	41,59	34,59

Figure 1. shows the average organic acid content of berries of *Vitis sylvestris* GMEL. accessions and *Vitis vinifera* L. cultivars in 2014 and in 2015.

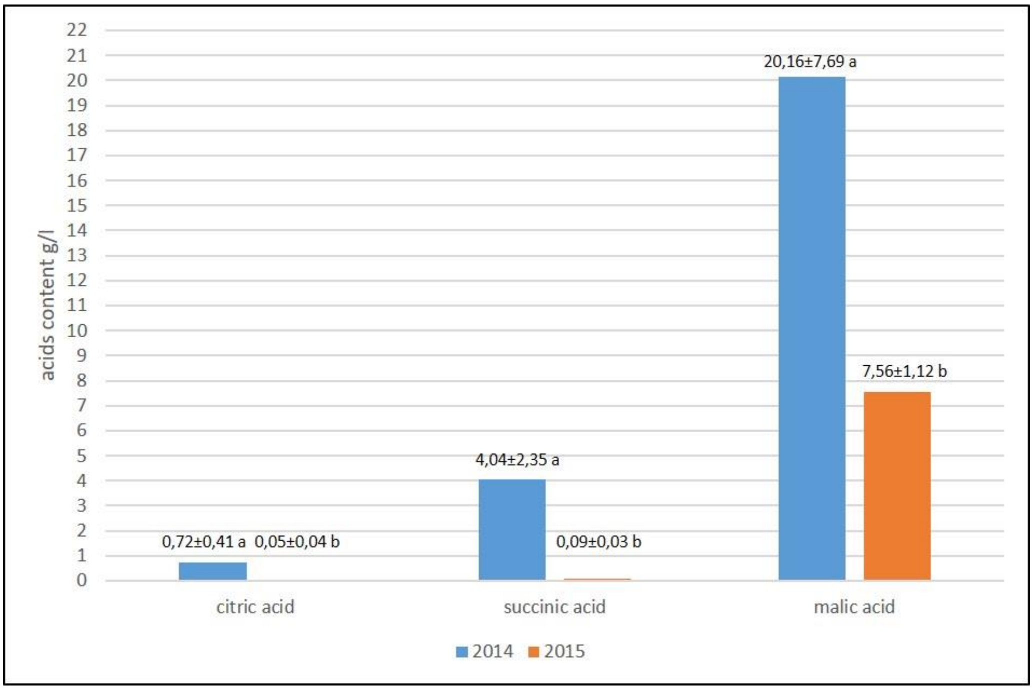

Figure 1.: Average organic acid content of berries of Vitis sylvestris GMEL: accessions and Vitis vinifera L. cultivars in 2014 and in 2015. Different letters mean significant differences between the investigated years of each organic acid (p< 0.05)

Figure 2. shows glucose and fructose contents of musts by years (2014 and 2015).

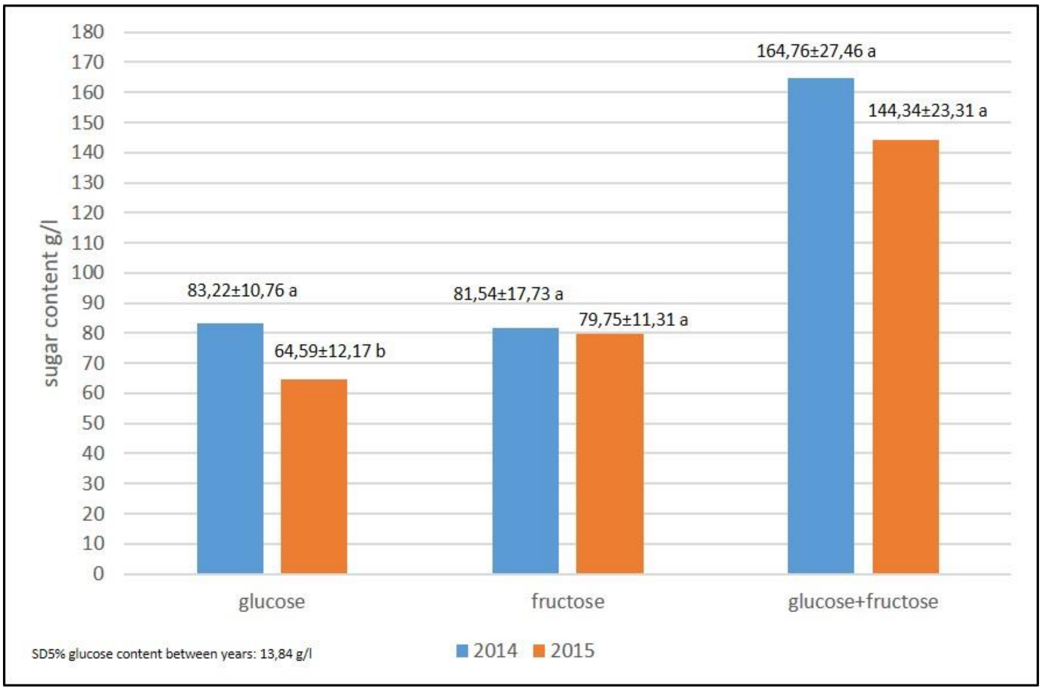

Figure 2.: Average glucose and fructose contents of musts by years (2014 and 2015) of Vitis sylvestris GMEL.accessions and Vitis vinifera L. cultivars. Different letters mean significant differences between the investigated years of each organic sugar ($p < 0.05$).

Figure 3. shows the glucose/fructose ratio (G/F) in grape berries in 2014 and 2015.

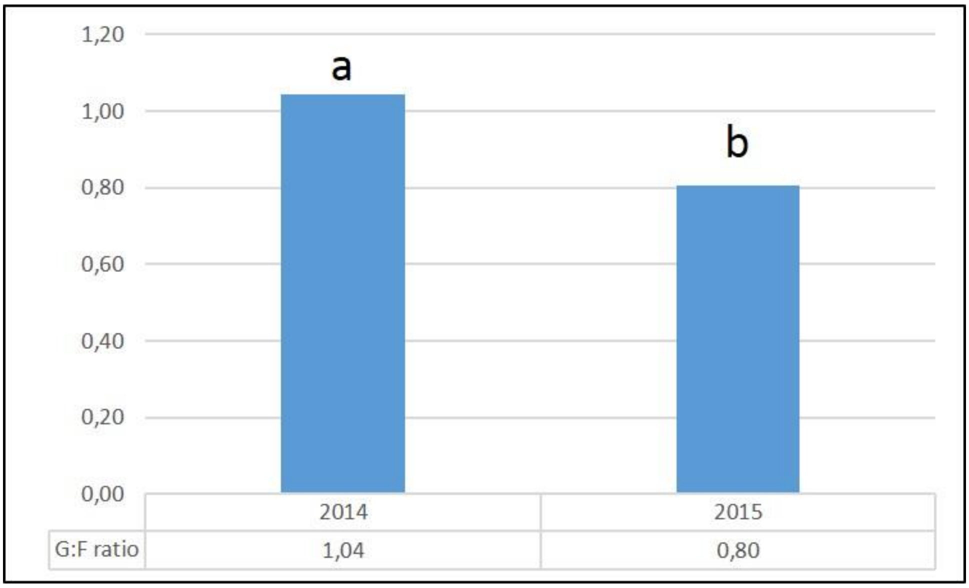

Figure 3. Glucose/fructose ratio (G/F) in grape berries in 2014 and 2015. Different letters mean significant differences ($p < 0.05$).

Figure 4. shows the Ca and Mg contents in grape berries in 2014-2015.

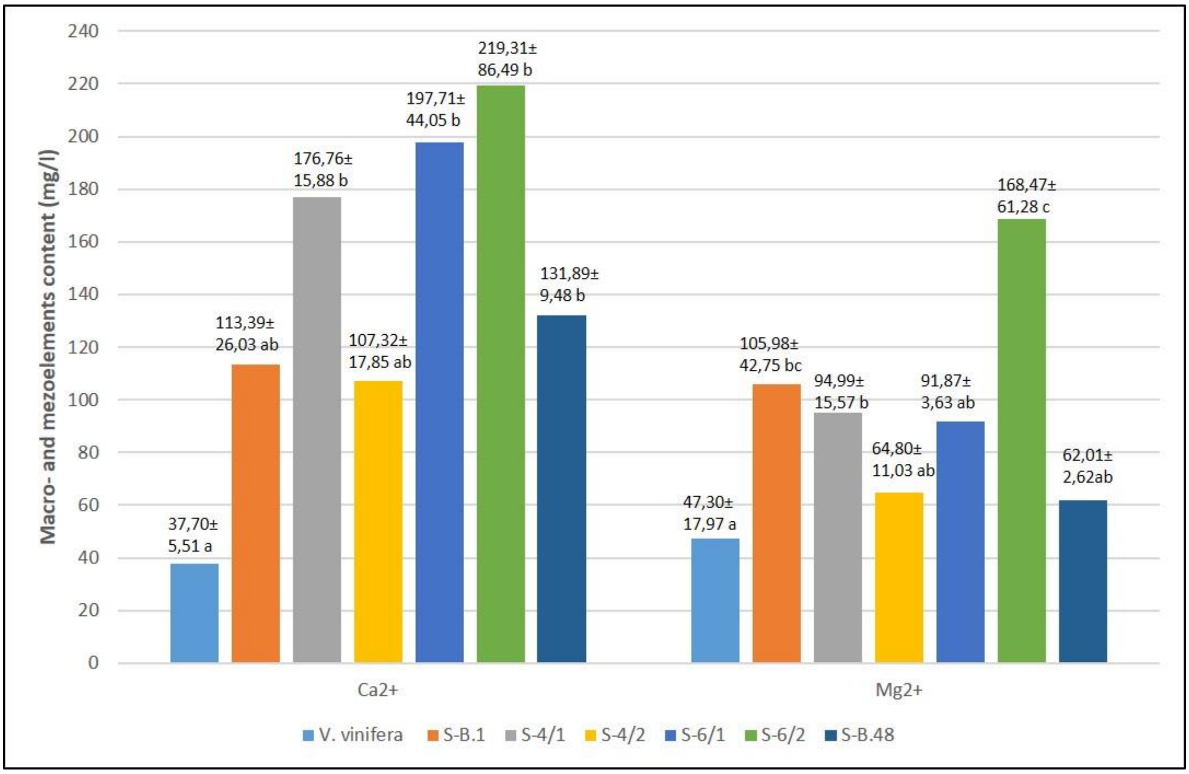

Figure 4. Ca and Mg contents in grape berries (Badacsony, 2014-2015). Different letters mean significant differences between the investigated genotypes and cultivars of each element (p< 0.05).

Discussion

The citric-, malic- and succinic acid content showed higher values in 2015 than in 2014, which was possibly caused by the difference in the weather conditions in the growing period (see Table 1. for the detailed meteorological data). The malic acid content in berries decreases during the ripening, while tartaric/malic acid ratio is in close connection with the maturation process itself. During the growing of the berries and even at veraison, malic acid content of the berries is prevalent, while tartaric/malic acid ratio is lower than 1 (Kliewer 1965, Kliewer 1967b). In 2014, the succinate content of the grape berries were considerably higher than in

2015 (Fig. 1.), which shows that the berries were less mature at harvest in 2014 than in 2015, due to the different weather conditions.

Musts of *Vitis vinifera* L. cultivars regularly contain 0,1-0,5 g L^{-1} citric acid, but musts originating from Botrytis-infected grapes can contain 1g L^{-1} (Kállay, 2010). The increased citric acid concentration of musts in 2014 can be traced back to the Botrytis infection.

The tartaric acid concentrations didn't show any significant difference between years or genotypes. The malic acid concentrations were significantly higher in 2014, than in 2015, because of the different weather conditions as mentioned before. No significant differences were found between genotypes regarding the malate concentrations of the musts.

The mature berries of the European grapevines contain a very low amount of succinate, but it always evolves during alcoholic fermentation (Pasteur 1859, 1969). The remarkable succinate content of woodland grape berries is a surprise.

The amount of fructose was almost the same in both years, but the glucose content showed significant differences (Fig. 2.). Significant difference was observed in the glucose/fructose ratio (G/F) between vintage years, the value was lower (0.80) in 2015 than in 2014 (1.04) (Fig. 3). As this ratio corresponds to the ripeness of the berries, and remarkable declines in G/F ratios can be observed up to veraison, this difference can be traced back to the different weather conditions in the years as well. During veraison, when the greater infiltration of sugars begins, the fructose content increases and the glucose/fructose ratio decreases rapidly. At the end of veraison, the glucose/fructose ratio approaches to 1 (Kliewer 1965, Kliewer, 1967a; Kliewer 1967b, Sabir, Kafkas & Tangolar, 2010.).

The musts of European grapevine cultivars contain small amounts of sodium (10-20 mg L^{-1}), which stagnate during the maturation (Table 2.). Plants take up potassium in high quantity, because it plays an important role in the regulation of the transport processes. The K concentration in must decreases in colder years and in case of stronger drought (Kállay, 2010). The observed pH of musts is primarily a reflection of the extent to which protons from the total acidity have been exchanged for potassium and sodium ions. During maturity, the uptake of potassium and sodium at constant total acidity can lead to a rise in pH of the must (Boulton, 1980).

According to the results, there were no significant differences in the sodium or potassium content of musts concerning the *Vitis sylvestris* GMEL. accessions and *Vitis vinifera* L. cultivars, or between the different vintage years (Table 2)

The amount of calcium and magnesium decreases in grape berries during ripening. Musts contain about 40-160 mg L^{-1} or 50-160 mg L^{-1} respectively (Kállay, 2010).

Magnesium ions have a positive effect on the protection of yeast cells against heat shock during fermentation (Birch & Walker, 2000).

In the measured values of Ca and Mg content, there were significant differences between genotypes. The *Vitis sylvestris* GMEL. accessions showed significantly higher values (Fig. 4.), which can be traced back to the partly immature state of the berries.

Conclusions

Based on the results, the following conclusion can be drown: at harvest time, the woodland grape (*Vitis sylvestris* GMEL.) accessions showed the same sugar content (glucose + fructose) and acidity as the European grapevine (*Vitis vinifera* L.), but the G/F ratio, acid composition (remarkable succinate in berries), and the significantly higher calcium and magnesium contents account for the unripe phenomenon in woodland grapes. Considering the climate change (global warming) processes, this phenomenon can be advantageous.

Acknowledgment

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INVESTIGATION OF THE LEACHING DYNAMICS OF A SUBMERSED MACROPHYTE (*MYRIOPHYLLUM SPICATUM*) IN THE AREA OF LAKE BALATON

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Abstract

The concentrations of the different nitrogen and phosphorus forms are key parameters in the ecological system, which can affect the aquatic organisms and the whole ecological balance in natural waters. In this study, from 22 September to 16 November 2017, we investigated the dynamics of nutrient dissolution during the degradation process of *Myriophyllum spictatum*, which is a dominant macrophyte in Lake Balaton. Glass bottles containing plant material and distilled water were incubated at natural temperature from which at specified intervals the liquid phase was removed. We measured the pH, conductivity, NO_3-N, NH_4-N and PO_4-P content of the water samples. The results showed that the NO_3-N and PO_4-P concentrations and the pH were the highest in the first 8 hours while the NH_4-N concentration reached the maximum on day 7. After the 14th day, all the tested parameters became permanent, only the conductivity was observed with greater variability. At the same time sampling was carried out from Lake Balaton and the change of these parameters was monitored.

Key Words: *Miriophyllum spicatum,* Lake Balaton, leaf litter decomposition, leaching

Összefoglalás

Az ökológiai rendszerben a különböző nitrogén- és foszforformák kulcsfontosságú paraméterek, amelyek hatással lehetnek a vízi élőlényekre és a teljes természetes ökológiai egyensúlyra. Kísérletünkben a Balatonban domináns hínárfaj, a *Myriophyllum spicatum* lebontási folyamata során végbemenő tápanyag kioldódás dinamikáját kísértük figyelemmel 2017. szeptember 22 és november 26 között. A növényi anyagot és vizet tartalmazó üvegpalackokat természetes hőmérsékleten inkubáltuk, melyről meghatározott időközönként a folyadékfázist eltávolítottuk. Mértük a víz pH-ját, vezetőképességét, NO_3-N, NH_4-N és PO_4-P tartalmát. Eredményeink azt mutatták, hogy az NO_3-N és PO_4-P koncentráció, illetve a pH az első 8 órában volt a legmagasabb, míg az NH_4-N koncentráció a 7. napon érte el maximumát. A vizsgált paraméterek a kioldódásának üteme egyenletessé vált a 14. napot követően, csupán a vezetőképességnél figyeltünk meg nagyobb változékonyságot. A mintavételekkel egy időben a Balatonból is vízmintát vettünk és figyelemmel kísértük e paraméterek alakulását.

Kulcsszavak: *Miriophyllum spicatum*, Balaton, avarlebontás, kioldódás

Introduction

When macrophytes die, the resulting decomposition processes can, in turn, substantially regulate the recycling of nutrients in fresh water ecosystems over an extended period of time (Pieczynska 1993, Shilla et al. 2006). The importance of each factor in regulating decomposition varies between ecosystems. During mineralization, organic nutrients, such as phosphorous and nitrogen are transformed into inorganic forms, which increases the internal nutrient loads (Ward et al. 2013). The growth of phytoplankton has been regarded as P-limited in many lakes worldwide (Schindler 1977, Correll 1998, Schindler et al. 2008). Shifts in N:P ratios can also shift the composition of phytoplankton species with flow-on effects (Elser et al. 2000).

The examination of the rate of nutrient leaching is important for the quality of the water in Lake Balaton.

Components released from the decomposition litter can contribute to the natural contamination of the water. In this study, the aim was to investigate the nutrient release from macrophyte litter (*Myriophyllum spicatum*) in Lake Balaton.

Materials and Methods

The study was conducted in Lake Balaton, which is a shallow, freshwater lake that lies in the southwestern Hungary. The sample site was in Keszthely Bay, in the littoral zone of the lake, 5 meters far from the bank (17°14'46.3" E and 46°43'32.1" N).

The leaching dynamics of the submersed macrophyte (*Myriophyllum spicatum*) were studied from 22 September to 16 November 2017, using the method of Gaudet and Muthuri

(1981). Release of nutrients during decomposition of the macrophyte litter was investigated *in situ* in Lake Balaton using 0.5 liter of volume glass bottles into which 0.45 liter destilled water and 25 g of oven dried macrophyte material were placed. The bottles were incubated *in situ* to 1 m depth in the water.

The changes in nutrient content of each bottle were analyzed after 0.4, 1, 3, 7, 14, 28 and 56 days by taking 450 ml of water from the triplicates for the determination of phosphorus, ammonium, nitrate, pH and conductivity. These parameters were also determined from the water samples taken from Lake Balaton. The physical andchemical variables (pH, conductivity, NO_3-N, NH_4-N and PO_4-P) were determined using a spectrophotometer (Lovibond MultiDirect) and pH and conductivity were determined with Neotek-Ponsel Odeon Digital Meter.

Results and Discussion

A decreasing trend was observed in the nitrate concentrations in the bottles (Figure 1.). The decrease in the first 14 days in the incubated bottles could be registered either due to the microbial utilization of nitrates by microbes for protein production or due to ammonia volatilation (Reddy and Sacco 1981), which was also evidenced by higher pH values recorded during the incubation in the bottles (Figure 4.). Subsequently, a slow increase in nitrate concentration was observed. The figure shows that nitrate dissolution (29.7 mg L^{-1}) is the fastest in the first 8 hours, and it is consistent from the seventh day (8-11 mg L^{-1}). Tamire et al. (2017) observed a similar tendency in the nitrate dissolution from *Arundo donax*, *Echinochloa colona*, *Potamogeton schweinfurthii*, *Cyperus articulatus*, *Typha latifolia*, *Cyperus papyrus* and *Nymphaea lotus*. In the littoral zone of Lake Balaton nitrate was not detectable (under 1 mgL^{-1}).

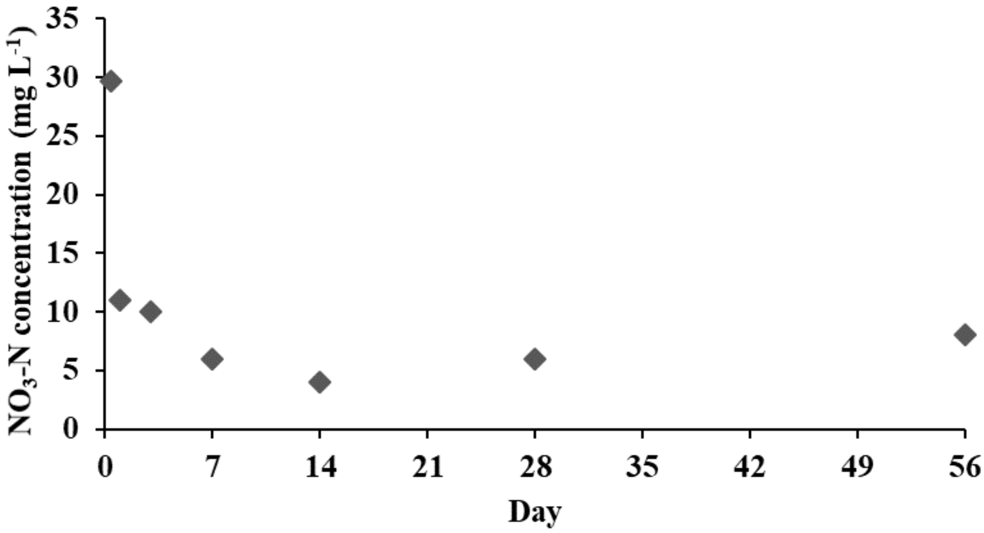

Figure 1. Change in NO_3-N concentrations in the water of the incubated bottles during the experimental period in Lake Balaton

The dissolution rate of ammonia is inversely proportional to nitrate. The concentrations of ammonia at each sampling time are shown in Figure 2. The concentration of ammonia increased intensively, peaked on the seventh day, which was followed by a sudden decrease. The average concentration of ammonia was 0.2±0.1 mg L^{-1}in Lake Balaton during the study period. The rapid increase of NH_4-N concentration at the beginning stage was mainly derived from releasing of the decomposition process, and the subsequent decrease was probably attributed to nitrification by nitrifying bacteria in the water. Wu et al. (2017) also observed a similar tendency . As well as their investigations showed that higher NH_3-N was obtained with higher dosage of the plant litter, for example, NH_3-N in the water increased from 2.58 to 14.30 mg L^{-1} when the dosage of *Eichhornia crassipes* litter increased from 0.1 to 1.0 g L^{-1}.

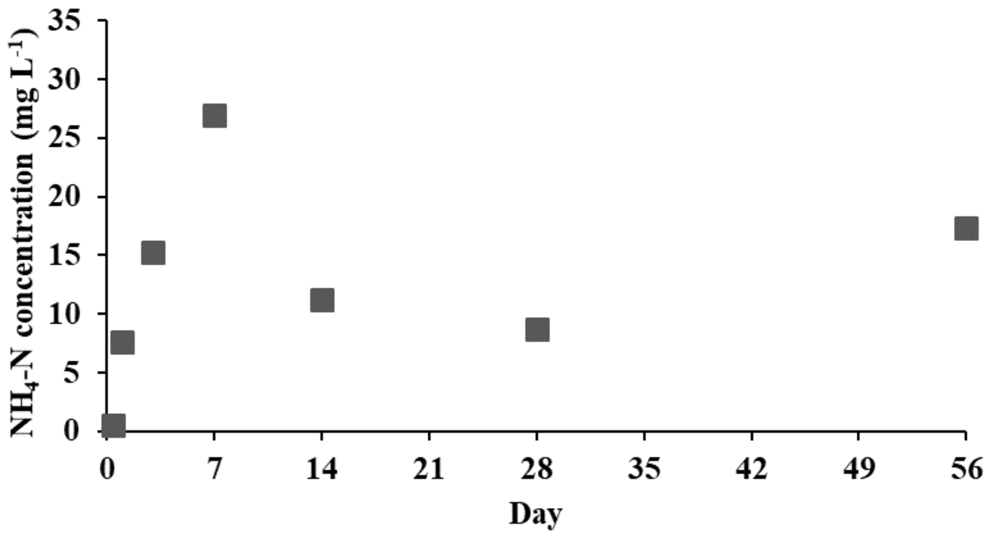

Figure 2. Change in NH_4-N concentrations in the water of the incubated bottles during the experimental period in Lake Balaton

Landers (1982) investigated the nutrient release of the submerged macrophyte, *Myriophyllum spicatum* during its annual dieback, and also demonstrated a greater impact on water column P than N. Park and Cho (2003) have reported that the leaching process of phosphorous from aquatic macrophyte litter can provide considerable contribution to the eutrophication of some aquatic ecosystems. According to our results, phosphorous concentration from the decomposition of macrophytes appears to be low in Lake Balaton (Figure 3.). The rate of phosphorous leaching is decreasing. The highest concentrations were measured on the first day (47.3 and 40.3 mg L^{-1}), from the 14th day degree of dissolution decreased by half (24.0-21.4 mg L^{-1}). The concentration of phosphorus was low (0.23±0.1 mg L^{-1}) in the littoral zone of the Lake Balaton from September to November. The change in the phosphorous concentration during the experiment was narrow. The increment in phosphate during this study, where the increase was from 21.4 to 47.3 mg l^{-1} in bottles, was much higher when compared with the experiment of Tamire et al. (2017) (with similar submerged macrophyte (*Potamogeton schweinfurthii*), where 3 g of dried macrophyte was incubated into 1 liter of water. They observed an increase from 35 µg L^{-1} to 160 µg L^{-1} of phosphate in Lake Ziway (Etihopia). Gibtan and Abera (2012) reported much higher phosphate concentration (212 µg l^{-1}) at the mouth of River Bulbula, than recorded in Lake Ziway.

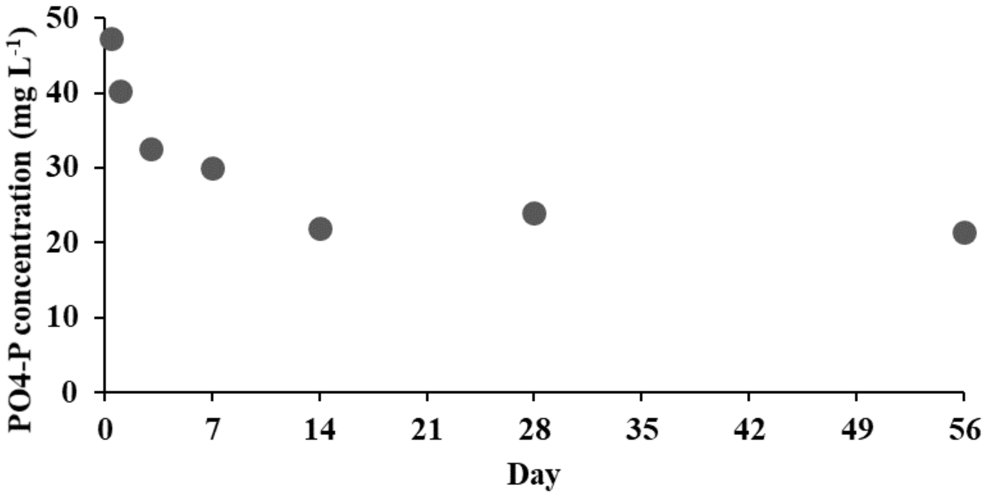

Figure 3. Change in PO_4- concentration in the incubated bottles water during the experiment period in Lake Balaton

The pH values of the water samples taken from the glasses are shown in Figure 4.a. At the first day, pH of the water grew rapidly and reached the maximum (pH=7.67), followed by the gradual decrease (1-7 day) and finally reached a stable value (14-56 day, pH= 6.3-6.5). The pH increment was probably due to the consumption of the organic acids by the microorganisms in the water (Gaudet & Muthuri, 1981). Later, pH became stable at the decomposition stage, it was likely that decomposition process was slowed down by lower leaching acids at this stage. The seasonal average of pH in Lake Balaton was 8.4±0.5 in the in investigation period.

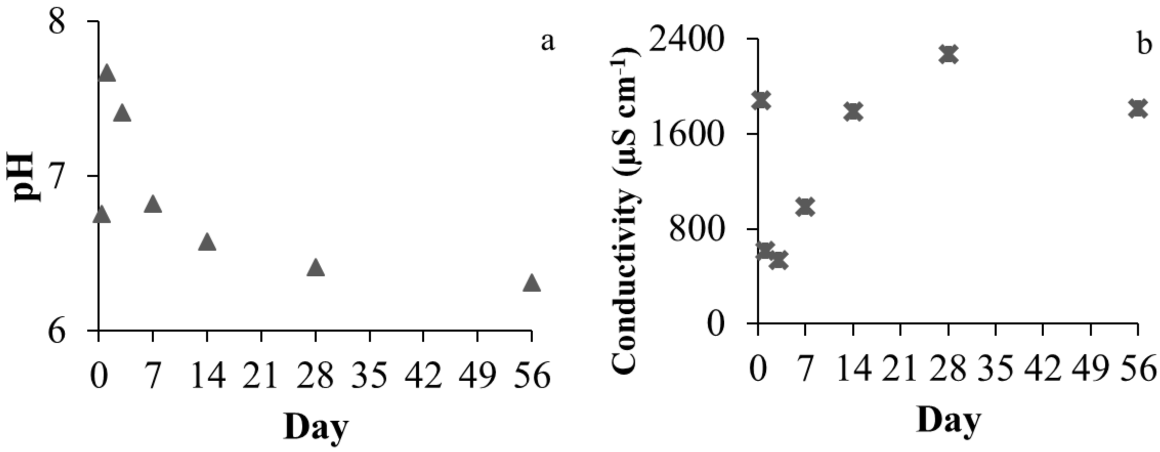

Figure 4. Change in pH (a) and conductivity (b) in the incubated bottles during the experiment period in Lake Balaton

Figure 4.b. shows the conductivity in the bottles. The high conductivity measured after the first sampling was followed by a sudden decrease in the first and third days, and after the third day it shows an upward trend. Dahroug et al. (2016) investigated the existence of temporal fluctuations of limnological parameters during *Eichhornia azurea* decomposition. The study showed that the conductivity significantly correlated with the density of water and bacterial biomass. According to Esteves (1988), conductivity values are related to the trophic state of the water. Thus, increased nutrient concentrations from the decomposition and subsequent release of ions affected the conductivity. The conductivity measured at natural conditions in Lake Balaton was 671.5±41.3 µS cm^{-1} during the investigation period.

Conclusions

In this study, decomposition processes of *Miriophyllum spicatum* litter were studied to determine the influence in aquatic environment, including pH, conuctivity, NH_4-N, NO_3-N, PO_4-P. In conclusion, this study showed that macrophyte species could release significant concentrations of nutrients when desiccated and rewetted. The relative high pH (8.4±0.5), low conductivity (671.5±41.3 µS cm^{-1}) and low NO_3-N (underrange), NH_4-N (0.23±0.1 mg L^{-1})

and PO_4-P (0.2±0.1 mg L^{-1}) concentration of the littoral zone of the lake could show the absence of significant effect of decomposition of the macrophyte on the water quality parameters of Lake Balaton in autumn.

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RENEWABLE ENERGY ALTERNATIVES IN CENTRAL AND EASTERN EUROPEAN COUNTRIES – THROUGH THE EXAMPLE OF HUNGARY

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Abstract

The European Union can be considered as the leader of the struggle for a new energy economy, in particular in the field of alternative energies and climate policy. The EU legislative and support framework for the promotion of the use of renewable energy has undergone significant development over the last few years. The feasibility and sustainability of the goals also lie in the productivity of the smaller territorial units and countries of the Union, thus, each country must find the development trends appropriate to their local circumstances, which must be materialized in sustainable investments. In the Central and Eastern European region (Hungary, Poland, the Czech Republic, Croatia, Slovakia, Slovenia), which has similar characteristics (historical, cultural, geographical regarding renewable energy forms), the renewable energy market is clearly underdeveloped compared to Western

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European countries. As a result, there are considerably more opportunities to develop green projects in the future. In our present examinations, we look at the directions of renewable energy development projects, which are already in process and are expected to accelerate in the future, through the example of Hungary.

Keywords: renewable energy market, East-Central Europe, Hungary, investments, green projects

Összefoglalás

Az Európai Unió megújuló energiaforrásokhoz kapcsolódó célkitűzéseinek teljesíthetősége és azok fenntarthatósága az Unió kisebb területi egységeinek, országainak teljesítőképességében is rejlik, így minden országnak meg kell találnia a helyi adottságaihoz illeszthető fejlesztési irányokat, melyeknek fenntartható beruházásokban kell testet ölteniük. Az Európai Unió több szempontból (történelmi, kulturális, földrajzi) hasonló adottságokkal rendelkező kelet-közép-európai térségének (Magyarország, Lengyelország, Csehország, Horvátország, Szlovákia, Szlovénia) megújulóenergia-piaca egyértelműen alulfejlett a nyugat-európai országokéhoz képest. Ebből adódóan lényegesen több lehetőség mutatkozik zöld projektek generálására a jövőben. Jelen vizsgálatainkban a már elindult, és a jövőben várhatóan felgyorsuló megújuló energia-ipari fejlesztések irányait tekintjük át Magyarország példáján keresztül. A témát felölelő vizsgálataink során a saját adatgyűjtéseken alapuló descriptív statisztikai módszerek mellett kvalitatív kutatás keretében 25 magyarországi szakértői/szakmai szervezeti véleményeket és tapasztalatokat feltáró strukturált mélyinterjú készült megújuló energiaforrások hasznosításának jelenére és jövőjére vonatkozóan, így tanulmányunkban az elmúlt időszakban felhalmozódott szakértői tapasztalatok, nézőpontok is megjelennek.

Kulcsszavak: megújuló energia-piac, Kelet-közép Európa, Magyarország, beruházások, zöld projektek

1. Introduction

1.1 Renewable Energy Objectives in the European Union

Following the enlargement of the EU in 2004, ambitious goals have been set for the EU-25. In March 2007, the European Council adopted the new EU Energy and Climate Package, which main objectives are sustainability, competitiveness and the security of supply. To implement the program, the EU has committed itself to increase the share of renewable energy sources to 20% in total energy consumption. Commitments should not be met at national levels, but at EU level on average, since the economic situation, the energy structure, the supply of resources and the energy consumption of the countries showed internationally significant differences. Directive 2009/28/EC on the promotion of energy from renewable energy sources sets a mandatory target for 2020 for all EU member states, which refers to the proportion of renewable energy sources within the final energy consumption.

In order to achieve this, the member states have adopted national action plans on renewable energy in 2010 and reported their national action plan on renewable energy sources to the European Commission. The targets of the national plans are between 10% (Malta) and 49% (Sweden). The European Commission regularly evaluates the progress achieved by the member states regarding the objectives of renewable energy by 2020 and has set even more ambitious targets[2].

[2] In June 2016, the European Parliament adopted an attitude to the Renewable Energy Report demanding the European Commission to come forward with a more ambitious package on climate and energy policies by 2030 that will raise the EU's renewable energy target to at least 30%, which is to be achieved through specific national targets.

According to the latest resources available at the time of the preparation of the present study (Eurostat, 2017), 13% of the energy used in the European Union in 2015 came from renewable energy sources, compared with 8.5% recorded in 2005, the starting year of data recording.

According to the statistics of Eurostat (2015), Croatia (joining the Union in 2013) has reached the target set for the country by 2020 in 2015, some other countries (Denmark, Lithuania, Italy) have a few percentage to complete the target. Almost all the other member states will have to make further efforts to reach the 2020 targets. However, it is important to emphasize that many other countries have set up very ambitious goals – depending on their economic development and other characteristics (Denmark 30%, Austria 34%, Finnland 38%, Latvia 40%, Sweden 49%).

2. Material and Methods

Future uncertainties in energy supply, economic efficiency issues, climate protection efforts have also raised the value of renewable energy in the Central and Eastern European region. In our study we will review the results regarding the realization of the EU commitments so far for the Central and Eastern European countries (Hungary, Poland, the Czech Republic, Croatia, Slovakia, Slovenia). The present essay is a 'snapshot' which gives a picture of the results of the recent period and shows the realization of EU development concepts and targets through the example of Hungary. We supplemented the data provided by the European Union and the member states with the available data provided by international and national renewable energy organisations, associations, statistics and own data collection. In this part of the research, we used mostly simple, descriptive statistical methods, such as distribution ratios, quantification of proportion changes and descriptive analysis of certain time series (Barna-Molnár 2004, Molnár 2015). In addition, in the framework of qualitative research, structured in-depth interviews with 25 Hungarian experts/professional organizations were made exploring their opinions and experiences on the present and future of the utilization of renewable energy sources. The sampling process was conducted using ’snowball’ method (based on expert recommendation), the survey was carried out between September 2017 and January 2018. In the present essay, the partial results of this exploratory research – as well as the accumulated expert experiences and perspectives – are published.

The global development of the renewable energy industry creates opportunities for the transformation of the national economy, the comprehensive production and market reforms, the production of marketable goods, job creation and job restructuring in the Central and Eastern European region – thus, also in Hungary –, which has similar characteristics in several aspects (historical, cultural, geographical regarding renewable energy forms). The results of our research, the conclusions on this topic can help regions with similar characteristics in investment decisions, in the development of a suitable renewable energy mix. The research was supported by the ÚNKP–17-4 New National Excellence Program of the Ministry of Human Capacities.

3. Results

3.1 Renewable Energy Sources in Central and Eastern European Countries

In the examined Central and Eastern European region, energy saving and the increase in the utilization rate of renewable energy sources are on the agenda not only due to the requirement to achieve the related EU objectives, but due to the fact that it also may decrease energy import significantly, which can bring environmental aspects to the fore. A significant part of the buildings in the region is outdated regarding energy efficiency, they waste energy. Recent targeted EU and national tender opportunities have contributed to energy efficiency improvements and the inclusion of renewable energy sources into energy systems significantly.

Table 1 shows the ratio of renewable energy and the distribution of renewable energy sources by categories in the wider region. The 2020 target ratio for renewable energy in the region is between 13.5% (the Czech Republic) and 25% (Slovenia). The commitments of Croatia (20%) and Slovenia (25%) are forward-looking, while those of the other countries are more modest, depending on their economic indicators and strategic objectives, between 13.5 and 15.5 %. Hungary (14.65%) is in the middle, ahead of the commitments of Slovakia and the Czech Republic.

Table 1: The typical values of the ratio of renewable energy and the distribution of renewable energy sources by categories in the countries surveyed in 2015

Denomination		**Hungary**	**Poland**	**Czech Republic**	**Croatia**	**Slovakia**	**Slovenia**
Share of renewable energy in 2005 (%)		4,3	7,2	6,1	12,8	6,7	16,0
Share of renewable energy in 2015 (%)		12,0	9,4	10,1	23,0	9,6	16,1
Target of share of renewable energy till 2020 (%)		14,65	15,5	13,5	20,0	14	25,0
Proportion of renewable energy by categories (2015, %)	Solar energy	1	1	5	1	3	3
	Biomass and waste	93	87	90	67	75	61
	Geothermal energy	3	0	0	0	0	4
	Hydropower	1	2	4	28	21	31
	Wind energy	2	11	1	3	0	0

Source: Own editing based on datas from REN21 (2016) and Eurostat Statistics Explained (2017)

It can be concluded that the progress of member states in the survey is partial and extremely uneven. Within the utilization of renewable energy, solid biomass has a predominant role in some countries (Hungary, Poland, the Czech Republic), while in others – due to their geographic features – water resources are the most important (Croatia, Slovakia, Slovenia). There are several reasons for the difficulties encountered in advancing further renewable energy forms. The higher costs of renewable energies seemed unaffordable to the population from the viewpoint of their income and also to the corporate sector that has emerged from the crises and transformation processes of the last decades. Inadequate, in some cases insufficient information, administrative problems, difficult licensing and commissioning procedures, as well as the long-term unpredictability of the delivery prices of small- and medium-sized power plants using renewable energy sources did not favour the spread of these solutions.

3.2 Renewable Energy Alternatives in Central and Eastern European countries – through the example of Hungary

In the current global industrial environment, the mining of fossil fuels is unprofitable in many cases in Hungary. Regarding the composition of primary energy production, the yield of natural gas, oil and coal has almost halved over the past fifteen years. Nowadays, one third of the resources to cover energy needs comes from domestic production and two thirds from imports.

Before the millennium, electricity supply was based on fossil and nuclear power plants almost exclusively, today renewable energy sources have also been involved, but most of the Hungarian production (40%) is the electricity from nuclear power[3].

The ratio of energy from renewable sources within the consumption is rising steadily, in 2015 it was 12%, approaching the EU average of 13% (Eurostat, 2017). The proportion of electricity produced from renewable sources was 7.3% in 2015. Looking at the data of the last ten years, it is clear that the increase in the ratio of renewable electricity stopped in 2011, then it started to grow again in 2013. A change in the legal environment of mixed combustion

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[3] The expansion of the Paks Nuclear Power Plant, signed on 14 January 2014, includes the construction of two new nuclear power plant blocks in Paks. The new nuclear power plant blocks will be constructed next to the four 500MW nuclear power plant reactors set up in Paks in 1982. As a result of the life-enhancing investment, which is a decisive element of the energy mix in Hungary, the capacity of the nuclear power plant will increase by 20% in the long run. (http://www.atomeromu.hu/teljesitmenynoveles, 2016)

power plants, and hence the fluctuations in biomass-based production play a role in this process. After a few years of stagnation, the renewable ratio of transport has also increased, it was between 6-7% in the last five years. The heating and cooling renewable ratio is around 20%. The increase in the ratio of renewable energy in recent years is due to the increase in the use of renewable energy and the decreasing gross final energy consumption (MEKH, 2017; Németh, 2017)

The expansion of usage of renewable energy sources in Hungary is hindered by a number of economic and social factors also (Varjú, 2013). The number and total performance of the renewable investments actually realized is steadily increasing, but there are definitely bigger opportunities than the current utilization. The commitments of the European Union and the EU funding opportunities for 2014-2020 also urge answering questions in the area and creating a more favorable and transparent situation.

3.2.1 Renewable Energy Market Situation Report – Solar Energy

Within the ratio of renewable energy, the utilization of solar energy was the most common in the Czech Republic (5%), Slovakia (3%) and Slovenia (3%) in the examined region in 2015. In Hungary, Croatia and Poland the ratio is around 1%. Despite the small ratio of solar energy, the region has been experiencing steady growth over the past ten years. This situation is due to the economies of scale of the solar cell market (retail prices have fallen considerably), the spread of incentive support schemes and the presence of more environmentally-conscious consumers. The latter are mainly from the younger generations and the wealthier older generation. The increase in solar panel systems is continuous, since their purchase and operation are relatively cheap, the payback period is short, maintenance is minimal and the scope of application is wide-ranging.

In recent years, the number of small-scale household power plants and their total installed capacity has also risen significantly in Hungary (Table 2). The positive trend experienced for several years continued in 2016, the installed capacity is doubled every year in this category.

Table 2: The amount and capacity of installed small-scale solar panel systems annually

SMALL-SCALE PHOTOVOLTAIC SYSTEMS	**YEAR**								
	2008	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
P < 50 kW number of household size small-scale photovoltaic systems (pieces)	107	165	292	629	1882	4855	8829	15131	20401
50 kW<P<500 kW non-household size small-scale photovoltaic systems (pieces)	0	0	0	2	5	13	33	60	88
P < 500 KW ∑ NUMBER OF SYSTEMS (pieces)	**107**	**165**	**292**	**631**	**187**	**4868**	**8862**	**15191**	**20489**
P < 500 KW ∑ CAPACITY kW	**363**	**465**	**992**	**3 339**	**13 840**	**34 916**	**76 984**	**143 299**	**190 800**

Source: own editing based on datas from Hungarian Energy and Public Utility Regulatory Authority (2017)

Half of the systems implemented so far are less than 5 kW, that is typically family houses. A quarter of them are between 5-10 kW, that is large consumer residential users or smaller municipial public institutions (eg. nursery schools, libraries, mayor’s offices), and a further quarter between 10-50 kW, which covers the consumption of smaller plants, businesses and middle-sized commercial units. Licensed power plants of over 500 kW performance have also started their operation (Pécs, 10 MW, Visonta, 15, 6 MW) in the past one or two years.

It is important to note that the actual solar panel capacity is larger in Hungary than what is mentioned above, since these numbers do not include off-grid solar panel systems. We do not have detailed information about these systems.

The solar energy market was characterized by relatively steady growth from the beginning of the millenium to the end of 2008. According to the data of the Solar Energy Association of the Hungarian Building Engineers, a solar system of approximately ten thousand square meters has been realized by this year. Subsequently, growth has been fluctuating, the breakpoints in the growth rate coincide with the changes in the quality of support. The size of solar panel systems installed in Hungary until 2015 is 269,000 sq meters (Varga, 2017).

3.3 Renewable Energy Market Situation Report – Wind Energy

Regarding wind energy, Poland has the highest share (11%) within the ratio of renewable energy in the Central and Eastern European region in 2015. The majority of the energy produced this way comes from over twenty large wind power plants in the northern part of the country.

Croatia and Hungary reached 3% and 2% ratio by 2015. Further investments have been announced in the former country, in Hungary no permission has been issued for a wind power plant since 2011. In the Czech Republic 1% is the ratio of wind power within renewable energy utilization. In Slovakia and Slovenia, the use of wind power is minimal, its ratio is not provable. In the future, in addition to the power plant size, efficient, manageable, automated low-powered wind turbines are expected to appear in the region as well.

In Hungary, the annual average wind speed is 2-4 m/s (10 meters above the surface). The Hungarian Energy Authority issued a license for 330 MW wind power capacity in 2006, this performance was installed in 2011 (Table 3). According to the National Renewable Energy Action Plan (2010), the Hungarian electricity network can control a power of approximately 740 MW. Due to the changing directions of winds, power plants independent from winds with the same capacity should be ready to use in case of the stillness of air, and controllability should be improved.

Table 3: Wind power plants in Hungary in 2016

Place (pieces)	**Number of towers** (pieces)	**Unit-power** (kW)	**Total power** (kW)	**Installation** (year)
32	172	225-3000	329 325	2000-2011

Source: Own editing based on datas from Hungarian Wind Energy Corporation (2016)

There are currently 172 wind turbines in the country. The number of towers is 1-19 at each location. The performance of the equipment ranges from 225 kW to 3 MW.

3.4 Renewable Energy Market Situation Report – Water Energy

Due to its geographic location, Slovenia had the highest ratio within renewable energy in the region (31%), followed by Croatia (28%) and Slovakia (21%) in 2015. In these countries, the potentials have been well exploited in the utilization of water energy. New developments focus primarily on the application of small to medium turbines. The latter type of developments can be of importance in the future in the Czech Republic, Poland and Hungary.

In Hungary, the vast majority of hydroelectric water plants operate on three major rivers and their tributaries. There are 29 miniature-, small- and medium capacity power plants, the total capacity of which is 53.297 MW (Table 4).

Table 4: Water power utilization bases in Hungary in 2016

Categories by built-in capacity	**Typical range of capacity**							
below 500 kW power plants (23 pieces) ∑ 4,617 MW								
Between 500 kW and 10 MW power plants (4 pieces) ∑ 8,77 MW								
Between 10 MW and 30 MW power plants (2								

pieces) ∑ 40,5MW								
∑ CAPACITY 53,297 MW	0-0,5 MW	0,5-1 MW	1-5 MW	5-10 MW	10-15 MW	15-20 MW	20-25 MW	25-30 MW

Source: own editing based on datas from Hungarian Energy and Public Utility Regulatory Authority and own collection

The total performance of the Tiszalök (12.5MW) and the Kisköre (28 MW) Hydroelectric Power Plants operating on Tisza provides more than two thirds of the power output. The Kenyeri Power Plant (1.5 MW), opened in 2009, was constructed after a 30-year break in hydropower plant construction, since the last hydroelectric power plant in Hungary was built in the 1980s.

In research and development, the primary goal is to reduce costs and increase efficiency. According to expert opinions, the revision of the current Hungarian practice in the utilization of hydropower is reasonable in parallel with the development of technologies.

3.5 Renewable Energy Market Situation Report – Biomass

Solid biomass has a significant, sometimes predominating role within renewable energy utilization. Its ratio was around 90% in Hungary, in Czech Republic and in Poland in 2015 while in Slovakia, Slovenia and Croatia 75% of renewable energy comes from bio energy. In addition to traditional wood-heating, modern biomass heating has appeared in both small-scale and power plant sizes in the region in the past 10-15 years. In addition to large-scale biomass utilization, the sustainable development, growth of forestry and forest-based industries have to be given a prominent goal in Central and Eastern Europe in the future. Besides the increase in the efficiency of traditional wood-heating, the spread of pellet and briquette-heating can also be observed.

Alongside biomass combustion, biogas plants are also present depending on the level of development and the concentration of agriculture – in a much smaller scale compared to Western European countries. In recent years, the biggest debate has been the environmentally-friendly nature and grounds of biofuels, which has led to a number of investments that have failed or been postponed for several years. In the future, it is expected that second-generation (utilizing by-products, timber) solutions will also gain ground.

Approximately 90% of the heat energy produced from renewable energy sources comes from solid biomass in Hungary. Half of the electricity produced from renewable energy sources comes from solid biomass, we currently utilize most of the potential quantity. Several places of utilization, power plants would intend to rely on a supplier base of straw or woody timber from short rotation plantations in the long run.

3.6 Renewable Energy Market Situation Report – Geothermal Energy

In most of the Central and Eastern European countries surveyed, geothermal-based district heating systems already operate. In Hungary and in the Pannon basin, which includes the region next to the Hungarian border, heat flow of 90–100 mW/m^2 is well above the average, which is accompanied by high geothermal gradient of 45°C/km on average (Hungarian Office for Mining and Geology - MBFH, 2012). Taking advantage of these factors, relating the utilization of geothermal energy a ratio of 4% in Slovenia and 3% in Hungary could be detected within renewable energy sources in 2015. The geothermal potentials in the region would enable further district heating projects to be realized in other countries as well (e.g. Poland, Slovakia).

There is no geothermal-based electricity generation in the examined region. The development rate of near-surface heat pump energy utilization is much slower in the region than in the western and northwestern countries.

In Hungary, geothermal energy has been utilized on more than twenty sites for several decades (Table 5). About two thirds of it is used in district heating systems, while one third of it is used in individual heating systems.

Table 5: Deep geothermal heat energy utilization in Hungary in 2015

Place*	**Year of implementation**	**Total capacity**	**Total annual energy production in 2014**	**Typical utilization mode**
pieces	year	**MW_t**	**GWh**	-
23	1958-2015	219,08	827,59	Settlement, institutional energy supply, agricultural utilization

*The place is not equal to the number of wells. The number of wells is between 1 and 32 pieces per location.
Source: Own editing based on datas of the European Geothermal Energy Council (2014, 2016) and own collections

Near-surface energy utilization using heat pumps has been known to the public since the 2000s. Today, only a few thousand households generate energy with heat pumps.

4. Discussion

An important new industry and breaking technology is emerging around the world in connection with renewable energy sources. This development will not and should not keep away from the countries with less economic strength in the Central and Eastern European region. The present essay is a ’snapshot’ which gives a picture of the results in the region in the recent period and shows the possible directions and opportunities of development through the example of Hungary.

Renewable energy utilization in the region is predominant in the form of biomass utilization. This is well-illustrated by the example of Hungary, where other renewable energy utilization methods have a much smaller role. As regional and Hungarian analyses show the development is indisputable, but its scale raises the need for further exploration, analysis and intervention in some cases.

The biggest advancement in the residential sector is in solar energy utilization, which is in line with global and Western European trends, it is on a smaller scale compared to the latter, but it already shows measurable progress. There is also progress in the field of heat pump investments and biomass combustion. The latter – in addition to the emergence of wood combustion in power plants and the increase in the efficiency of traditional wood heating – is slightly due to the spread of pellet- and briquette-heating.

Recently, most of the higher volume investments in the region have been realized with the involvement of tender sources. The number of self-financed investments is far less. Several renewable energy investment companies are seeking to make use of increased market interest in the stock market in the form of equity and bond issues. In the future, this may also be an increasingly popular investment form for small investors. In addition to favorable natural conditions, investments will be located in regions with favorable incentive systems and predictable energy policies, where investors are sure that the favorable support system for renewable energy will be stable and predictable. In many cases, the termination of the administrative and financial constraints that are currently delaying the realization of investments may be a major challenge.

With many similarities in the Central and Eastern European region, barriers that prevent consumers from being more energy-conscious and renewable energy sources from being more widespread have been known for decades, however, they remain until today and the practical application is not popular with the people. In addition to low incomes, old habits, the lack of information and the simplicity of good practices, the ’fear’ from the new all hinder the wider spread. There have been a number of attempts – mainly linked to tenders – to form and develop energy- and environment-conscious approach in the region, but the actual possibilities in this regard are – in our opinion – unexploited for the time being.

In the countries of the region – in line with the commitments of the European Union – an important national strategy objective is to achieve the highest degree of energy independence, as well as to find environmentally-friendly, economically and socially sustainable solutions, therefore the role of renewable energy is even more crucial.

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