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COLIFORM BACTERIA CONTAMINATION MEASUREMENT OF CELL PHONES AND USERS' HANDS

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Abstract

Several environmental problem and diseases should be faced by mankind nowadays. Some of them are consequences of our high-speed social life. Recently handwashing has got highlight and several diseases could be avoided by it. This study aims to examine the presence of *coliform* bacteria on the surface of cell phones and the users' hands, as these devices are essential part of human life now. Association was supposed between the contamination level of the telephone parts and owners' hands. More than 90% of the samples from the right and left hands were

infected by *coliform* bacteria and 76.1% of the samples from the screen and back mobile phone were infected by *coliform* bacteria.

The percentage of negative cultures from mobile phone was 20.5% (average percent). The negative samples of the right and left hands were only 12% (average percent). It should be emphasized that the present study is only a case study to highlight the problem and to draw attention to the necessity and accuracy of handwashing, but it is not a representative survey.

Keywords: mobile phone; hands; bacteria contamination

Összefoglalás

Napjainkban számos környezeti problémával és betegséggel kell szembenéznünk. Némelyik igen nagy jelentőséggel bír életünkre. A kézmosásra és higiéniára, szerencsénkre egyre nagyobb hangsúly kerül hazánkban is. Jelen kutatásunkban, célul tűztük ki a *koliform* baktérium kimutatását mobiltelefonokon és használóik kezén egyaránt. A jobb és bal kézből származó minták több, mint 90%-a *koliform* baktérium által fertőzött volt, és kevesebb, mint 10%-a volt csak negatív a mintáknak. A mobiltelefonok 76,1%-a volt pozitív - amelyek esetében *koliform* baktériumot mutattunk ki. Szeretnénk hangsúlyozni, hogy kutatásunk a probléma feltárására, valamint a kézmosás szükségességére és annak pontosságára kívánja felhívni a figyelmet, de nem reprezentatív elemzés.

Kulcsszavak: mobiltelefon, kéz, baktérium szennyeződés

Introduction

Mobile phones (smart phone) have become one of the essential devices used for communication in daily life and are being used almost everywhere (Al-Abdalall and Amira, 2010). Brady et al. (2006) have been reported that mobile phones can harbor more microorganisms than a man's lavatory seat, the sole of a shoe or the door handle. Microorganisms can be transferred from a person to another or from inanimate objects to hand, and vice versa (Brady et al., 2007). Cell phones can be contaminated through various sources, such as human skin or hand, bags, pockets food particles. Mobile phone can spread infectious diseases by frequent contact with hands (Kilic et al., 2009;Tagoe et al, 2011). These sources are links, through which microorganisms can colonize the phone, thus causing diseases that could range from mild to chronic. According to studies, the most common type of microorganism that occupies the hand phones are the coagulase negative *Staphylococcus*, followed by *Staphylococcus aureus*, *Escerichia coli* and *Enterococcus fecalis*, followed by other microorganisms like *Klebsiella pneumonia*, *Bacillus* spp. and *P. aeruginosa* (Sichani and Karbasizadeh, 2011; Al-Abdalall and Amira, 2010). Another study to determine the transfer efficiency of microorganisms by formites suggests that the Gram-positive bacteria are transmitted most readily followed by virus and Gram-negative bacteria (Rusin et al., 2002).

Coliform bacteria are defined as facultatively anaerobic, Gram-negative, non-spore-forming rods that ferment lactose vigorously to acid and gas at 35 ± 2 °C within 24 or 48 h. Coliform bacteria generally belong to four genera of the *Enterobacteriaceae*: *Citrobacter freundii, Enterobacter cloacae, Enterobacter aerogenes, E. coli*, and *Klebsiella pneumoniae* (Eckner, 1998). Coliform bacteria have been used for many years to determine the quality and safety of water for human consumption. Escherichia coli and other groups of coliforms may be present

where there has been faecal contamination originating from warm- blooded animals (Choa et al, 2003)

There are different types of *E. coli* bacteria, from which some strains are way more pathogenic than other ones and it has the potential to cause serious food poisoning and even death. Infections spread through the fecal-oral route, for example by touching contaminated hands with the mouth after using the bathroom or touching fecal matter. In such situations, hand washing is the simplest and also the most effective measure to prevent the spread of agents responsible for communicable diseases (Brandl et al., 2006). Researchers at the London School of Hygiene & Tropical Medicine found fecal matter on one out of every six smartphones in a 2011 study (Hafner, 2017).

Ustun and Cihangiroglu (2012) reported that 97.8% of culture-positive specimens isolated from mobile phones from which 9.5% of phones had MRSA, (Methicillin-rezisztens Staphylococcus aureus) 11.2% had ESBL-producing *Escherichia coli*, which can cause nosocomial infections. According to Cuttler et al. (2018), in the general population, one of six mobile phones in Britain is contaminated with fecal matter. The study reported, that 16% of hands and 16% of phones were found to harbour bacteria of a faecal origin, where those who had bacteria on their hands were more likely to have bacteria on their phone as well (Cuttler et al., 2018). Another study found that the microbial contamination frequency of mobile phones of college students was 98%: Gram-positive bacilli (30%), Gram-negative bacilli (8%), *Staphylococcus* spp. (14%), *Escheria coli* (16%), *Enterococcus* (18%), coliforms (8%), *Micrococcus* spp. (1%) (Jagadeesan et al., 2013). Study of Reynolds et al. (2005) reported that more than 90% of health care workers' cell phones were contaminated with microorganisms and more than 14% of them carried pathogenic bacteria that commonly cause nosocomial infection. A study examining the phones of 20 hospital staff members found that 94.5% of the phones were contaminated with

some kind of bacteria and many of which were resistant to multiple antibiotics (Ulger et al., 2009). A study carried out in an Indian Dental school revealed that the mobile phones may act as an important source of nosocomial pathogens in the dental setting. The most common organisms isolated from the mobiles from the above study were coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, *Bacillus* spp., *Acinetobacter*, *Pseudomonas*, micrococci, *Staphylococcus citreus* (Singh et al.,2010).

Recently handwashing has got highlight and several diseases could be avoided by it. This study aims to examine the presence of *Escherichia coli* bacteria on the surface of cell phones and the users' hands, as these devices are essential part of human life now. It should be emphasized that the present study is only a case study to highlight the problem and to draw attention to the necessity and accuracy of handwashing, but it is not a representative survey.

Materials and methods

In this case study, 201 mobile phones and 201 pair of hands were examined with standard microbiology methods. Sampling was carried out at Budapest Business School. Samples were taken from the students of the School, aged between 19 and 25. Therefore the results of this study are not representative, only a case study.

The laboratory study was conducted at Budapest Business School, between October 2017 and June 2018 by the Department of Catering.

Microbiological methods

Samples were obtained from cell phones of all participants using sterile cotton swabs. Prior to sample collection swab were moistened in sterile water and were rotated over the front screen and back of the cell phones, and after repeated their right and left hand also. Swab are then

inoculated onto Lactose broth tubes (formula in g/l: pepton 5 g; meat extract 3 g; lactose 5 g; final pH 6.9±0.2 /25 °C/) containing Durham tubes and incubated 48 hours at 37°C. 4 different tubes were used by every student. The color of the samples in the tubes containing *coliform* bacteria after the incubation period changed to yellow, with presence of gas. The negative samples – not containing *Escherichia coli* nor c*oliform* bacteria – kept their green color (Fig.1.). If bacteria utilize carbohydrates as energy source from the nutrient solution, there may be two end products, a gas and acid. The substrate formed from the metabolism of carbohydrate is either glucose or lactose. Even if bacteria releases enzymes that enable to use carbohydrates through fermentation and oxidation, gas may or may not be produced. Fermentation is noted by acid production which can be observed by a color change in Durham tubes aka carbohydrate fermentation tube.

Figure 1. Positive (yellow with gas) and negative samples (green without gas) of Coliform bacteria (source: own picture)

Statistical methods

Data of 201 telephone owners was examined as a pilot experiment. Telephone owners are the graduate students of the Budapest Business School, therefore this research is not representative. The results must be considered as results of a case study. Association was supposed between the contamination level of the telephone parts and owners' hands. Cross tables were generated and the χ^2 independency test was applied to detect the relationship between the variables: telephone screen, telephone back, right hand and left hand, respectively. In case of each variable two levels of contamination was examined as variations: negative and coliform contamination, respectively. The χ^2 independency test is a non-parametric hypothesis test to decide whether two variables are independent (null-hypothesis H_0) or not (alternative hypothesis H_a). If the empirical significance level (p-value) of the test is lower than the chosen significance level (α) H_a should be accepted, and the variables are not independent, there is significant association between the variables. The chosen significance level was 5%. Cramer's V is an indicator of the tightness of the relationship, and its value should be higher than 0 and less than 1 (0 means independency, 1 means complete functional dependency). Computations were made by SPSS v.24 of IBM. Dataset was divided in two parts according to the owners' gender, as it was supposed that there should be some differences in case of then association of the contamination level of the telephone parts and owners' hands. χ^2 independency test was applied from the separated dataset. The ratios of the genders in the sample are 38.8% of male and 61.2% of female.

Results

201 samples were obtained from both the right and left hands. 92.5% of the right samples and 90% of the other hands were infected by *coliform* bacteria and only 7.5% and 10% of the samples were negative (*Fig. 2.*).

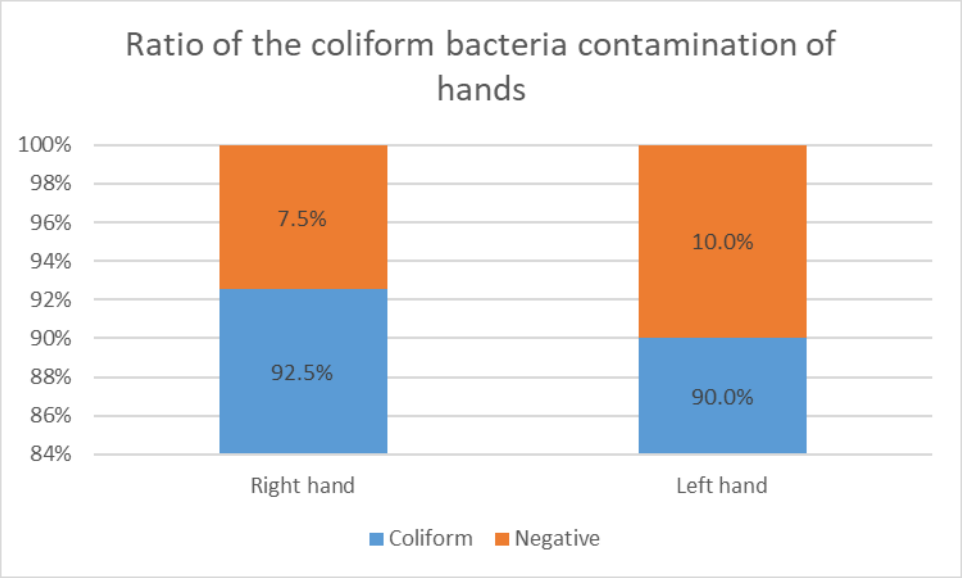

Figure 2. Ratio of the coliform bacteria contamination of hands

201 samples were obtained from both the screen and back of mobile phones.78.1% of the screen and 81.1% of back were infected with *coliform* bacteria and 21.9% of the screen and 18.9% of back samples were negative (*Fig. 3.).*

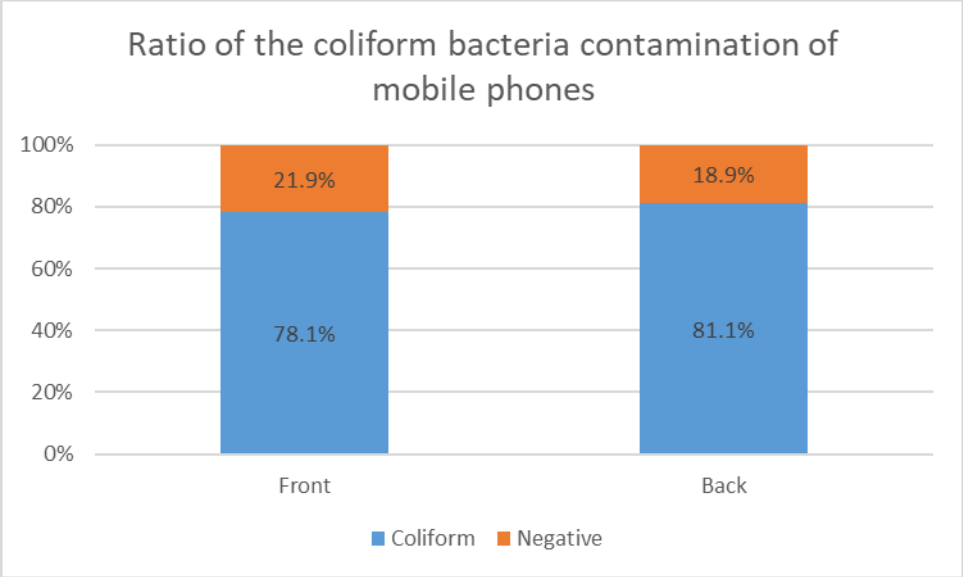

Figure 3. Ratio of the coliform bacteria contamination of mobile phones

We assessed the results of the samples from the mobile phones and hands by gender as well. Female's right and left hand samples included more negative samples (right hand 7.5% - left hand 5.5% negative), than samples from male (right hand 2.5% - left hand 2% negative), however samples of female's left hands had slightly higher *coliform* bacteria contamination level, and right hands had more *coliform* bacteria than the samples from male (female: 55.7%, male: 36.8%).(*Table 1.)*

Table 1. Ratio of the hand contamination by genders (*source: own data)*

	Right hand		**Left hand**	
	negative (%)	**Coliform (%)**	**negative (%)**	**Coliform (%)**
male	2.5%	36.8%	2.0%	36.3%
female	7.5%	55.7%	5.5%	53.7%

The female's mobile phones were cleaner than male's, but interestingly, female's samples had more coliform bacteria (screen: 45.8%; back: 48.3%) (*Table 2*.).

Table 2. Mobile phone contamination by genders

	Screen of mobile phone		**Back of mobile phone**	
	negative (%)	**coliform (%)**	**negative (%)**	**coliform (%)**
male	6.5%	32.3%	6.0%	32.8%
female	15.4%	45.8%	12.9%	48.3%

No association can be demonstrated between the gender and the contamination level of the front of the cellphone, the back of the cell phone, neither of the left and right hand, respectively (*Table 3.)* Gender and the level of contamination are significantly independent, no significant relationship can be found at 5% significance level.

Table 3. P-values of the χ^2 independency test

	Front of the mobile phone	**Back of the mobile phone**	**Right hand**	**Left hand**
Gender	0.154	0.310	0.316	0.182

Table 4. and *5.* summarizes all the p-values of the χ^2 independency tests performed separated by genders. In *Table 6.* and. *7.* the Cramer's V value are given separately for male and female, and where significant association can be demonstrated the values are typed in bold. Cramer's V indicate association in most cases, but differences can be seen between the genders.

The separated dataset of the male owners was analyzed and according to the matrix of p-values in *Table 4.* significant relationship can be shown in almost all cases, except two ones. There is

no significant association according to the hypothesis test between the contamination of the screen of the telephone and the contamination of male owners' left hand, and between the two hands. Significant values of Cramer's V in *Table 6.* rages between 0.32 and 0.47. These values suggest weak and medium level of association between infection of the examined surfaces in case of the male owners.

It can be concluded that in the separated dataset of the female owners, significant association can be found between the telephone parts (screen and back side, respectively) and the level of contamination of the owners' hands (right and left hand, respectively). The matrix of the p-values in *Table 5.* shows that significant relationship can be found between all examined variables. Cramer's V in *Table 7.* explains that there is significant relationship between the contamination of the front and back of the phones and of the hands. The range of the value is 0.18-0.29. These values suggest weak association between infection of the examined surfaces in case of the female owners.

Table 4. P-values of the χ^2 independency test in case of males (significant values are in bold)

	Front of the mobile phone	**Back of the mobile phone**	**Right hand**	**Left hand**
Front of the mobile phone	-			
Back of the mobile phone	**0.000**	-		
Right hand	**0.001**	**0.001**	-	
Left hand	0.148	**0.004**	0.119	-

Table 5. P-values of the χ^2 independency test in case of females (significant values are in bold)

	Front of the mobile phone	**Back of the mobile phone**	**Right hand**	**Left hand**
Front of the mobile phone	-			
Back of the mobile phone	**0.024**	-		
Right hand	**0.019**	**0.041**	-	
Left hand	**0.005**	**0.001**	**0.010**	-

Table 6. The values of Cramer's V in case of males (significant values of relationship are in bold)

	Front of the mobile phone	**Back of the mobile phone**	**Right hand**	**Left hand**
Front of the mobile phone	-			
Back of the mobile phone	**0.477**	-		
Right hand	**0.364**	**0.384**	-	
Left hand	0.164	**0.324**	0.177	-

Table 7. The values of Cramer's V in case of females (significant values of relationship are in bold)

	Front of the mobile phone	**Back of the mobile phone**	**Right hand**	**Left hand**

Front of the mobile phone	-			
Back of the mobile phone	**0.204**	-		
Right hand	**0.212**	**0.184**	-	
Left hand	**0.256**	**0.294**	**0.232**	-

There is stronger association between the contamination of the right hand and the front and the back side of the telephone and the contamination of the two sides of the cell phone in case of male owners compared to the female owners. The association between the infection of the left hand and of the cell phone's front side is significant only in case of the females. This can be concluded also for the pollution of the two hands. Another study found that the students of Faculty of Health Sciences, University of Ljubljana, Slovenia had shown that there was a statistically significant relationship between gender and microbiological contamination of the mobile phones, such as mobile from female users were highly colonized with bacteria compared with those mobiles from male students (Nwanko et al., 2014).

Discussion

The results show that mobile phone and users' hands were relatively higher contaminated with *coliform* bacteria.

The coliform group, as defined above, includes species of the genera *Citrobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, Serratia* and *Yersinia*. They are a commonly used indicator of sanitary quality of foods and water. *Coliforms* can be found in the aquatic environment, in soil

and on vegetation; they are universally present in large numbers in the feces of warm-blooded animals. While coliforms themselves are not normally causes of serious illness, they are easy to culture, and their presence is used to indicate that other pathogenic organisms of fecal origin may be present.

The overall percentage of positive cultures (*coliform bacteria*) from mobile phone was 79.5% (average percent), compared with 20.5% (average percent) for negative cultures.

The overall percentage of positive cultures (*coliform bacteria*) from right and left hands was more than 90% (average percent), compared with 8.5% (average percent) for negative cultures. This is somewhat higher than the figures reached by studies conducted in Nigeria (14.3%) (Andrej et al., 2012), India (16) %) (Tankhiwale et al., 2012), but lower than the findings of another study from Iraq with 25% (Husam, 2013) and Morocoo with 33.3% of positive samples (Abdellatif et al., 2017).

Our opinion is that keeping hands clean is one of the most important steps we can take to avoid infections and spreading germs. Many diseases and conditions are spread due to improper washing of hands with or without soap and clean, running water. A single gram of human feces — which is about the weight of a paper clip — can contain one trillion germs (Franks et al.,1998). Germs can also get onto hands by people touching any object that has germs on it as a result of being coughed or sneezed on it, or being touched by other contaminated object.

Maxine and colleagues (2011) found that bacteria of potential fecal origin (mostly *Enterococcus* and *Enterobacter spp*.) were found in 44% of samples took without handwashing. Handwashing with water alone reduced the presence of bacteria to 23% (p-value < 0.001). Handwashing with plain soap and water reduced the presence of bacteria to 8 % (Burton et al.,2011).

Hoque and Briend (1995) found that a wide variety of hand cleaning means in poor settings (soap, ash, mud) are effective in reducing the contamination with coliform bacteria on hands. The same author reported that soap may be more effective than water in reducing the presence of coliform bacteria on hands.

Conclusions

Based on our opinion that the education of handwashing and phone disinfection is indispensable, and we believe that this education should be started at childhood. Smart phones are being extremely broadly, but their purity is rarely taken into consideration. We have to accept smart tools will dominate our future; therefore, lot of new surface will present further risk of catching variety of bacteria, and only one of these will be the smart phones (Soto et al., 2006). It will be important to pay attention to the cleaning and disinfection of these tools, surfaces. In parallel the good personal hygiene and handwashing will remain critical, because mobile phones are primarily being infected from hands. These bacteria can cause reinfection from the mobile tools.

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RELATIONSHIP BETWEEN FIBER HUMIDITY AND FIBER PERMEABILITY IN PVDF-BASED MEMBRANES

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Abstract

Fiber humidity and fiber permeability were measured in 678 samples of polyvinylidene-difluoride based fiber membranes, and then their relationship was investigated. We found that this can be described by a three-variable regression function. For 220 samples, we examined the effect of rewetting prior to permeability measurement. The result was that soaking for at least 12 hours was required for the membrane to reach maximum permeability.

Keywords: PVDF membrane; membrane drying; membrane permeability; membrane humidity

Összefoglalás

678 polivinilidén-difluorid alapú membránszálon mértük a szálnedvességtartalmat és a szálpermeabilitást, majd ezek kapcsolatát vizsgáltuk. Megállapítottuk, hogy az összefüggés leírható egy háromváltozós regressziós függvénnyel. 220 minta esetében kutattuk az

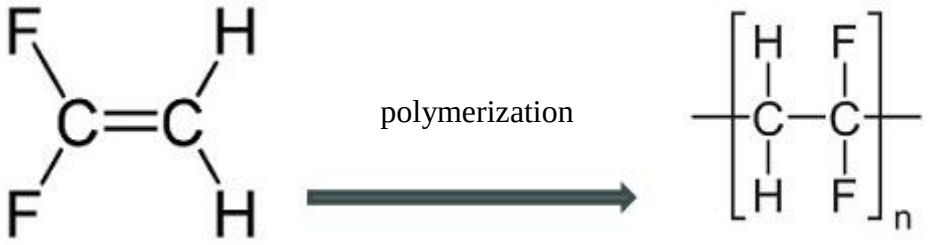
újranedvesítés hatását a szálpermeabilitás méréseket megelőzően és azt tapasztaltuk, hogy legalább 12 óra áztatás szükséges annak érdekében, hogy a membránszál elérje a maximális permeabilitását.

Kulcsszavak: PVDF membrán, membrán kiszáradás, membrán permeabilitás, membrán nedvességtartalom

Introduction

The use of membrane water treatment technology has been popular since the 1940s and continues up-to-now *(Pendergast and Hoek, 2011)*. At present, organic polymer-based membranes are used in almost all industrial sized applications and the most widely used of them is polyvinylidene-difluoride (PVDF) due to its outstanding properties *(Liu et al., 2011).* In this case PVDF ultrafiltration membranes were chosen for the research.

PVDF is a semi-crystalline substance composed of – (CH_2CF_2) – units (Fig. 1.). It has a high mechanical and chemical resistance, quite stable under higher temperatures and extremely resistant against ageing. Its use is not limited to micro- and ultrafiltration, but includes bioreactors, gas separation, and desalinating equipment, too *(Kang, et al., 2014).* All of these features make this material a superb raw material for sheet and fiber membranes, too *(Liu, et al., 2011).*



1,1-difluoroetilene PVDF

Fig. 1. Structure of 1,1-difluoretilene and PVDF (SpecialChem, 2020)

The main objective of the study is to validate or discard the need of rewetting before membrane fiber permeability measurements on fibers with different water content. From the obtained data, we would like to deduce whether the membrane drying is reversible and how the permeability of the membrane changes as a function of the rewetting time. I would like to find a reasonable rewetting time, what is enough to get a realistic picture of the expected operating filtration performance after the fiber permeability measurements. If such exists, I would like to describe the relationship between fiber humidity and fiber permeability

Materials and methods

Membrane type

Polyvinylidene-difluoride (PVDF) based wastewater treatment membranes were used in the study, with pore size between 0.02-0.04 μm, so this was in the ultrafiltration range (0.01-0.1 μm). This membrane is a tubular, supported type, what means that it has an inner polyethylene terephthalate (PET) yarn frame. To this shoelace-like skeleton comes the membrane layer during the production, what was thermally induced phase separation (TIPS). The final membrane fiber has approximately 2000 μm diameter (Fig. 2.). From water solutions this membrane can separate macro and micro particles, macro molecules, parasites, bacteria,

suspended solids, some viruses, proteins and colloids *(Morao et. al., 2001).* Practically it means that this is for cleaning of municipal and industrial wastewaters (*Szabó and Anda, 2018*).

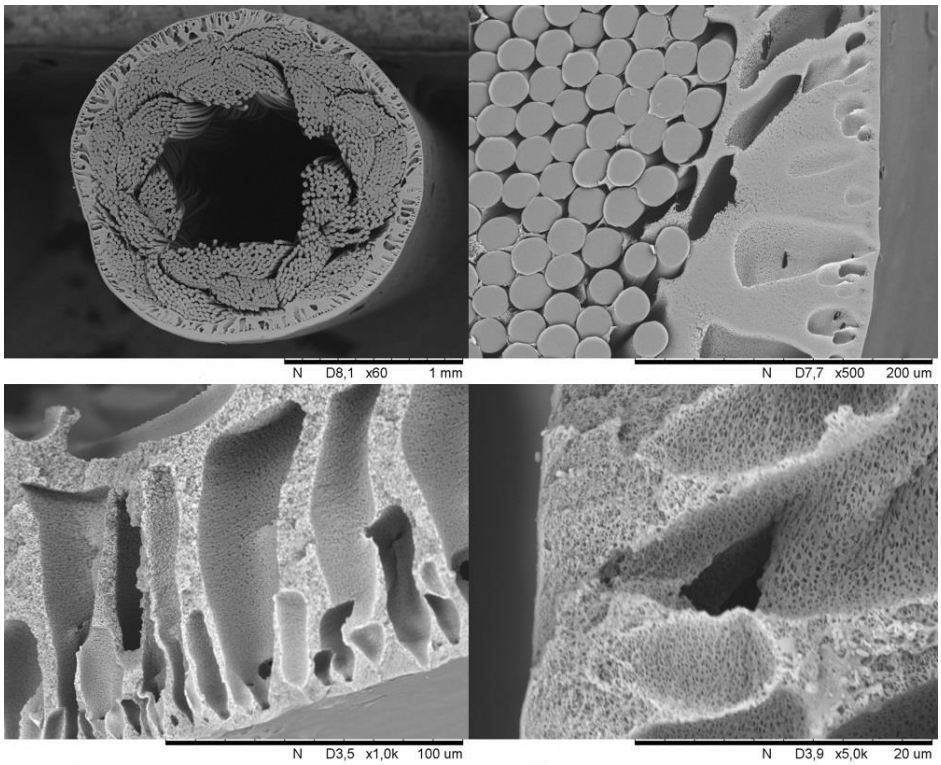


Fig. 2. SEM pictures of PVDF based supported membranes made with a Hitachi S-3000N microscope

Accelerated drying

During the experiments, the samples were dried in a temperature-adjustable VWR VENTI-Line oven at 60°C. The drying time varied depending on how low fiber humidity was to be prepared.

Fiber humidity measurement

The fiber humidity was measured with a Sartorius MA 35 moisture meter equipment (Fig. 3.). The device consists of a heating unit, a weight scale and a combined display and control panel. The humidity determination method is based on the thermogravimetric principle, the resolution of the device is 0.01%. In the first step of the measurement, the wet weight of the membrane fiber was measured, then the apparatus was heated to 110°C and the fiber was dried

for 5 minutes. During this time, the fiber dries to constant weight, so after another measurement, the dry mass can be obtained and thus the moisture content can be calculated in a simple division.

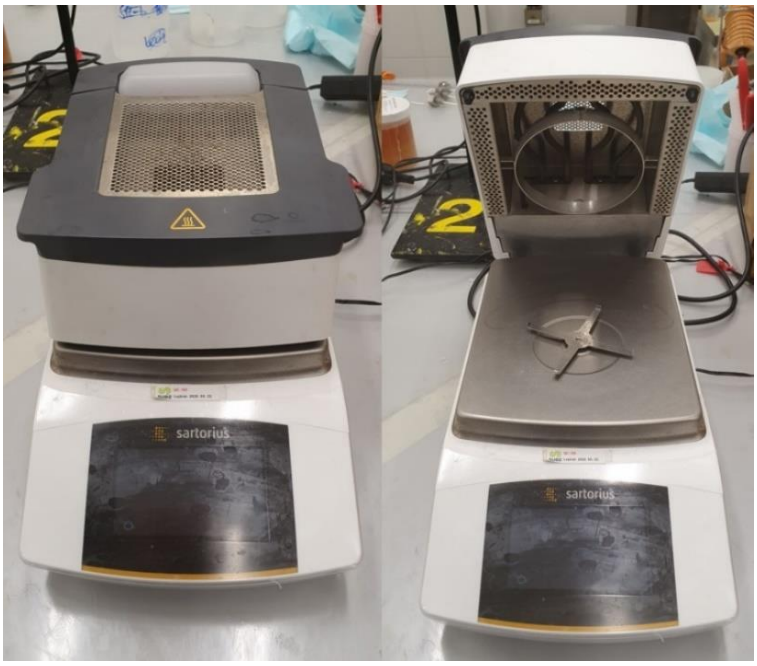


Fig. 3. Sartorius MA35 moisture meter equipment

Fiber permeability measurement

Fiber permeability was measured in low-ionic content water over a period of 5 min and at a pressure of 5 PSI using a custom-made equipment manufactured and calibrated by the manufacturer. The device consists of pipes that can be pressurized and filled with low-ionic water. During the measurement, the fiber must be placed in the machine in such a way that a piece of fiber of known length is located inside one of the tubes, surrounded by water. Based on the length and knowing the diameter of the fiber, the surface can be calculated. The two open ends of the membrane fiber stick out from the tube - this can be solved by first gluing them to a connector part - and after the pressure has been built up, the liquid entering the fiber flows through them. This is because water flows through the pores towards the lower pressure

space, i.e. to the lumen of the fiber. The effluent permeate can be collected in a vessel and the amount can be measured (Fig. 4.).

Fig. 4. Membrane sample prepared for measurement, permeability measuring device and measuring cup for permeate water

Fiber permeability was calculated with the next formulas:

$$J = \frac{\delta V}{A \cdot \delta t} \qquad (1)$$

Where J is the flux measured through the membrane surface A, δV is the amount of permeate and δt is the flow time of the liquid.

$$\Phi = \frac{\frac{J}{\frac{\delta P}{\mu_1}}}{\mu_0} \qquad (2)$$

Where Φ is the permeability, J is the flux at δP pressure, μ_1 is the viscosity of water at the temperature of the measurement, and μ_0 is water viscosity at 20 °C. Flux was calculated by the next formula

Statistical methods

During the drying and rewetting experiments, it was necessary to determine the equality or difference of the data group means and for this we chose the one-way ANOVA method.

To use the ANOVA test, the examined data must meet two conditions, on one hand, they must be normally distributed, and on the other the variances of the examined groups must be homogenous. For the normality test, the Kolmogorov – Smirnoff test and the Anderson – Darling tests were performed. The Levene test was used for the homogeneity test.

We used a three-variable regression function to describe the relationship between fiber humidity and fiber permeability. The parameters of the regression function were found by an iteration method using the Gauss-Newton algorithm.

Minitab 18.1 statistical software was used to perform detailed statistical studies *(Minitab, 2017)*. The choice was made because it is widely used and contains a large selection of the necessary statistical methods.

Results and discussion

Relationship between fiber humidity and fiber permeability

After studying 678 samples, the relationship between fiber humidity and fiber permeability was evaluated. The following approximation function was used:

$$\varphi_i = \varphi_{max} - a_1 * e^{-a_2 * x} \qquad (3)$$

Where φ_i is the measured membrane permeability value, φ_{max} is the maximum permeability value of freshly manufactured membranes, a_1 and a_2 are the parameters of the regression function determined by Gauss-Newton iteration method and x is the fiber humidity of the measured membrane sample.

Determining the value of φ_{max} as a constant – in our case it was 65 gfd / psi – and identifying this with the empirical maximum value of the membrane fiber, we reduced the number of variables of the three-variable nonlinear functions to two. The maximum value was determined by taking the top 5% of the historical data and averaging it and then rounding it to the nearest whole number. In determining the values of the variables, we examined the stability and convergence of the iteration. The acceptance criterion was always the minimum of the calculated sum of squares errors (SSE) from the possible function forms.

The initial values of the iteration were determined by forming the natural logarithm of the differences of φ_{max} and φ_i and plotted against the corresponding fiber humidity values, i.e. x. A negative directional tangent was fitted to the points with the least squares method. From the obtained axis section and directional tangent, the searched parameters could be determined. A suitable range was determined around the initial values obtained and iteration was performed. From the obtained functions, the one with the smallest SSE of the regression was chosen as the best. This function is shown on Figure 5.

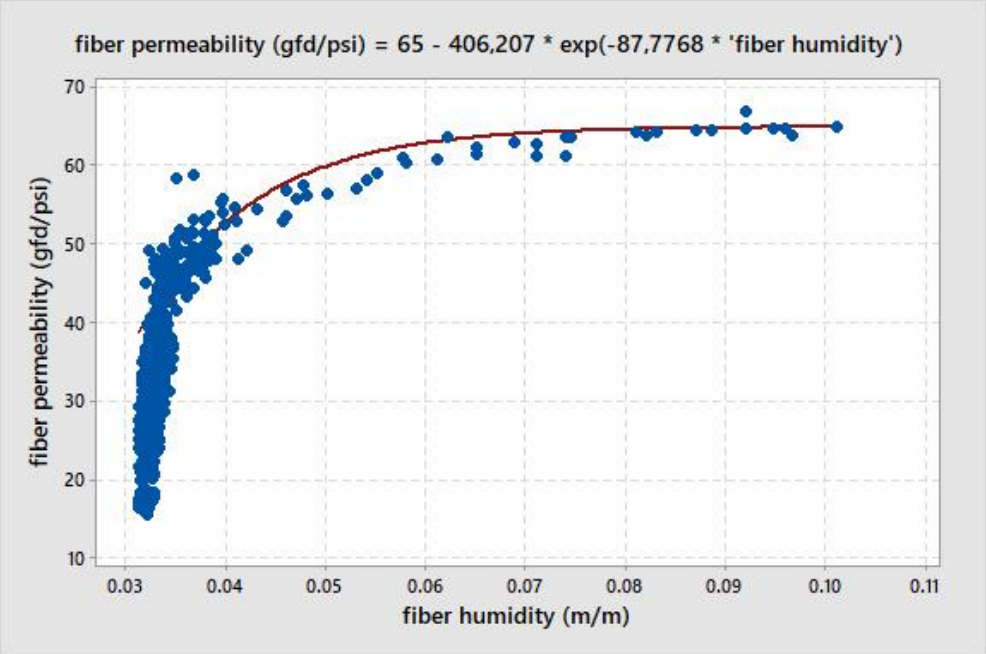


Figure 5. A function describing the relationship between fiber permeability and fiber humidity

Membrane rewetting measurements

During the experiment, 220 samples were dried, fiber humidity was measured, then the membranes were rewetted, and permeability was measured. The oven-dried samples with a given moisture content were rewetted by soaking in low-ionic water for 2, 12, 24 and 48 hours and then their permeability was measured. As a starting point, non-soaking fibers were also measured, which were subjected to permeability measurement immediately after drying.

Permeability values of the membrane samples after different rewetting times are shown on Figure 6.

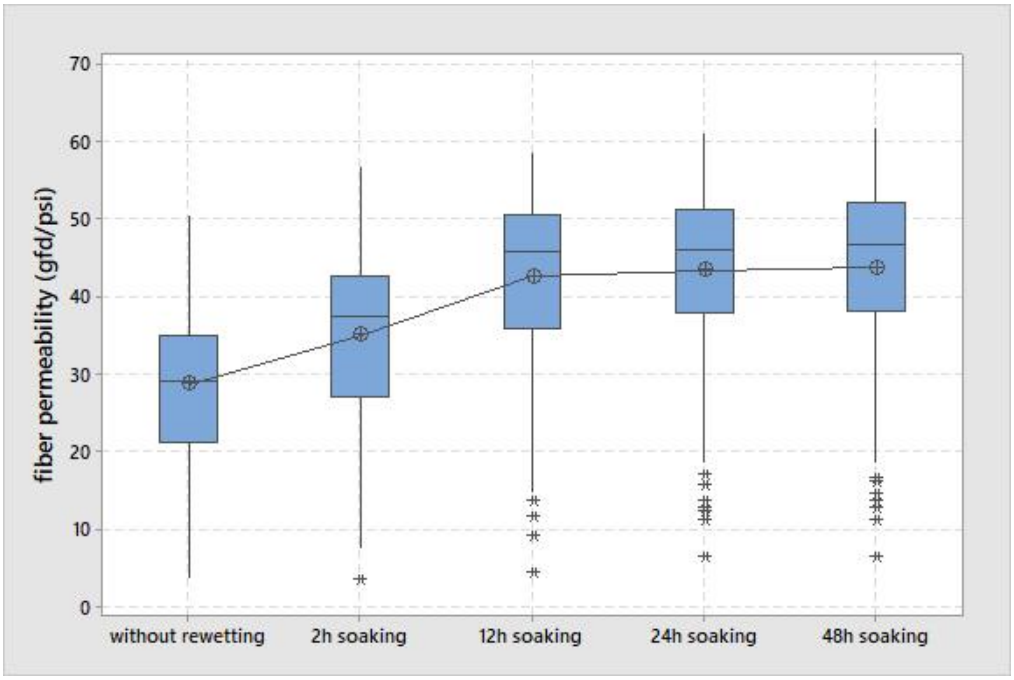


Fig. 6. Comparison of permeabilities of membranes with different rewetting times

Analysis of variance (ANOVA) was performed to determine if the average permeability of samples for a given soaking period varied as a function of rewetting time. Before performing ANOVA, we verified its applicability. Normality was examined in all cases, and since they showed a normal distribution and the standard deviations could be considered identical, there was no theoretical obstacle to performing the analysis of variance. For the former, ANOVA was considered valid for the entire population. We can state that the individual groups cannot

be considered the same - since the value of p (x) became 0.00, which is lower than the value of p = 0.05 at the 95% significance level - and thus the alternative hypothesis is true, i.e. there is a difference between the averages.

The previously described 3-parameter functions, together with the equations, fitted to the points showing the fiber humidity – fiber permeability relationship measured on the membranes, together with the equations, are presented below. Figure 7. shows the fibers without soaking, Figure 8. shows the values measured on the membranes after 2 hours, Figure 9. after 12 hours, Figure 10. after 24 hours and Figure 11. the values measured on the membranes after 48 hours.

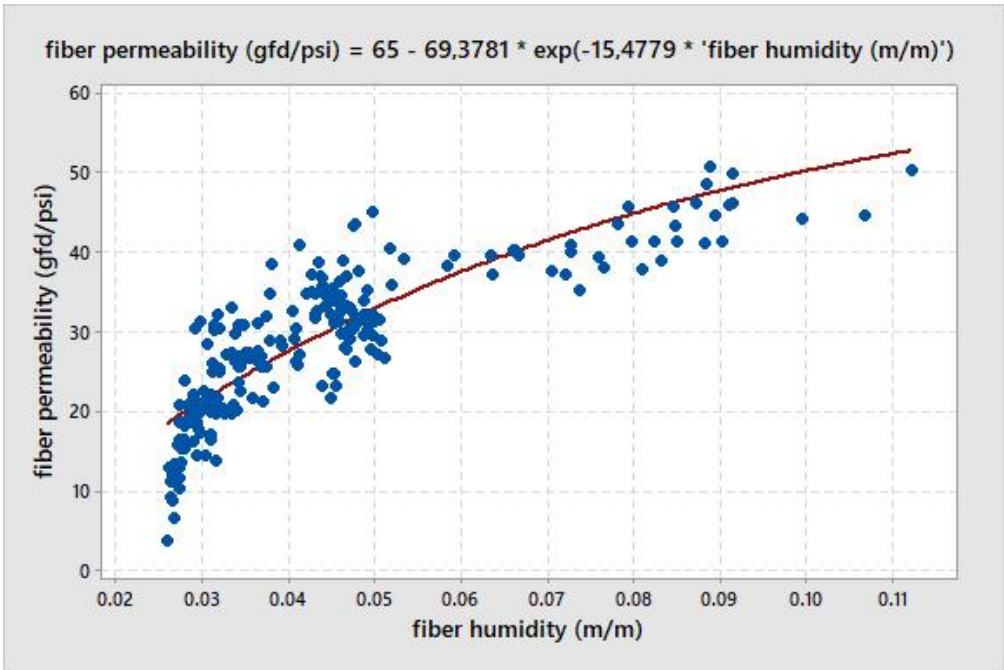


Fig. 7. Relationship between fiber permeability and fiber humidity without rewetting

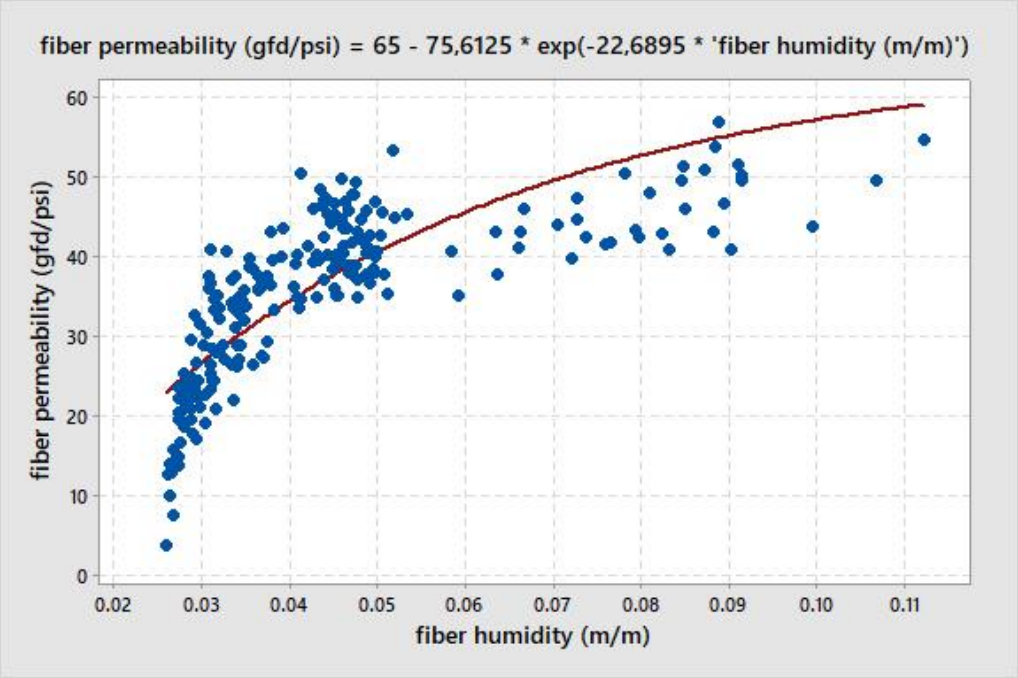


Fig. 8. Relationship between fiber permeability and fiber humidity after 2h soaking

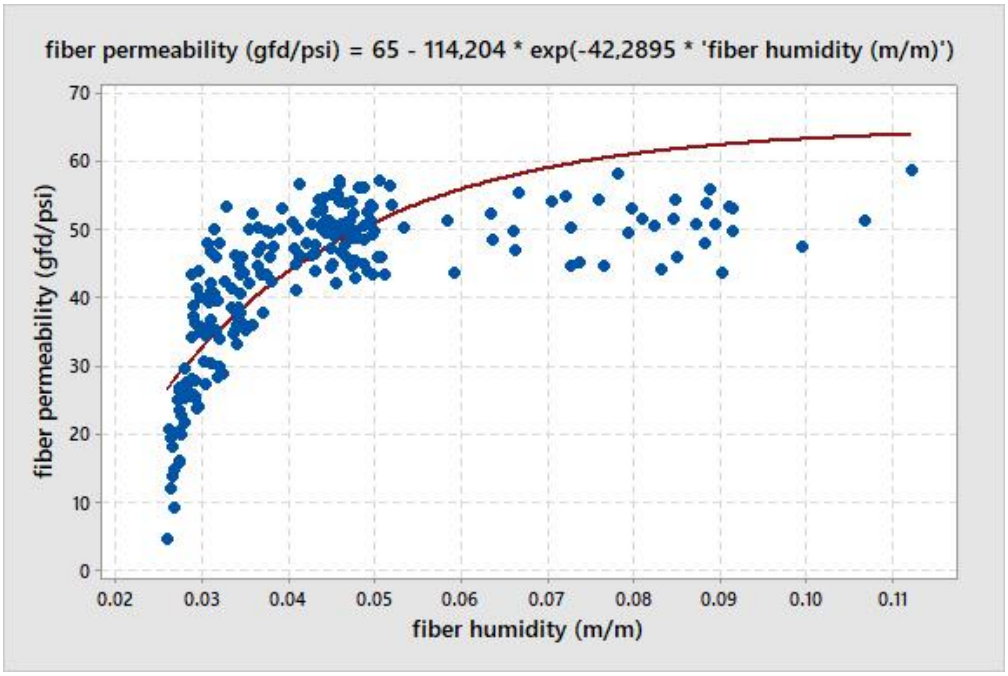


Fig. 9. Relationship between fiber permeability and fiber humidity after 12h soaking

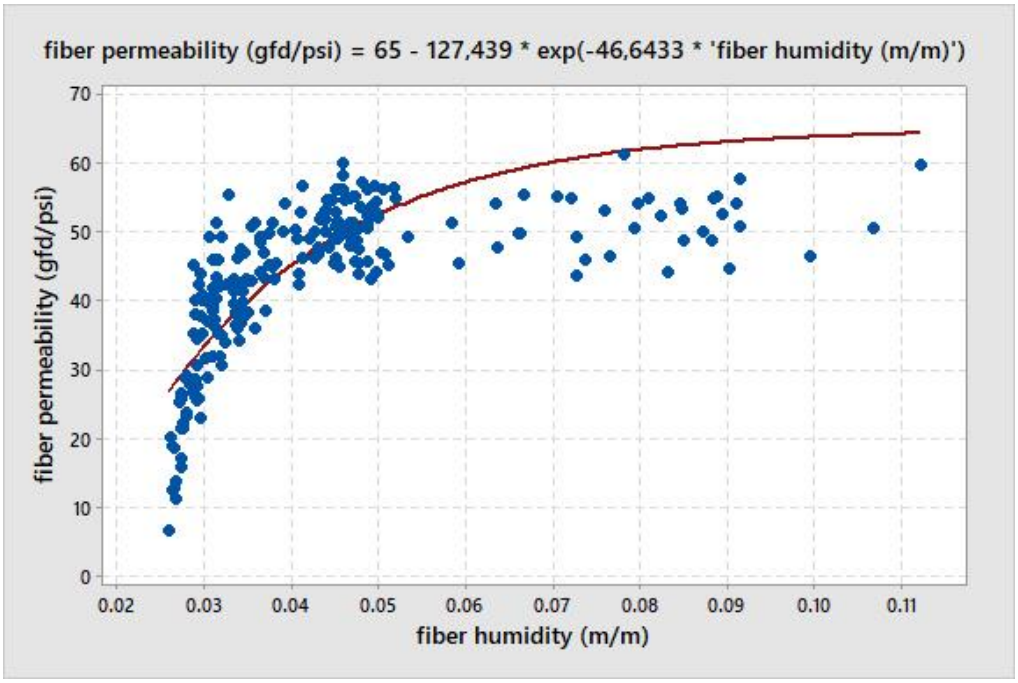


Fig. 10. Relationship between fiber permeability and fiber humidity after 24h soaking

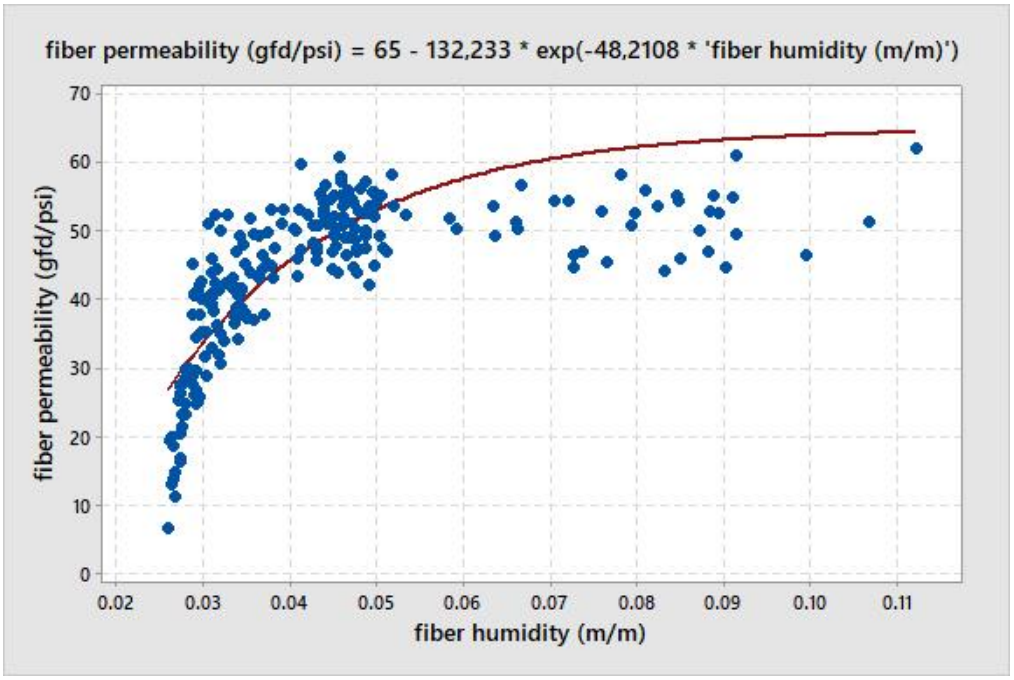


Fig. 11. Relationship between fiber permeability and fiber humidity after 48h soaking

The patterns shown in the figures confirmed the results of ANOVA, i.e. the permeability measured on membranes without soaking was lower than where the measurements were preceded by rewetting, i.e. drying is reversible.

We compared the two extreme groups of the experiment, i.e., the samples without soaking, with those that had spent 48h in low-ionic water prior to the permeability measurement. The aim was to prove our hypothesis that rewetting plays a role in the increase of permeability.

Comparing the two graphs in Figure 12., the difference in the distribution of permeability values can be clearly seen. After 48 hours of soaking, the permeability definitely increased compared to the values without soaking, which can be attributed to the fact that the membrane pores became more permeable under the influence of water. This is explained by the fact that the glycerol remaining in them absorbs water due to its hygroscopicity, even though the hydrophobic membrane makes this difficult.

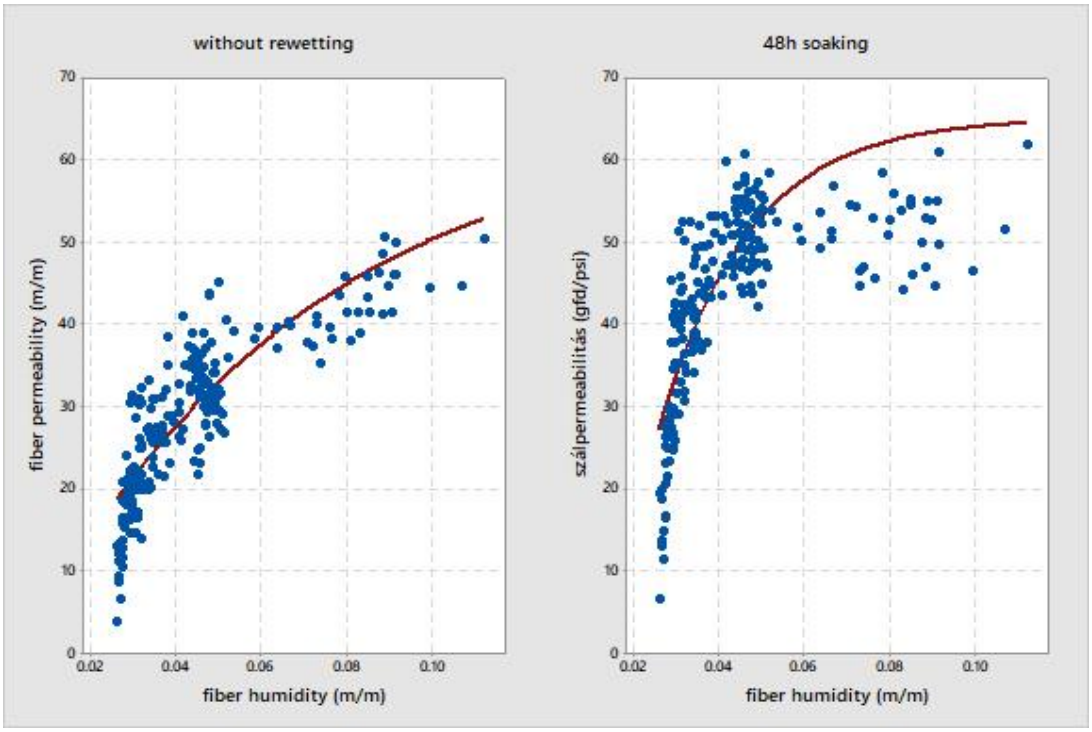


Fig. 12. Comparison of samples measured without rewetting and after 48h soaking

The change in permeability can be seen even more clearly on Figure 13., where the values without rewetting were subtracted from the permeability values measured after 48 hours of soaking. The differences obtained were plotted as an increase in permeability as a function of fiber humidity. It can be seen that after 48 hours of soaking, the permeability of samples with initially lower fiber humidity increased to a greater extent. This can be explained by the fact that the pore diameter decreased due to the evaporating water, more evaporation resulted in smaller pores and lower fiber moisture content, and the measurements showed lower permeability. The glycerin remaining in the pores of the membranes with lower fiber humidity absorbed water during wetting, the higher the glycerin concentration, the more absorbed water, thereby increasing the pore size to the greatest extent, resulting in the highest increase in permeability.

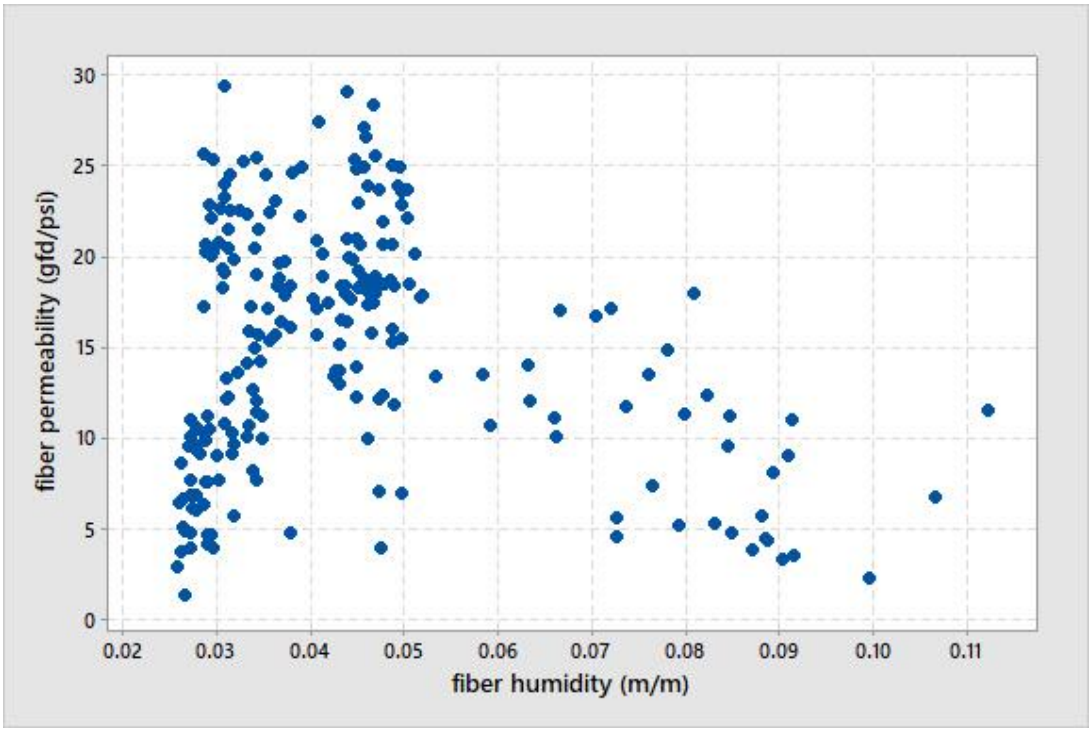


Fig. 13. Difference in permeability of membranes without rewetting and soaking for 48 hours as a function of membrane moisture

Conclusions

We described the relationship between fiber permeability and fiber humidity with an approximate equation that can be written as follows:

$$\varphi_i = \varphi_{max} - a_1 * e^{-a_2 * x}$$

The significance of the determination of the equation comes from the fact that with its help it is possible to approximate the fiber permeability only by performing in-situ fiber humidity measurements, from which we can deduce the expected operating performance, i.e. permeability.

We found that soaking prior to permeability measurements had an important role in the values obtained. After our tests we consider it necessary to soak for at least 12 hours, so we recommend this as a minimum rewetting time before measurement.

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EXAMINATION OF THE DECOMPOSITION OF WILLOW, POPLAR AND MIXED LEAF LITTER WITH LITTERBAG TECHNIQUE

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Abstract

Leaf litter decomposition is one of the most important ecological material cycle processes. For saprophytic water organisms, allochtonous plant parts (especially leaves) represent the main source of energy and nutrients. As a consequence of their shredding activity, the organic nutrients of detritus can return into soil, and can be uptaken by plants again. In our study, decomposition rates of willow *(Salix* sp.*)*, poplar *(Populus* sp.*)* and mixed leaf litter were monitorized, with litterbag method in a class „A" evaporation pan. Litterbags were used with two different mesh sizes. With the 500 µm mesh-sized bag we were able to examine decomposition with the exclusion of macroinvertebrates, and with the 3 mm mesh-sized bag we got information in the presence of them. The study took place between 15. June 2019. and

24. October 2019. Based on our results, we did not notice any remarkable differences between the decomposition of willow, poplar and mixed leaf litter. All of them fell into „medium" decomposition category. Furthermore, we did not measure notable differences between the two different devices. During each and every sampling, water samples were also taken, and their pH, conductivity, NH_4^+, PO_4^{3-}, SO_4^{2-} and Cl^--ion content were determined. There weren't any considerable changes in the quality of water, during the experimental period. The main aim of the study was to examine the process of decomposition and the changes of water parameters, furthermore, to compare the evaporation of the modified experimental pan (sediment, decomposing leaf litter), with the standard class „A" evaporation pan's (control pan). From that, it was able to determine the effect of decomposing leaf litter on evaporation. From the results of this experiment, we found that, the presence of sludge, and decomposing leaf litter, placed in the modified pan, increased the rate of evaporation in 2019.

Keywords: decomposition, willow, poplar, litterbag, class „A" evaporation pan

Összefoglalás

Az avarlebontás egyike a legfontosabb ökológiai anyagkörforgalmi folyamatoknak. A szaprofita vízi élőlényeknek és a vízi szervezeteknek a külső forrásból érkező növényi részek (főleg falevelek) jelentik a fő energia és tápanyagforrást. Az aprító tevékenységük következtében a detritusz szerves anyagai visszajutnak a talajba és ismét felvehetővé válnak a producensek számára. Kutatásunkban a fűz *(Salix* sp.*)*, nyár *(Populus* sp.*)* és kevert avar lebontását vizsgáltuk, avarzsákos módszerrel, „A" típusú párolgásmérő kádban. Kétféle avarzsák típust használtunk. Az 500 µm lyukbőségű avarzsák segítségével ki tudtuk zárni a

makrogerinctelen szervezeteket a rendszerből, míg a 3 mm lyukbőségű avarzsákkal ezek jelenlétében vizsgálhattuk a lebontást. A kutatás 2019. június 15. és 2019. október 24. között zajlott. Eredményeinket tekintve nem tapasztaltunk meghatározó különbségeket a fűz, nyár, és kevert avar lebontási ütemében. Továbbá nem tapasztaltunk kimagasló eltérést a 2 különböző eszköz esetében sem. A mintavételek alkalmával a vízminták vétele is minden esetben megtörtént, melyekből a pH-t, vezetőképességet, NH_4^+, PO_4^{3-}, SO_4^{2-} és Cl^--ion tartalmat határoztuk meg. A vízkémiai paraméterek tekintetében sem volt nagyobb mérvű változás a kísérleti időszak alatt. Kutatásunk fő célja az avarlebontás ütemének vizsgálata volt, emellett a vízkémiai paraméterek változásának figyelemmel kísérése, továbbá az avarlebontásnak helyet adó módosított kád (iszap, bomló avar), standard „A" típusú kád (kontroll kád) párolgásával való összehasonlítása. Ez utóbbiból meghatározhattuk a bomló avar párolgásra gyakorolt hatását is. Ennek a kísérletnek eredményeiből megállapítottuk, hogy a módosított kádba kihelyezett iszap, és bomló avar jelenléte növelte a párolgás ütemét 2019-ben.

Kulcsszavak: lebontás, fűz, nyár, avarzsák, „A" típusú párolgásmérő kád

Introduction

In the floodplain of rivers and highland and lowland streams the dominant plant communities are willow and poplar forests (Bagi et al., 1996). Plant parts can get into the water body from two main sources. The first and most important one is the allochtonous source which also contains branches, twigs, cones, crust, fruits etc., but depending on the vegetation, leaves represent 42-98% of it (Abelho, 2001). Leaf fall means approximately 1000 to 7000 kg dry weight per hectar, so it is obvious, that we speak about a significant amount (Mátyás, 1997). Autochtonous source means the inner source, which is the organic material, produced by

aquatic plants. Leaf litter decomposition is a multifactorial process, in which a number of micro- and macroinvertebrates take part. It is a long-term period process, which can be separated into three main parts. The first part is leaching, where leaves may lose up to 25% of their soluble organic material content (Webster et al., 1986). The second section is microbial colonisation, in which bacteria and microbial fungi colonise leaves and tenderize their structure. In the third part macroinvertebrates play the main role as they shred leaves into smaller pieces (Abelho, 2001). As a consequence of mechanical and biological processing CPOM (Coarse Particulate Organic Matter) transforms into FPOM (Fine Particulate Organic Matter) (Wurzbacher et al., 2016). CPOM is the fraction, which is bigger than 1 mm. FPOM is between 1 mm and 5 μm, and there is also a further category, DOM (Dissolved Organic Matter) which is smaller than 5 μm (Allan and Castillo, 2007). With shredding CPOM into FPOM, decomposers prepare usage form of mineral nutrition for other organisms (Santonja et al., 2018). The speed of decomposition depends on many factors, such as environmental conditions, like water temperature and flow conditions, litter input, which is limited by the nearby vegetation, chemical composition of leaves and the concentration of chemical compounds in water. Fungi have a prominent role in decomposition, because they do the breakdown of large molecular polymers, such as cellulose, kitin and lignin (Moorhead and Reynolds, 1992). According to our knowledge, there are approximately 600 water fungi from which about 300 belong to Ingoldian hyphomycetes (Goh and Hyde, 1996). PH is also an important factor because most of the decomposers are narrow tolerant species, and prefer neutral pH value. Aquatic microbes are micro-algae, bacteria, viruses, fungi, protozoas and archeas in less than 200 μm size range (Sigee, 2005). Macroinvertebrates are bigger than 200 μm and the main taxons are snails, shells, leeches, crustaceans, dragonflies, bugs and water beetles. These listed creatures disengage the bonded organic compounds of dead plant material, so it can be consumable for the aquatic and

terrestrial producers again. In natural waters their function is not only decomposing. They also serve as preys for fishes and other insectivorous aquatic animals (Ward et al., 1995). As time goes by, water parameters will also change with the progresses of decomposition, because more and more dissolved substances will be released into the water.

Materials and Methods

The usage of litterbag method

Our experiment was set up at the Agrometeorological Research Station at University of Pannonia, Keszthely (N: 46° 44' 7.93", E: 17° 14' 16.65"). Leaf litter was collected from two different venues: willow from the lakeside of Lake Balaton and poplar from the area of Kis-Balaton. After that, it was let to dry to constant weight, then 10-10 g of willow, poplar and mixed leaf litter was filled into tiny litterbags. To examine decomposition rates we have chosen the litterbag technique, which is a widely acknowledged method, firstly described by Singh and Gupta (1977). The litterbags were made in two different mesh-sizes and were made out of non-biodegradable materials. It is expedient to use litterbags in the two different, upper mentioned mesh-size because with the exclusion of macroinvertebrates we can easily underestimate decomposition rates (Robertson et al., 1999). The experiment was launched in 05. 06. 2019. The size and arrangement of the pans fitted the world-wide accepted standards (inside and outside white, 120 cm diameter, 25 cm deep, layed on double wooden lattice, filled with tap water) (Gombos, 2011). The bottom of the modified class „A" evaporation pan was filled with sediment from Lake Balaton in 3 cm layer, in order to let decomposers in the system, then it was filled up with tap water. (In this research, tap water was used, instead of water from Lake Balaton, because we had to compare the evaporation of the experimental pan with the standard

evaporation pan's.) The litterbags were tied to storage bins and were weighted to the bottom of the pan with greater stones to avoid their displacement *(Figure 1).*

Figure 1.: Class „A" evaporation pan with litterbags

Sampling and processing of the samples

The first sampling happened 64 days after the launch of the experiment, then further samplings took place every 2 weeks. On each occasion 12 samples were processed: 2 willow, 2 poplar, 2 mixed samples with 3 mm and 500 µm mesh sized litterbags. The bags weren't put into natural water, however, we could have assumed that, macroinvertebrates get into the system from the sediment or from the air. That is the reason, why we used 2 different mesh sizes. With the litterbags, water samples were also taken for subsequent water analytical tests. Under laboratory conditions, litter samples were unwrapped and depurated from contaminations with laboratory sieve and tap water *(Figure 2)*, then they were left to dry until their constant weight, for usually 2 weeks.

Figure 2.: Depurate of leaf litter samples

In the next step, we measured the remaining dry weights on digital analytical scales, and got information about weight loss. To define the decomposition rates, Graca et al's. (2005) widely acknowledged and used exponential decay model was applied:

$$\mathbf{Mt = M_0 \cdot e^{-kt}}, \qquad (1)$$

where „Mt" (g) is the mass at time, „M_0" (g) is the mass at time 0, „k" (day^{-1}) is the exponential decay coefficient and „t" (day) is time. With the exponential decay coefficient decomposition can be classified into three speed categories. If k<0.005 it is slow, if k=0.005-0.01 it is medium, and if k>0.01, we can speak about fast decomposition (Graca et al., 2005). The halving times were also measured, based on another formula of Graca el al. (2005):

$$\mathbf{TH = ln2 \cdot k^{-1}} \qquad (2)$$

It shows that how many days does it take for the samples to lose the half of their weight.

Measurement of water physical and chemical parameters

To define pH, conductivity and NH_4^+, PO_4^{3-}, SO_4^{2-} and Cl^- -ion-content of the water samples Adwa AD110 pH and thermometer, Adwa AD310 conductivity-temp portable meter and Lovibond Multidirect Spectrophotometer were used.

Measurement of evaporation

Next to the modified evaporation pan, a standard „A" pan was also settled, to compare their evaporation results. The water level of the pans had to be 5 centimetres under the edge of the pan, and it wasn't allowed to go 7.5 centimetres under it (Brouwer & Heibloem 1986). It is extremely important to maintain the water level, because if it falls 10 centimetres under the required level, measurement errors can be as high as 15 percent (Brouwer & Heibloem 1986). Wire mesh was stretched around the class „A" evaporation pans to prevent animals (birds and smaller mammals) from drinking the pan's water. It could have increased the measured values by up to 7% (Gifford et al. 2005). The measurement was performed according to the meteorological practice, during which the hole of the measuring cylinder placed in the tub was opened with a screw, and then, following the law of the moving vessels, the water level in the cylinder stopped at the level, that was in the tub. After the water level had levelled off, to determine the height of the water column, the hole was closed, then water was filled into 0.1 mm scaled glass measuring cylinder. After the measurement, it was filled back to the pan. The amount of daily evaporation was given by the difference in water column heights, measured on two consecutive days. During the experiment, actual evaporation was determined by subtracting the daily precipitation. The measurement was performed at the usual 7 a.m. observation time, in the morning (WMO, 1966, 1976). Tap water was used to replace evaporated water. It was stored in 120 l white tanks, in order to replace evaporated water, with water of the same temperature (Anda et al., 2016, 2018). Daily evaporation of the treatments was analyzed by paired t-test at the significance level of 0.05.

Results and Discussions

Water chemical parameters

The parameters, and dissolved material-, and ion-content of the pan's water were slowly, but constantly changing as time went by. Nevertheless, we did not notice any outstanding changes during the experimental period. Only conductivity and NH_4^+ content has shown less variability.

Table 1. Water quality parameters during the experimental period

pH	7.93±0.35
Conductivity (µS/cm)	325.93±218.45
NH_4^+ (mg/l)	0.367±0.16
SO_4^{2-} (mg/l)	14.71±8.62
PO_4^{3-} (mg/l)	0.187±0.121
Cl^- (mg/l)	8.24±1.099

The pH of the pan's water (7,93±0,35) was slightly alkaline, which is favourable for most of the saprophytes. In an experiment after the well-known red mud catastrophe, Hubai et al., (2007) were carrying out research by the Torna-stream. They reported that on strongly alkaline pH each and every evincible creature have disappeared, and they emerged only after the decrease of pH value again. In their study, Tripole et al. (2008) examined the tolerance spectrum of aquatic macroinvertebrates, depending on acidity, in the Grande-river. Their results have shown that there weren't any macroinvertebrates under 5.5 pH. The increase in conductivity can be traced back to leaching, in which more and more ions were released as time passed (Hasanuzzaman and Hossain, 2014). From that, it is obvious that leaf litter decomposition depends on a number of influencing factors. For example, Ágoston-Szabó et al., (2014)

documented 69% mass loss by willow leaf litter, also in a 140 day experimental period. It is more than our 57.3%, and the reason of the difference could be the diversity of environmental, water temperature and flow conditions. In the absence of drift, degraded materials and sediments may accumulate in litterbags which, if not flushed or removed, may reduce colonisation of microorganisms, thereby reducing the rate of degradation (Chauvet, 1987). Meentemeyer (1978) mentioned water temperature as the main influencing factor in leaf litter decomposition. Other researchers, such as Liu and Sun (2013) emphasises C:N ratio of leaves as the main factor. I believe that, all of these mentioned factors take part in decomposition and their influencing force depends on the local environmental conditions, and human interventions.

Leaf litter decomposition

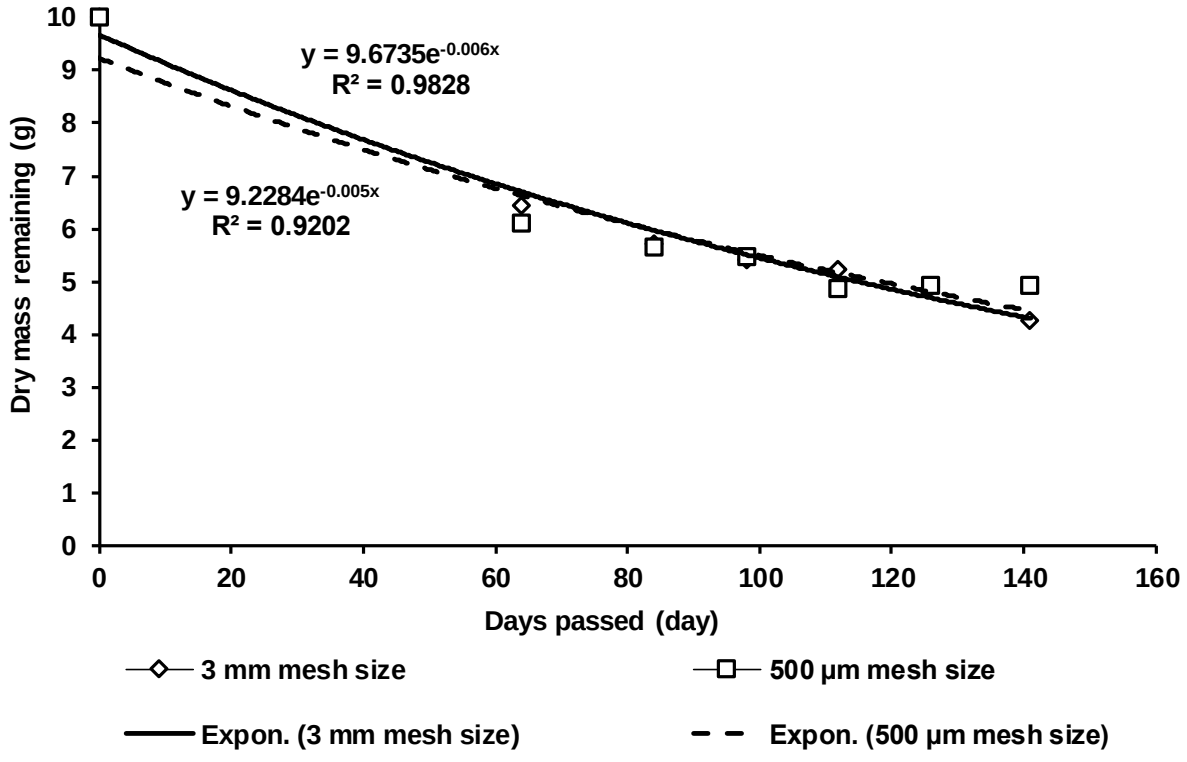

Figure 3. Decomposition rate of willow (Salix sp.*) in class ,,A'' evaporation pan*

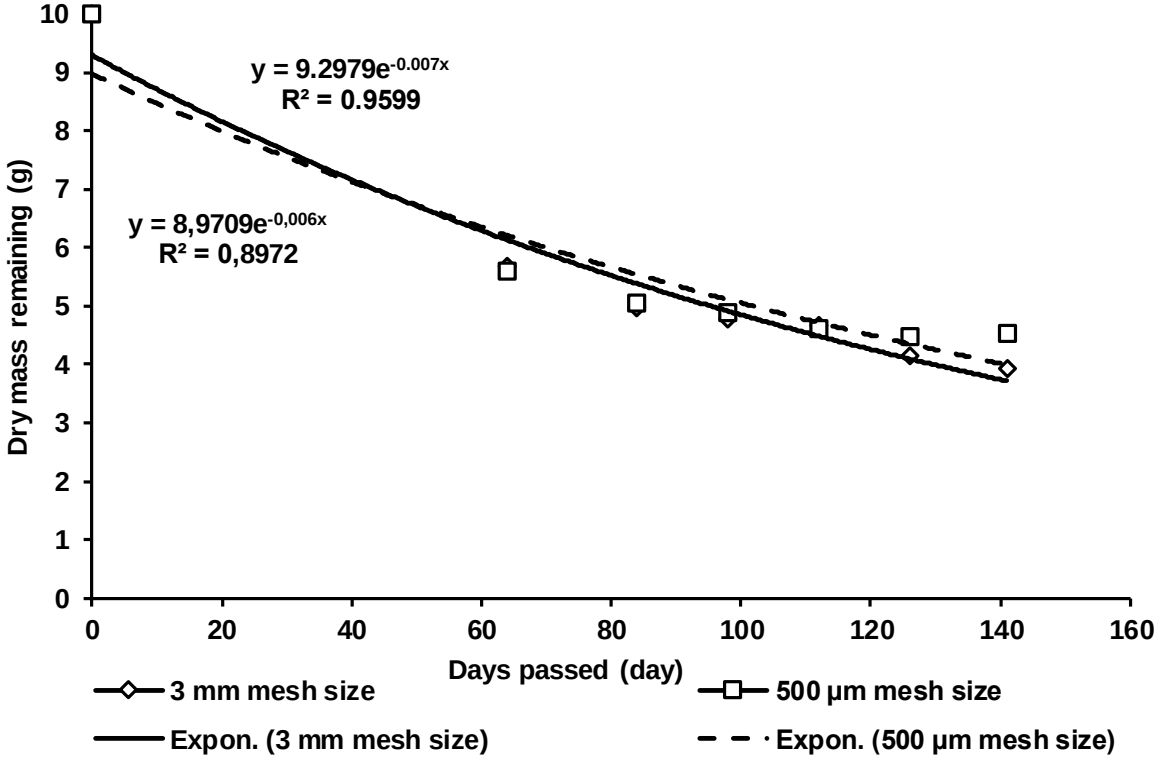

Figure 4. Decomposition rate of poplar (Populus sp.*) in class ,,A" evaporation pan*

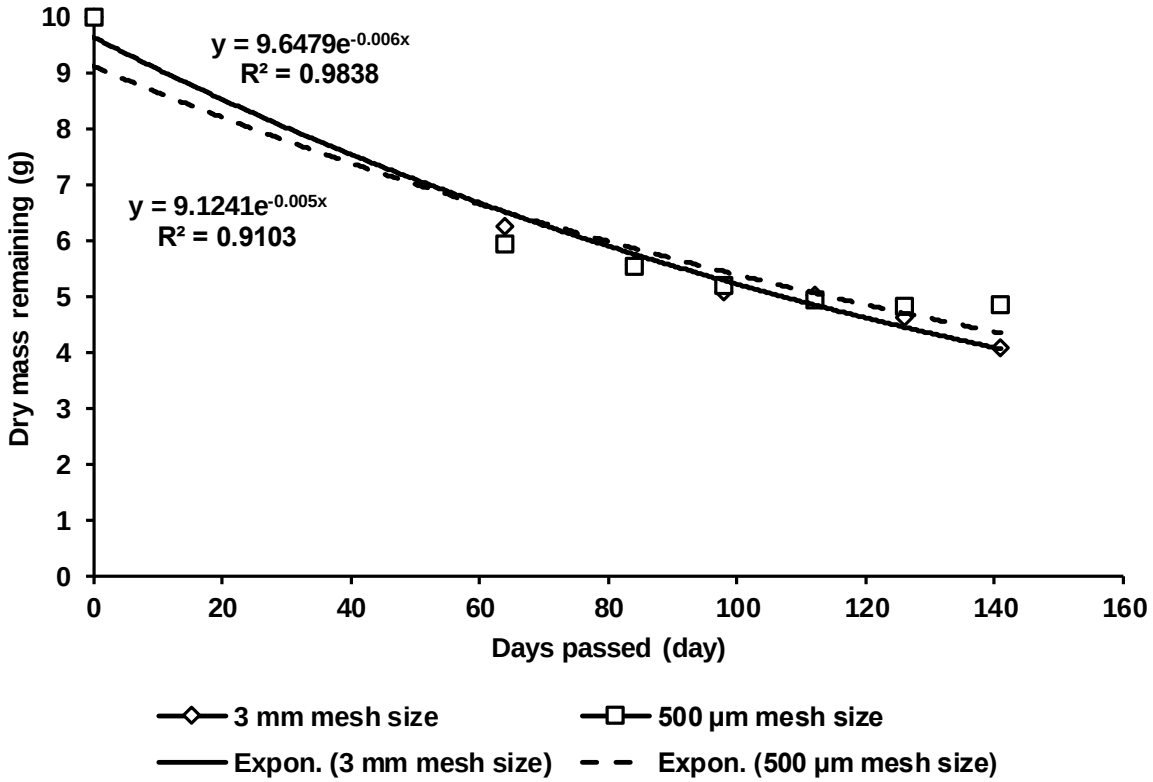

Figure 5. Decomposition rate of mixed leaves in class ,,A" evaporation pan

Decomposition followed exponential curve in every case (Figure 3-5). On the 112nd day the line of wide mesh-sized samples overcame the 500 µm mesh-sized litterbag's line by every litter type. The probably reason may be the hatching and shredding activity of macroinvertebrates.

Table 2.: The halving times of the investigated leaves from 15 June 2019. to 24 October 2019.

Leaf litter type	**Litterbag mesh-size**	**Halving times (day)**
Willow	3 mm	111.6
Willow	500 μm	110.3
Poplar	3 mm	92.3
Poplar	500 μm	95.9
Mixed	3 mm	104.62
Mixed	500 μm	106.5

Concerning to halving times, there weren't any considerable differences between the three litter types, and the two distinct litterbags. Zhai et al. (2019) also examined the decomposition of willow and poplar in the University of Bejing with litterbag technique, and in their results, there weren't significant differences in the speed of decomposition between the two different mesh-sized bags. The longest halving time was observed by willow, and the shortest by poplar. Mixed samples were between the two upper mentioned tree species.

The k values were expressed from the exponential decay model (Figure 6).

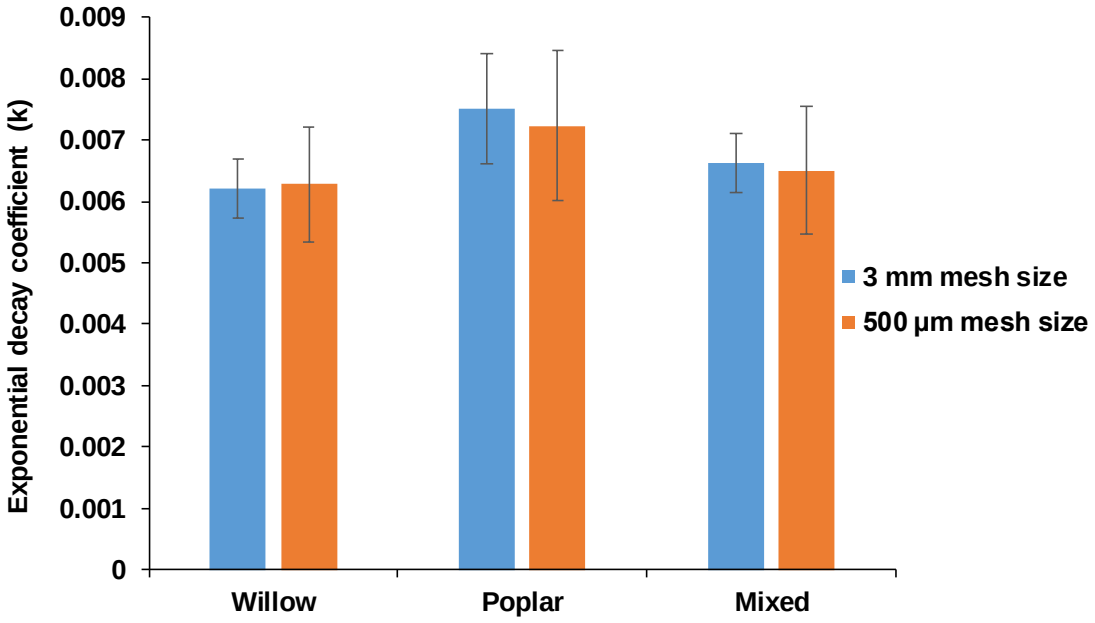

Figure 6. The distribution of exponential decay coefficient among our samples

On the diagram we can see that all „k" coefficients fell between 0.005 and 0.01 unit, so all of them were classified into „medium" decomposition category. Furthermore, there weren't any outstanding differences in the speed of decomposition, between the two different mesh-sized devices. In their study Markus and Gessner (2009) said, that the usage of mixed leaves tend to accelerate decomposition. The mixture of leaves containing more than one species could provide more homogenous nutrients for the decomposers, even species with narrow demands (Chapman and Koch, 2007). With our results, these statements cannot be proven. The highest value (k=0.0075±0.0009) was measured on poplar, with wide mesh-size, and the lowest value (k=0.0062±0.0005) on willow, also with wide mesh-sized bags. The results of mixed leaf litter were almost exactly the average of the two other litter types.

Evaporation from the pans

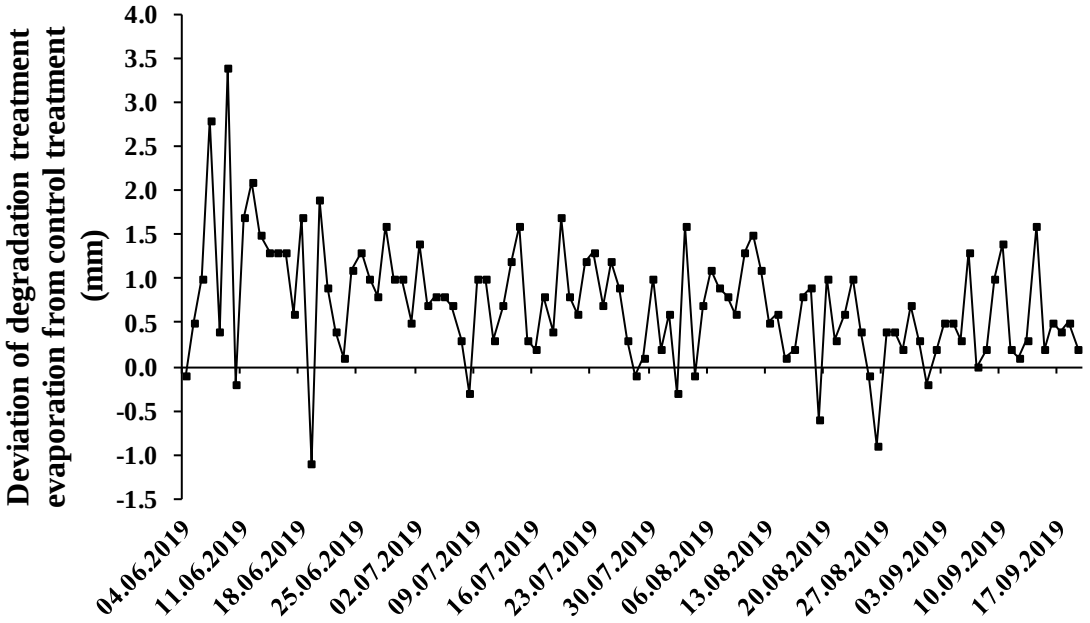

Figure 7. Differences in evaporation, between the modified and the control class „A" evaporation pans

The seasonal average daily evaporation of the control pan was 3.6±1.3 mm*day^{-1}, and the degradation treatment's result was 4.3±1.6 mm*day^{-1}. 19 % higher ($p<0.001$) evaporation rate in modified pan's evaporation was observed, which means that sediment and leaf litter placed in the pans increased the evaporation in 2019. The probably reason may be the higher energy absorption of filled pan, due to the darker color of the sediment (the standard tub is white), and the heat releases during the decomposition processes.

Conclusions

Taking everything into account, leaf litter decomposition is a complex multifactorial process, which is influenced by a number of natural and human factors. There are many other studies dealing with the decomposition of the same species, among different conditions. They all get conspicuously different results. Therefore, it can be stated, that the speed of decomposition is mostly influenced by water temperature, water quality (in connection with decomposers), the variety of saprophytes, decomposed plant species, and the chemical structure, and nutrient content of leaves. There are plenty of studies investigating decomposition rates in great lakes, rivers and disaster-weighted venues, but as far as I am concerned, it would be expedient to assess the decomposition rates of the Country's smaller streams too, to get a clearer picture about the condition of our waters, which could be useful to find out, if there is any deficiency, which may require human intervention. Temperature is also one of the main factors of decomposition, so global warming may bring unforeseen changes, so it would be advantageous to start researches examining the effects of global warming, on leaf litter decomposition. Furthermore, while leaf litter represent the main source of nutrients and energy for aquatic microorganisms, the protection of waterfront plant communities would also be inevitable.

Acknowledgements

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STRUCTURAL ANALYSIS OF THE COMMON RAGWEED (*AMBROSIA ARTEMISIIFOLIA* L.) CHLOROPLAST GENOME

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Abstract

Chloroplast is an essential organelle in plants. Besides its key role in photosynthesis fatty acids and many amino acids are synthesized in chloroplasts. Due to the endosymbiotic origin chloroplasts have their own DNA, the cpDNA that called also plastome. The chloroplast genome has well defined structure and due to its conservative nature slow evolution is characteristic for it. In the present study we analyzed the plastome structure of common ragweed (*Ambrosia artemisiifolia*), the most widespread and highly allergenic weed in many parts in the world. Compared to other chloroplast genomes, the analysis indicates the differences in start codon of some genes, in gene content and structure, in intron content, as well as the repeated sequence motifs are identified, with special attention on the potential microsatellites. Results of this study can be utilized in future phylogenetic analyses and genotyping.

Keywords: common ragweed, chloroplast genome, plastome genes, repeats, microsatellites

Összefoglalás

A kloroplasztisz a növények létfontosságú sejtszervecskéje, mely a fotoszintézisben betöltött kulcsfontosságú szerepe mellett a zsírsavak és több aminosav bioszintézisének helye is egyben. Endoszimbionta származásának köszönhetően a kloroplasztisz saját DNS-el, a cpDNS-el rendelkezik, melyet plasztomnak is nevezünk. A kloroplasztisz genom meghatározott szerkezeti felépítése és konzervatív természete miatt lassú evolúció jellemzi. A jelen tanulmányban a világ számos régiója leggyakoribb gyomnövényének és legfőbb allergén növényének számító ürömlevelű parlagfű (*Ambrosia artemisiifolia*) plasztomjának szerkezetét elemeztük. Más kloroplasztisz genomokhoz hasonlítva a parlagfű plasztom egyes gének esetében eltérő start kódonnal rendelkezik, különbségek tapasztalhatók a géntartalomban és génszerkezetben, illetve az intron tartalomban. Továbbá meghatároztuk a parlagfű plasztom ismétlődő szekvencia motívumait, különös tekintettel a lehetséges mikroszatellitekre. A jelen tanulmány eredményei jól hasznosíthatók a továbbiakban filogenetikai és genotipizálási vizsgálatokban egyaránt.

Kulcsszavak: ürömlevelű parlagfű, kloroplasztisz genom, plasztom gének, ismétlődések, mikroszatellitek

Introduction

The chloroplast is an essential organelle of green plants that converts the energy of sunlight into energy storing organic molecules by using carbon dioxide and water. Besides capturing and utilizing the light-energy, in chloroplasts such essential molecules as the fatty acids and many of the amino acids are synthesized (Alberts et al., 2015). In today's agriculture chemicals

blocking either the photosynthetic or the biosynthetic processes which take place in chloroplast are widely used for weed control (Délye et al., 2013). However, chloroplasts have their own genome, processes in chloroplast are under control of the nuclear genome of the plant cell. Chloroplasts are able to divide in the plant cell, and generally they are inherited only maternally. A photosynthesizing plant cell contains about 100 chloroplasts, and each of them has 10-100 chloroplast DNA (cpDNA) molecules (Alberts et al., 2015). This high copy number on the one hand ensures high gene dosage and the possibility of variation that occasionally occurs in low copy number and is called heteroplasmy. On the other hand, since many genes of the chloroplast are essential for the life of the plant, the effect of mutations which are deleterious for the function of that gene will be alleviated or neutralized by the high number normal alleles (Birky, 2001).

The cpDNA or plastome, is generally visualized in circular form (Sakamoto and Takami, 2018). It has conservative structure with a determined order of genes. It is considered, that chloroplasts originate from a single endosymbiotic event, when a eukaryotic cell engulfed a photosynthetic cyanobacterium. This common ancestry, the highly conservative nature and the maternal inheritance are the reasons for the widespread use of cpDNA in phylogenetic studies (Alberts et al., 2015).

Recently, we published the chloroplast genome sequence of *Ambrosia artemisiifolia* (Nagy et al. 2017), the most widespread and highly allergenic weed in many parts in the world. In that article the main structure of the *A. artemisiifolia* plastome was determined and the gene content was characterized. In the present study we analyzed the gene structure and sequence repeats of the common ragweed cpDNA and identified the microsatellites of it. Our findings can be utilized in future evolutionary studies and chloroplast genome based genotyping.

Materials and methods

The assembled common ragweed chloroplast genome which we used in this study was uploaded and can be found at the NCBI (National Centre for Biotechnology Information, USA) MF362689.1 GenBank identification number.

The *A. artemisiifolia* plastome was compared to the *Helianthus annuus* cpDNA sequence.

Search for repeats was performed with the REPuter (Kurtz et al., 2001) and Tandem Repeat Finder (Benson, 1999) programs. The use of both programs was necessary, because the REPuter identifies just those repeats which are scattered on the sequence of analysis, while the Tandem Repeat Finder identifies repeats which are neighboring each other. For REPuter, the set parameters were as follows: Hamming distance: 3, number of hits 5000, minimum repeat length 30 bp, and all repeat types were marked. Results of REPuter were filtered with a self-developed program, called Clean Repeats, which we made freely accessible to the scientific community on the following link: http://cleanrepeats.georgikon.hu/. Tandem Repeat Finder was used with default parameters. For exploring the variability of the identified repeats plastome sequences of *Helianthus annuus* (NC_007977.1), *Arabidopsis thaliana* (NC_000932.1), *Artemisia argyi (*KM386991.1), *Ambrosia trifida* (MG029118.1) and of *Taraxacum officinale* (KU361241.1) were downloaded and sequence motives similar to *A. artemisiifolia* tandem repeats were identified.

The occurrence of repeats with length from 1 to 6 nucleotides, which are considered as potential microsatellites or SSR (simple sequence repeat) markers was investigated with the Msatcommander (Faircloth, 2008) and the WebSat (Martins et al., 2009) programs. The minimal number of repeats of mono-, di-, tri-, tetra-, penta- and hexanucleotides was adjusted to 10, 5, 4, 3, 3, 3 for both program. That means that a sequence motive should be constituted

from at least 10 mononucleotides, five dinucleotides, four trinucleotides or three tetra-, penta- or hexanucleotides to be recognized by either program as a microsatellite.

Results and discussion

Analysis of plastome genes of the common ragweed

In the common ragweed plastome from among the 80 protein coding genes three genes were identified which have different start codon from the ATG, which is the start codon of eukaryotic organisms. These start codons and genes are the followings: ACG in *ndhD*, ATC in *rpl16* and GTG in *rps19*. In other plants similar phenomenon was already reported for the *atpE, psaI, rps11, petB* (Wiegert et al., 2012), *rps19* (Kim et al., 2014), *ndhD, psbL, rps19* (Curci et al., 2015) chloroplast genes. These alternative start codons indicate to the prokaryotic origin of the chloroplast.

Compared to *A. thaliana*, the model plant in plant genetics, *A. artemisiifolia* has two more protein coding genes. These are the *infA*, which is a translation initiation factor, and the *ycf15*, which codes for a protein with unknown function.

In the IRA and IRB regions six protein coding genes (*rpl2, rpl23, ycf2, ndhB, rps17, ycf15*) as well as the 3'end of the *rps12* gene are repeated.

Compared to the *H. annuus* reference genome length differences in 11 common ragweed plastome genes were detected. Among these, the *trnT-GGU, trnA-UGC, psbH, rpoC2, rps16,* and the *ycf2* genes were larger, while the *rrn23, ccsA, matK, rpoA* and the *ycf1* genes were smaller in *A. artemisiifolia* than in sunflower. Length variations in these chloroplast genes were already reported in several studies. Timme et al. (2007) described sequence variations in the sunflower *ccsA, matK, psbH, ycf1* genes when compared to *Lactuca sativa*. In rice, *rpoC2*

length variations were detected (Shimada et al., 1990). Further, comparing 84 chloroplast protein and rRNA coding genes of 17 plant species significant sequence variations were registered for the *ycf2, rps16, rpoA* and *psbH* genes (Kim and Lee, 2004). Due to their length variation in different plant species it is suggested to analyze the usefulness of the above genes of *A. artemisiifolia* in phylogenetic and evolutionary studies. Whether the length variation of these genes is affecting their function needs investigation.

One intron was detected in the *atpF, petB, petD, ndhA, ndhB, rpl2, rps16, rpoC1* protein coding genes and in the *trnA-UGC, trnG-UCC, trnI-GAU, trnK-UUU, trnL-UAA, trnV-UAC* tRNA genes of the common ragweed. Two introns were detected in the *ycf3* and *clpP* genes. *A. thaliana* plastome has three more intron containing genes, the *rpl12, rpl16* and *rps12* genes, than the *A. artemisiifolia* (Sato et al., 1999), while *H. annuus* has one more intron containing gene, the *rps12* (Timme et al., 2007).

Further, the *trnE-UUC* gene of *A. artemisiifolia* is missing from the *H. annuus* plastome. The presence/absence of this gene is very variable in species of the *Asteraceae* family (Wang et al., 2018, Kim et al., 2017, Chen et al., 2018).

It is concluded, that except the above the *A. artemisiifolia* protein coding plastome genes do not show significant structural differences compared to related species or to *A. thaliana*.

Detection of repeats in the common ragweed plastome

The REPuter program with the adjusted parameters provided more than 2000 hits. Nevertheless, these hits were overwhelmingly overlapping repeats. To eliminate overlaps, we have created a program called Clean Repeats (http://cleanrepeats.georgikon.hu/) that will be published elsewhere. The filtered REPuter results were combined with results of the Tandem Repeat Finder program. In total 67 repeats were detected, from which one third is scattered throughout

of the plastome, and the two-third is constituted from neighboring repeats, which follow each other in the plastome.

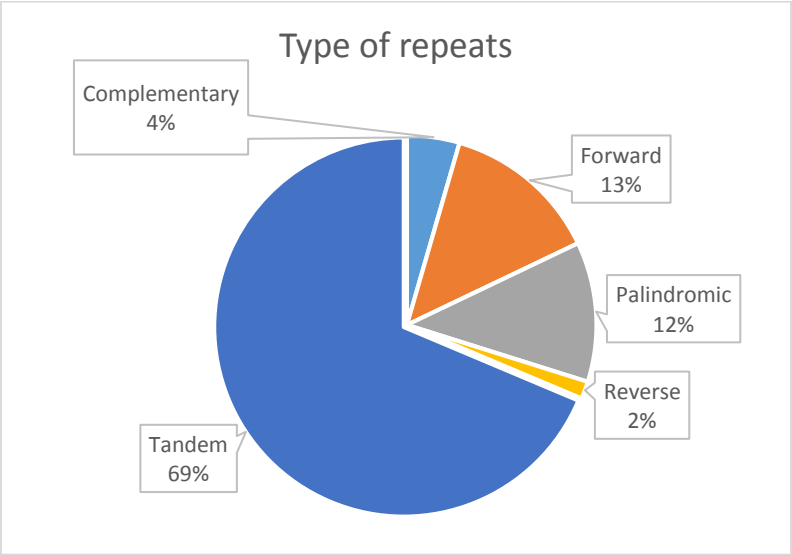

Figure 1: Distribution of the five repeat types in plastome of the common ragweed. Values are calculated from the combined results of the Tandem Repeat Finder and the filtered REPuter program.

Most of the repeats, 69%, belong to the tandem type, while the percentage of forward and palindromic repeats is just slightly above 10%. The reverse and complementary repeats have the lowest proportion with less than 5%. (Fig. 1.). Most repeats (52%) belong to the 15-30 bp long category (Fig. 2.). The number of smaller (<15 bp) and larger (>30 bp) repeats was almost similar. Besides the two inverted repeat regions two repeats above 100 bp were detected.

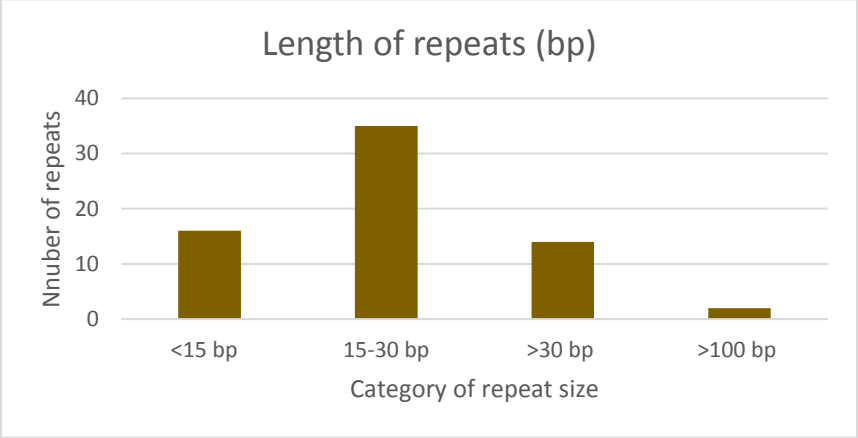

Figure 2: Repeat size categories in plastome of the common ragweed. Values are calculated from the combined results of the Tandem Repeat Finder and the filtered REPuter program.

However, the LSC region accounts for about 55% of the common ragweed plastome, 48 repeats (72%) occurred in the LSC region and three in LSC – inverted repeat region junctions (Fig. 3.).

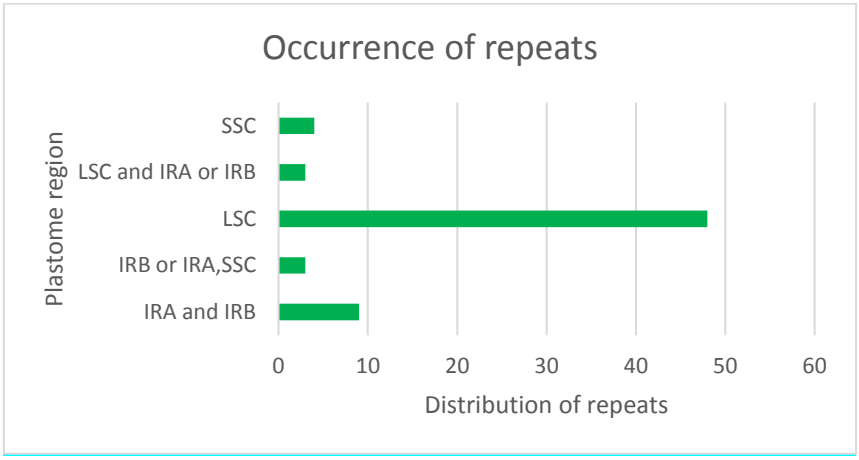

Figure 3: Number of repeats in different plastome regions of the common ragweed. Values are calculated from the combined results of the Tandem Repeat Finder and the filtered REPuter program. SSC (Small Single-Copy region, LSC (Large Single-Copy region), IRA and IRB (Inverse Repeat regions A and B)

Regarding the distribution, 47 repeats (70%) were found in intergenic spacer regions, and just six (9%) were identified in exons and three repeats (4%) in introns (Fig.4.). Interestingly, seven

repeats (10%) occurred in intergenic spacer region – intron junctions, and four (6%) in intergenic spacer region – exon junctions.

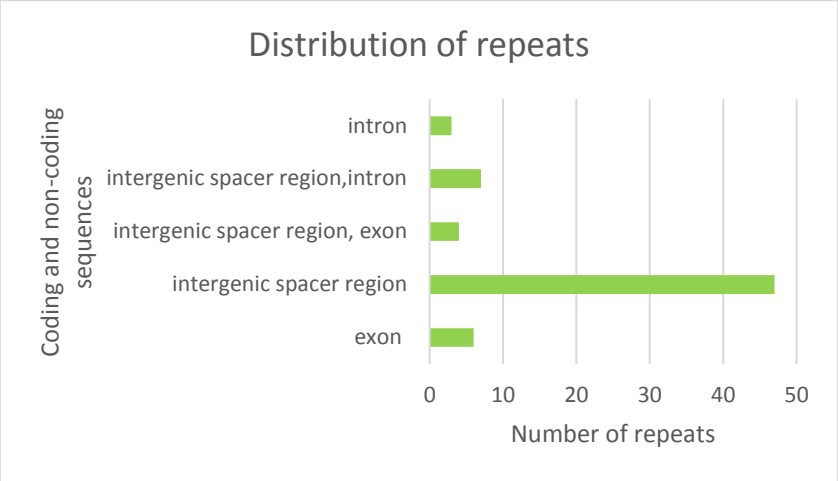

Figure 4: Number of repeats in different types of coding and non-coding sequences in plastome of the common ragweed. Values are calculated from the combined results of the Tandem Repeat Finder and the filtered REPuter program.

Comparing tandem repeats of *Ambrosia artemisiifolia*, *Helianthus annuus*, *Arabidopsis thaliana*, *Artemisia argyi*, *Ambrosia trifida* and *Taraxacum officinale* sequence similarity was detected in 45 cases. Only the *ycf2* gene occurred in all of these species with at least 70% similarity. Except *A. thaliana* repeats in the *atpI-atpH*, *rbcL-accD* and *rrn5-rrn4.5* intergenic non-coding regions and in *rps8* gene were common among the analyzed species. Except one of the remaining repeats of *A. artemisiifolia* all repeats could be found in *A. trifida*, four in *H. annuus*, four in *T. officinale*, and three in *A. argyi* while in *A. thaliana* no similar motif of these repeats could be detected. These results are in harmony with the evolutionary distances of *A. artemisiifolia* and the analyzed species, and reveal the variability of tandem repeats and their applicability in evolutionary studies.

Identification and characterization of microsatellites

With the adjusted parameters 43, as well as 53 microsatellites were detected with the Msatcommander and with the WebSat program, respectively (Table 1.).

Table 1. List of microsatellites in the common ragweed chloroplast genome

Start position (bp)	**Repeat motive**	**Repeat number**	**Repeat type**	**Position of the microsatellite**			**program**
41	A	10	mono	*rpl2-trnH-GUG*	IGS	LSC	W, M
2147	T	17	mono	*trnK-UUU-matK*	IGS	LSC	W, M
2490	T	11	mono	*matK*	exon	LSC	W, M
4696	A	30	mono	*trnK-UUU-rps16*	IGS	LSC	W, M
4897	A	77	mono	*trnK-UUU-rps16*	IGS	LSC	W, M
11220	T	22	mono	*psbM-trnD-GUC*	IGS	LSC	W, M
13129	T	16	mono	*trnE-UUC-rpoB*	IGS	LSC	W, M
13251	T	16	mono	*trnE-UUC-rpoB*	IGS	LSC	W, M
13940	A	10	mono	*rpoB*	exon	LSC	W, M
17170	A	11	mono	*rpoC1*	intron	LSC	W, M
17714	G	10	mono	*rpoC1*	intron	LSC	W, M
19025	A	10	mono	*rpoC1*	exon	LSC	W, M
21485	T	10	mono	*rpoC2*	exon	LSC	W, M
26699	T	10	mono	*atpI-atpH*	IGS	LSC	W, M
28640	T	18	mono	*atpF-atpA*	IGS	LSC	W, M
30438	A	11	mono	*trnR-UCU-trnG-UCC*	IGS	LSC	W, M
30792	A	12	mono	*trnG-UCC*	intron	LSC	W, M
35374	T	18	mono	*psbC-trnS-UGA*	IGS	LSC	W, M
44414	A	20	mono	*ycf3*	intron	LSC	W, M

47168	A	20	mono	*trnT-UGU-trnL-UAA*	IGS	LSC	W, M
54715	T	25	mono	*atpB-rbcL*	IGS	LSC	W, M
70612	T	23	mono	*clpP*	intron	LSC	W, M
71234	A	13	mono	*clpP*	intron	LSC	W, M
74255	A	10	mono	*psbT-psbN*	IGS	LSC	W, M
74837	A	21	mono	*psbH-petB*	IGS	LSC	W, M
75450	A	36	mono	*petB*	intron	LSC	W, M
78191	T	10	mono	*petD-rpoA*	IGS	LSC	W, M
80639	T	36	mono	*rps8-rpl14*	IGS	LSC	W, M
81171	T	15	mono	*rpl14-rpl16*	IGS	LSC	W, M
84349	T	10	mono	*rps19-rpl2*	IGS	LSC, IRA	W, M
109673	T	12	mono	*ycf1*	exon	SSC	W, M
110286	A	11	mono	*ycf1*	exon	SSC	W, M
111364	A	16	mono	*ycf1*	exon	SSC	W, M
112589	A	10	mono	*ycf1*	exon	SSC	W, M
120420	T	11	mono	*psaC-ndhD*	IGS	SSC	W, M
122012	A	13	mono	*ndhD-ccsA*	IGS	SSC	W, M
124427	A	12	mono	*rpl32-ndhF*	IGS	SSC	W, M
19249	AT	5	di	*rpoC1*	exon	LSC	W, M
20248	AT	5	di	*rpoC2*	exon	LSC	W, M
46687	TA	5	di	*rps4-trnT-UGU*	IGS	LSC	W
59205	TA	5	di	*accD-psaI*	IGS	LSC	W
117148	TA	5	di	*ndhA*	intron	SSC	W
35161	TTC	4	tri	*psbC*	exon	LSC	W
60959	ATT	4	tri	*ycf4-cemA*	IGS	LSC	W, M
109561	AGA	5	tri	*ycf1*	exon	SSC	W
109563	AAG	4	tri	*ycf1.*	exon	SSC	M
4882	ATAA	3	tetra	*trnK-UUU-rps16*	IGS	LSC	W

63329	TTCT	3	tetra	*petA-psbJ*	IGS	LSC	W
63331	CTTT	3	tetra	*petA-psbJ*	IGS	LSC	M
67995	TATT	3	tetra	*rpl33-rps18*	IGS	LSC	W
117209	TATC	3	tetra	*ndhA*	intron	SSC	W
121797	TATT	3	tetra	*ndhD*	exon	SSC	W
123754	ATTT	3	tetra	*trnL-UAG-rpl32*	IGS	SSC	W, M
68056	AACCA	3	penta	*rpl33-rps18*	IGS	LSC	W
56532	GGATAA	3	hexa	*rbcL*	exon	LSC	W

IGS= Intergenic spacer region; LSC= Large Single Copy; SSC= Small Single Copy; IRA, IRB=Inverted Repeat A, B; W=WebSat; M= Msatcommander

Mononucleotide microsatellites accounted for 86% and 70% of all SSR-s with the Msatcommander and WebSat program, respectively (Fig. 5. and 6.).

However, penta- and hexanucleotide type microsatellites were not detected by the Msatcommander (Fig. 5.), with the WebSat one of each of these type SSR-s could be detected (Fig. 6.). The difference between the numbers of detected microsatellites is due to the differing algorithm of the two programs.

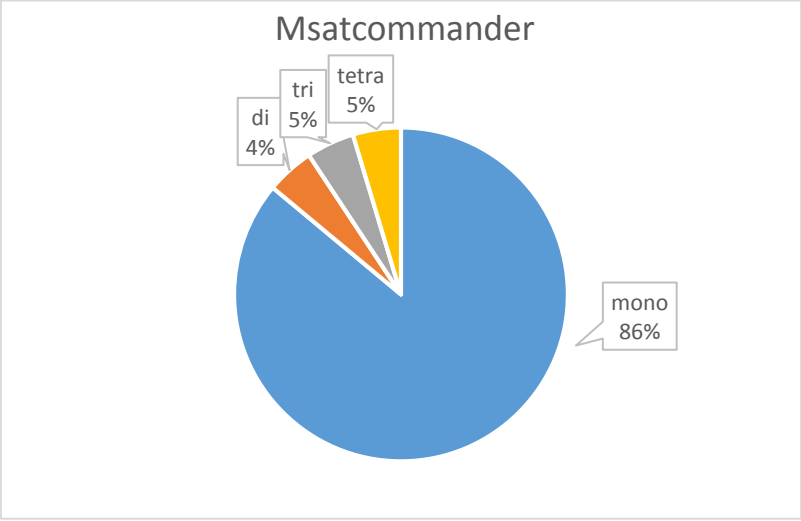

Figure 5: Distribution of microsatellites detected with the Msatcommander program in plastome of the common ragweed.

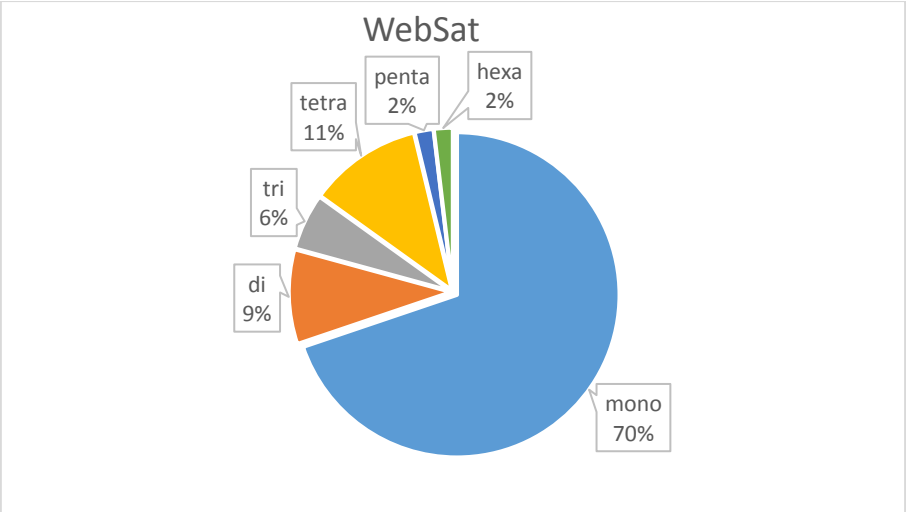

Figure 6: Distribution of microsatellites detected with the WebSat program in plastome of the common ragweed.

With one exception, only A (adenine) and T (thymine) mononucleotide microsatellites were found in the common ragweed plastome. Similar observation was already reported for other *Asteraceae* species, such as artichoke (Curci et al., 2015), *Jacobaea vulgaris* (Doorduin et al., 2011), and *Artemisia frigida* (Liu et al., 2013).

The microsatellites reported in the present publication could be efficient tools in future phylogenetic analyses and genotyping studies.

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THE ROLE OF AVIATION IN THE LIFE OF SPA CITIES LOCATED IN ZALA COUNTY

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Abstract

Tourism and transportation are sectors that are inseparable from many aspects. The paper focuses on the detection of correlations between the spa towns of Zala County and the international airport of the region. The spas of the county, offering services at different levels, are very popular with international tourists, for whom an aspect of selected importance is accessibility, when choosing destination. The spas of Zala are accessible, in addition to road, by air, due to Hévíz-Balaton International Airport operating in Sármellék. The authors in their paper examine the volume of guests – approximately 75% of whom are foreigners – in the major four-star hotels of Hévíz, a resort of international recognition, and the changes in the volume of guests using the services of the airport. The impressive airport development announced for Sármellék in the recent years have not been implemented yet, no significant increase can be seen in the number of air passengers. The impact of the definitely busier airports of the wider region – Budapest, Vienna and Zagreb – can be evidently felt in the health tourism of the region. The further development and sustainable operation of the Hévíz-Balaton

International Airport are a must, as foreign tourists play a very important role in the tourism of two spa towns with international significance: Hévíz and Zalakaros.

Keywords: spa, tourist attraction, aviation, Zala County

Összefoglalás

A turizmus és a közlekedés sok szempontból egymástól elválaszthatatlan ágazatok. Tanulmányunkban a Zala megyei fürdővárosok és a régió nemzetközi repülőtere közötti összefüggések keresésére koncentráltunk. A megye különböző szintű szolgáltatásokat nyújtó fürdőhelyei nagyon népszerűek a nemzetközi turisták körében, akiknek a cél desztináció kiválasztásánál kiemelt szempont a megközelíthetőség. A zalai fürdők a közút mellett légi úton is elérhetőek, köszönhetően a sármelléki Hévíz-Balaton nemzetközi repülőtérnek is. Munkánkban a nemzetközi hírű Hévíz legjelentősebb négycsillagos szállodáinak – melyek vendégköre kb. 75%-ban külföldi – idegenfogalmi adatait, valamint a repülőtér légi utasforgalmának változásait vizsgáltuk meg. Megállapítható, hogy a térség idegenforgalmában, az utóbbi években jelentős szereppel bíró orosz vendégkör (Budapestről) és az izraeli vendégek döntő része (Bécsből) a légi közlekedést választva érkezik hazánkba, azonban nem közvetlenül Sármellékre. A német vendégkör negyede viszont ide érkezik, két jelentős utazási irodának köszönhetően. Az utóbbi évek hangzatos repülőtér fejlesztései Sármelléken ez idáig nem valósultak meg, a légi utaslétszám alakulásában szignifikáns változás nem tapasztalható. A környék lényegesen nagyobb spektrumú nemzetközi repülőtereinek súlya – Budapest, Bécs, Zágráb – a térség beutazó gyógyturizmusában markánsan érzékelhető. A repülőtér további fejlesztése és fenntartható üzemeltetése mindenképp szükséges, hiszen a két nemzetközi jelentőségű fürdőváros, Hévíz és Zalakaros turizmusában is fontos szerepet töltenek be a

külföldi turisták. Egy stabilan működő, korszerű infrastruktúrával rendelkező repülőtér óriási versenyelőnyt biztosíthat a zalai fürdőknek, és a hatásai az egész megye turizmusát kedvezően befolyásolhatják.

Kulcsszavak: gyógyfürdő, turisztikai látványosság, légi közlekedés, Zala megye

Introduction

Zala County possesses outstanding tourism potential. The diverse supply is based on valuable touristic traditions. One of the major products is tourism of Lake Balaton in the hinterland of Keszthely, which generates significant numbers of tourist arrivals especially in the summer months, attracting both Hungarian and international guests. Important attractions are city and cultural tourism concentrated in the major cities (Zalaegerszeg, Nagykanizsa, Keszthely), ecotourism in the continuously developing landscapes of the Little Balaton reserve, and rural or mild tourism more and more popular in the Göcsej areas. As regards the attractions of Zala County, however, it is clearly the spa towns that are dominant, where guests are treated with high quality health tourism services.

The spas of Zala include medical resorts with international significance and long traditions, such as Hévíz or Zalakaros; spas continuously developing, with national level recognition, like Kehidakustány and Lenti; and also quality spas serving the population of the respective region and the neighbouring areas in the first place. Due to health tourism, successful spa towns dispose of significant revenues and resources, giving them a considerable economic advantage, and so the attraction of the largest possible pool of tourists is their vested interest. Hungarian health tourism is very successful among the solvent tourists of Western Europe

(Smith and Puczkó, 2010), also, the recent decade saw the appearance of masses of Russian tourists, which is unique in Hungary (Köbli, 2015).

The number one attraction for international markets, in addition to the quality of the medical water and the level of services, is the issue of accessibility. A significant role in this can be played by the third largest airport of Hungary, Hévíz-Balaton International Airport that can be found in Sármellék. The airport can promote the inclusion of new clienteles in the health tourism sector of Zala County; its implementation is not an easy task, however. The huge potential lying in the airport is in vain as long as the local experts are continuously struggling with problems during implementation, as they have done in the last decades. The stable operation of the airport, anyway, would be a sound basis among other things for the operation of health tourism in Zala County.

Materials and methods

The aim of this paper is the detailed analysis of the role of the airport, with special regard to the demonstration of the impacts on the clientele of the spa towns of Zala County. The significance of the spas of Zala is briefly discussed, and the history of the airport, rich in turning points, and its present state are introduced. The recent impact of the airport on the spa towns is demonstrated by the example of the most popular medical resort, Hévíz, where guest surveys in the hotels were used to assess the proportions of domestic and international guests, the range of countries generating the largest numbers of guests, and the share of international tourists arriving by air. Also, the travel habits of these guests and the deviations thereof from other tourists were looked at. The findings are compared to each other in an Excel table, and are evaluated. For this purpose interviews were made in three high quality four-star hotels of Hévíz:

in Ensana Thermal Hévíz, Ensana Thermal Aqua and Hotel Európa Fit. These hotels are the largest accommodations used by international tourists arriving at Hévíz, interested in the success of the airport also in the long run. The answer was sought in the case of each accommodation to the questions above. The findings of the interviews assist the understanding of the recent role of the airport and its future development potential in the tourism sector of the county, and also the exploration of the possibilities of close cooperation of the two sectors: health tourism and aviation.

Results

Health tourism in Zala County

Nowadays those settlements can be considered as spa cities successful in the long run that, with their former and recent developments, have acquired a significant domestic and international clientele by now (Jónás-Berki et al., 2014). For sick patients the possibility of healing is the primary motivation, for healthy ones it is prevention; both generate a constant demand. In addition, the growing popularity of health-conscious way of life, the penetration of civilisation diseases and the more widespread use of alternative medicine will all increase this demand (Connell 2013).

The main locations of the excellent medical tourism endowments of Hungary and the quality services built thereon are the continuously developing spa cities. Owing to different spa developments, a very large number of settlements possess spas and the related touristic facilities now, but in the view of Michalkó and Rátz (2011) there are few spa cities in the classic sense of the word in Hungary, as the dominance of medical tourism has essential, complex municipal impacts in a few settlements, only. The spas of Zala have excellent endowments, and

consequently they have leading positions in the supply of health tourism in Hungary. The unique composition of waters, the traditions of many decades – centuries in Hévíz –, the favourable geographical position in West Hungary give the Zala spas a basis on which continuous development is possible. Local developments lead to the improvement of the quality of services, and of the general living standards and quality of life, which is of outstanding importance for local society, besides guests. Urban and tourism developments, the new services and infrastructure investments have a combined and multiplier effect.

The Hungarian National Health Tourism Development Strategy (OES in Hungarian) categorised Hungarian spa facilities and the settlements giving home to spas at three levels. In 385 settlements of Hungary there are spas of some quality, of which 187 facilities, suitable for the implementation of development objectives, were categorised by researchers. Three categories were defined (OES 2007):

- Spa resorts of international significance, historical and monument spas, medical places
- Touristic service providers of national and regional significance
- Spas of local significance

Figure 1 demonstrates the location of Zala County settlements with spas of various qualities, and the airports of the county. The county has excellent geothermal endowments, as a consequence of which spa developments are continuously undertaken. Even spas of local significance have a stable clientele, is many cases attracting guests from the neighbouring countries as well (from Austria and Slovenia), but the bulk of the clientele is made by visitors from Zala and the neighbouring counties. The spas in Bázakerettye and Lovászi make the tourism supply of the regional more diverse, but they play no role in international tourism arriving at the airports.

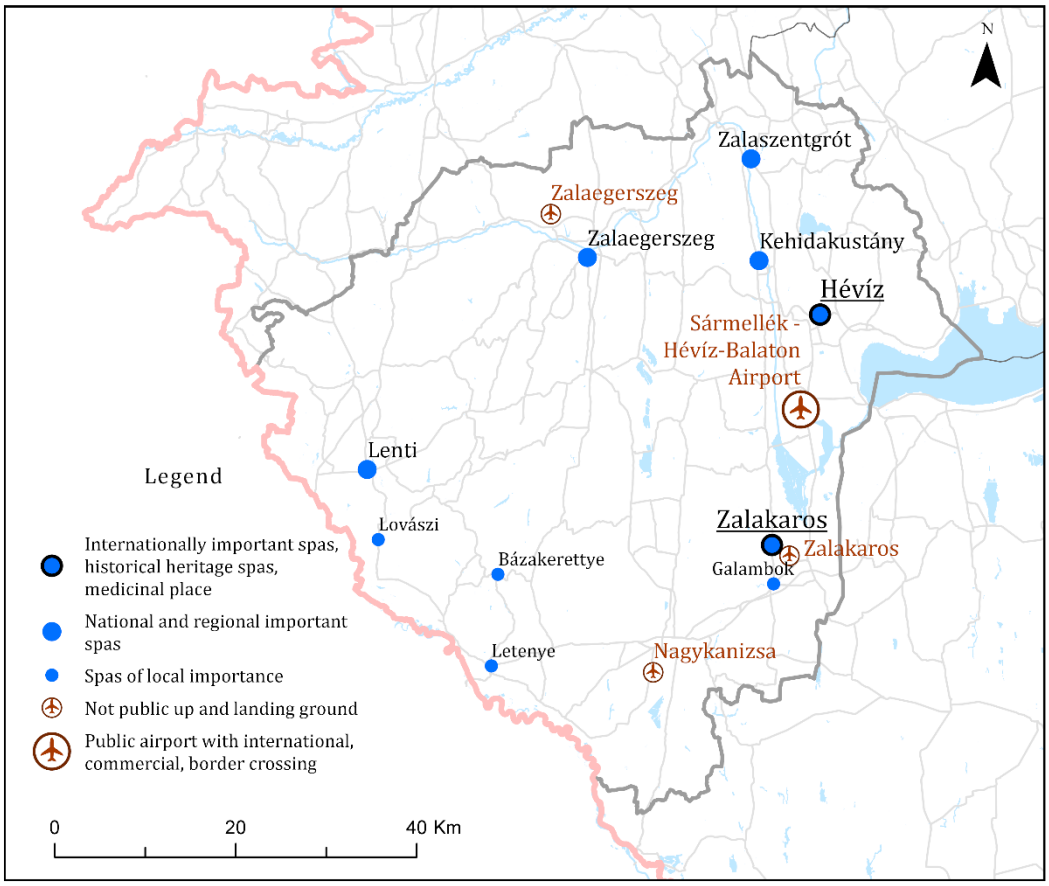

Figure 1: Spas and airports located in Zala County

Source: Edited by Morva and Köbli

Spas with national attraction too have a growing number of guests, the spa developments of the so-called Széchenyi Plan allowed investments of considerable scale, attracting international clientele to the spas (Mundruczó and Szennyessy, 2005). Kehidakustány and Lenti too have become important actors on the market of the county, attracting guests, especially those in search of wellness services, from all over Hungary due to the effective marketing activity. Foreign tourists visiting these spas are usually Austrian, German and Croatian tourists travelling in their own organisation, still, the majority of the guests of the spas are Hungarians. The airport of Sármellék has considerable impact on the lives of the international spas, mainly, and by the multiplier effects of tourism it can have a positive influence on the tourism sector of

Zala County as a whole. There are two spas of international recognition in Zala: Hévíz and Zalakaros.

The foundation of tourism in Hévíz is the world famous and unique Hévíz Lake and the medical tourism services built thereon. The surface area of the lake is 44,400 square metres, making it the largest natural, biologically active hot water lake of the world. The medical water erupts from the crater well at a depth of 38 metres, its average temperature in summer is 33-35 °C, and does not sink below 23 °C even in wintertime, so it is suitable for open air bathing all year long (UDC of Hévíz, 2015). The sulphuric water contains alkaline hydrogen-carbonate and is slightly radioactive, especially suitable for healing different rheumatic, locomotory, muscular and nervous system diseases and gynaecological disorders. Hévíz has long been the spa town of Hungary with the highest quality and the most diverse range of services, an outstanding venue of international tourism in the rural Hungary. The dominant part of the clientele is from the traditional German and Austrian market, supplemented in recent years by Russian and Ukrainian tourists showing a great interest in health tourism in the recent decades. Also, guest numbers from the Czech Republic, Israel and Switzerland are considerable.

Zalakaros is one of the least populated towns in Hungary for the time being, where, however, both tourists and local inhabitants have access to very high quality of life, due to the achievements of health tourism. Accommodation and attraction developments have been continuously implemented in the small spa town of Zala, making it the fourth most visited classical spa destination of Hungary now. Although the significance of international tourism is increasing year by year, the basic clientele is still the Hungarian guests.

These two settlements evidently have a vested interest in the as successful operation of the airport as possible, and the most possible effective access to foreign markets. Figure 2 shows

the numbers of guest nights in the two Zala County spa towns of international significance, taking both domestic and international guests into consideration.

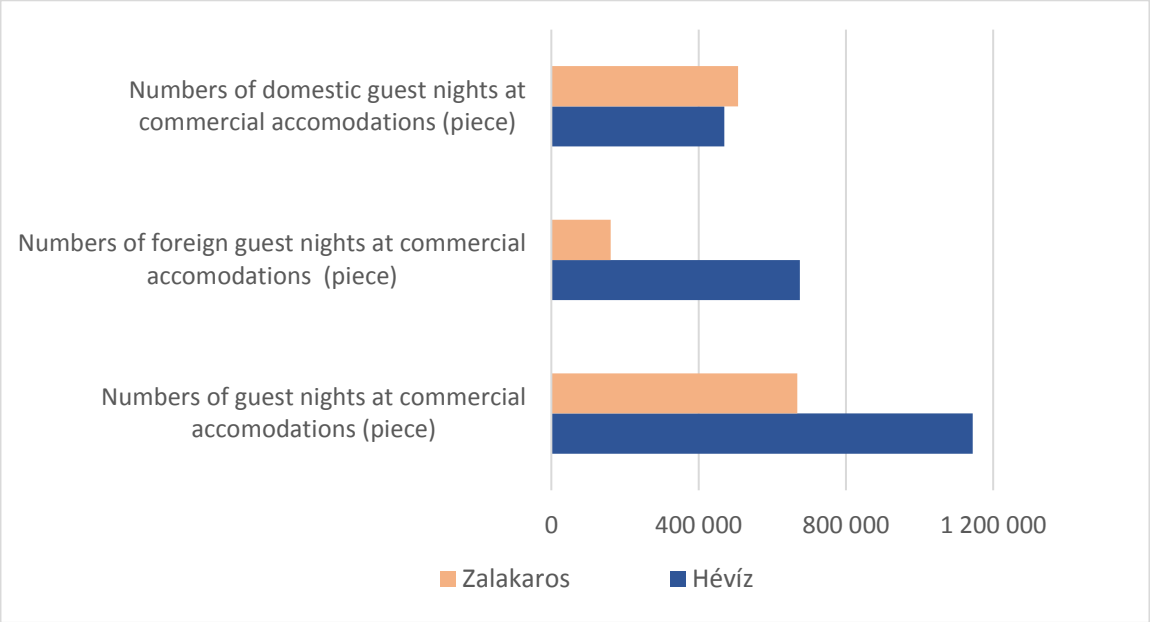

Figure 2: Numbers of guest nights spent in Zalakaros and Hévíz, at commercial accommodations 2019

Source: Database of HCSO, 2020.

The close connection between tourism and transportation is evident: in order to reach a tourism destination and visit an attraction, one must travel to the location (Jászberényi and Pálfalvi, 2009). There is not tourism without transportation; on the other hand, several tools and means of transportation would also be devoid of purpose without tourism. In the acceleration of transportation and the birth of globalisation, from the aspect of tourism it was the mass use of aircrafts that made the real breakthrough. As a result of this, European and overseas distances shrank; the majority of destinations are now much faster and more comfortable to reach (Pintér, 2013). Further touristic effects of Hungarian airports have been pointed out in the previous papers of other authors (Jászberényi and Ásványi, 2013, 2016).

Hungary has a total of 5 airports that have managed international air passenger traffic, at least temporarily, in recent years (Tiner, 2011). By the data of the websites of airports in

Hungary, the order in the volume of air passenger traffic in 2018 was as follows: Budapest (14.8 million persons), Debrecen (376 thousand persons) Győr-Pér (20 thousand persons), Sármellék (11.5 thousand persons), Pécs-Pogány (5 thousand persons).

Hévíz-Balaton International Airport

From the aspect of tourism the most important airport of the region is evidently Hévíz-Balaton (before 2012: FlyBalaton) Airport between Sármellék and Zalavár municipalities. The originally military airport built in 1953 has operated in the service of civil aviation since 1991. The runway, made of good quality basalt concrete, has a length of 2,500 metres and a width of 60 metres. The airport is perfectly suited for the reception of aircrafts with 150-200 passenger capacity.

The operation of the airport showed only a slow progress during 1991-2005. The reason for this was the lack of runway lights and modern air traffic control equipment, due to which aviation was only possible in daylight, in good visibility conditions (Pintér, 2009; Pintér and Makai, 2013). Also, there were no foreign investors who could make adequate profit from the operation of the airport. It is well known that the profit from the operation of regional airports can only be realised after several years (Zóka, 2006). The ownerships of the airport were settled by the turn of the millennium, and the real development started in 2004 when the management of the airport was done by an Irish-Hungarian company, Cape Clear Aviation. Low-cost airlines showed up, a new, up-to-date passenger terminal, restaurant and parking place were built, and so the interest in the airport continuously grew. The operator of the airport at that time, in order to cover the high costs – especially the expense of the operation of the air control and passenger safety system – made a marketing contract in 2007 and 2008 with the major commercial accommodations in the municipalities of the region receiving larger numbers of guests; these

were larger hotels in Hévíz, Keszthely, Zalakaros, Kehida and Tapolca (Lukács, 2009). The airport had the largest number of passengers in 2008, serving almost 110,000 thousands passengers that year. The most popular flight was the one to London (Stansted), followed by the German destination Frankfurt am Main (Hahn). Besides these cities, important airports with considerable passenger numbers to FlyBalaton were those of Düsseldorf, Berlin, Hamburg, Cologne, Zurich and Amsterdam (Pintér, 2009). However, the majority of low-cost airlines present at the airport – of which Ryanair carried the largest number of passengers – left by the end of 2008 and the airport had to be closed in the winter (Pintér, 2014).

The airport has been operated since March 2012 by Hévíz-Balaton Airport Ltd., 100% owned by the municipality of Hévíz. No significant increase in the number of air passengers has occurred since 2009 to date, according to statistics, the number of passengers is between 15,000 and 25,000 annually. In 2019 there was even a decline (6,620 persons), due to the five-week closedown in summer. A major increase in the number of air passengers cannot be expected in 2020, either, for the same reason, and the situation is further exacerbated by the global recession of tourism owing to the corona virus pandemics. The plans calculate with an improvement after 2021 (Hévíz-Balaton airport, verbal information).

The majority of passengers flying to Hévíz-Balaton airport are guests from Germany. The airport has connections to 7 German cities now (Frankfurt am Main, Berlin, Düsseldorf, Dresden, Hamburg, Leipzig, Erfurt) in the period from spring to autumn, with a weekly frequency (www.hevizairport.hu). The air passengers arriving here, dominantly from the senior generation, are guests interested in medical tourism for 2-3 weeks in Hévíz and the surrounding settlements.

Two German travel agencies organise the air transportation of spa guests to the region: the company Mutsch Ungarn Reisen transports passengers from Berlin, Düsseldorf, Frankfurt

and Hamburg, another one called Salamon Reisen from Dresden, Erfurt and Leipzig to the Hévíz-Balaton international airport at Sármellék, from spring to autumn. As regards Salamon Reisen, the latter three German cities also have seasonal air connection to the International Airport of Debrecen (www.termalonline.hu). The number of air passengers at Hévíz-Balaton airport in recent years can be seen in Figure 3.

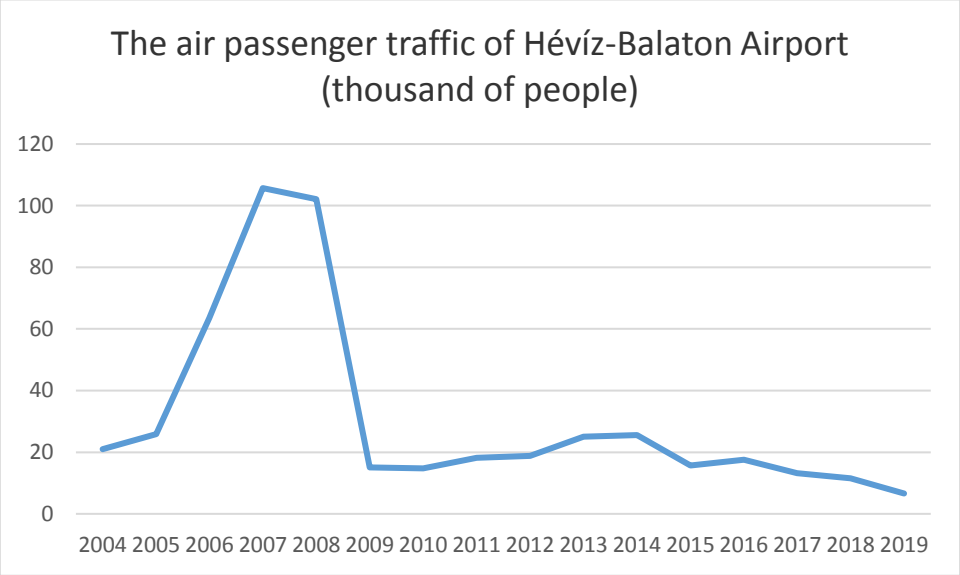

Figure 3: Number of air passengers at Hévíz-Balaton Airport

Source: Edited by Pintér, Á., using the statistical database of the Hévíz-Balaton airport (2020).

Findings of the hotel survey

Our research was focused on the four-star medical hotels of Hévíz, as these are the accommodations most popular with the foreign tourists arriving at the region. We assessed the characteristic features of the clientele in three selected locations – Ensana Thermal Hévíz, Ensana Thermal Aqua, Hotel Európa Fit – in connection with air transportation.

The findings reveal that the largest accommodations of Hévíz operate with 75-80% capacity utilisation on an annual average. Of course, there are some times with higher capacity utilisation (holiday periods, long weekends, summer months), but, due to the peculiarity of

health tourism, i.e. that medical water and the related services are available all year long, by and large to the same extent, seasonality is much less typical than at other touristic attractions. As for their capacity, the three hotels are quite similar to each other in this respect (210-234 rooms) – they are among the largest capacity accommodations in the whole of Zala County, anyway.

The breakdowns of domestic and foreign guests are also almost the same in all three hotels. On the average, 74 per cent of tourists are from abroad annually, leaving 26% to the domestic market. Of course, international guests participate in longer holidays and treatments and so their average duration of stay is significantly longer. A foreign guest spends 6 days on the average in a medical hotel of Hévíz – among them the longest stays, approximately 10 days, are typical of the Russian, Ukrainian and Israeli guests –, while the duration of stay of a Hungarian guest is exactly half of this, 3 days. Hungarians prefer shorter, wellness motivated travels, with a very large demand at the times of holidays and long weekends. A basic trend in today's tourism is the more frequent travels of guests, but for shorter periods of 2 to 4 days. Health tourism is perfect for such short sessions of a few days. Hungarian travellers are young couples and families with small children in higher proportions; they are more open to programmes and experiences. Almost one-third of international guests arrive at this macro-region – especially Budapest and Vienna – by air, Hévíz-Balaton airport for the time being only serves the travels of a part of the German health tourists to this area.

As regards foreign guests, totally different travel habits and target groups can be observed. In this group a typical guest is older and wants to be healed, they will usually use the services and treatments of the Hévízi Tófürdő (Hévíz Lake Spa). The breakdown of international guests at the three accommodations shows very considerable similarities and trends. The two main countries of origin are Germany and Russia. They are supplemented by guests from other countries, in smaller numbers: other German speaking countries – Austria

and Switzerland –, Israel, the Ukraine and Slovakia; and there are the one-day Chinese tourists. Foreign guests are typically seniors, mainly from the German speaking countries; they often arrive in groups, and stay for a week or two. What is determining for them is the attractive environment of the towns, the professionalism and kindness of the staff, and the price/value ratio of services. Germans were the most dominant clientele in the town for decades, and local service providers always paid special attention to meeting the demands of this solvent target group. Presently the generations aged over 60, often 70 arrive from Germany, for whom accessibility and the speed of that are of utmost importance. According to statistics, approximately one quarter of the German guests use Hévíz-Balaton airport as air passengers, from where they use shuttle service to get to Hévíz.

The huge increase in the number of Russian guests is a very interesting phenomenon. In the early 2010s both Hévíz and the tourism management of Hungary recognised the giant potential in the solvent Russian market. In Russia health tourism has significant traditions, and parallel the strengthening of the economy of Russia it seemed to be a logical conclusion that Hévíz, the highest quality and most prestigious Hungarian spa town, will be an attractive environment for them. In Hungary it is still only Budapest and Hévíz where we find Russian guests in very high numbers. The boom of the Russian clientele – most of whom arrive at Budapest by air and then use a shuttle service to travel to the area – was tremendous, as a consequence of which they totally took over the position of the main country of origin by 2015. The Russian-Ukrainian conflict and the crisis of the Russian Rouble caused a sudden drop in their number. The market has calmed down and stabilised by now (right until the COVID pandemics that burst out in early 2020 and has changed everything fundamentally), and now the number of Russian guests is balanced year after year, approaching the number of German guests. The dominance of German or Russian guests varies hotel by hotel, but the two clienteles

can get along with each other well now, after the initial hardships. As opposed to the Germans, from Russia it is typically younger guests, aged 30-50 often with their families that arrive, supplemented with the classic senior health tourists.

Conclusion

The spas of Zala County, offering services at different levels, are very popular with the international guests. When selecting the destination, accessibility is an aspect of selected importance. The spas of Zala are accessible not only by road but also by air, owing to Hévíz-Balaton International Airport at Sármellék. The busiest period of the airport, the largest volumes of guests were typical in the years from 2006 to 2008. The turnover of guests has stabilised at a low level – 15 thousand to 25 thousand air passengers annually – since then, due to the drastic decline, for several reasons. We can see that the dominant part of the Russian guests, making a significant proportion of clientele of the region in recent years, and also the guests from Israel arrive at Hungary by air, although not directly at Sármellék (they prefer Budapest and Vienna, respectively). A quarter of the German guests, however, arrive here, owing to two major travel agencies. In the transportation of air passengers from more distant destinations, the larger airports, especially in the neighbouring capital cities, play a dominant role. The paper highlights the fact that for senior medical tourists comfort and the time factor are of utmost significance. A financially stable airport with state-of-the-art infrastructure can be a huge competitive advantage for the spas of Zala County, and its operation may have a favourable impact on the tourism of the whole of the county. The economy of the region may be in a disadvantageous position in comparison to its competitors, if the underutilisation of the airport capacities continues.

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LEGISLATIVE ASPECTS OF AGRICULTURAL LAND USE AND SOIL CONTAMINATION

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Abstract

This paper analyses the institutional soil protection system regards the links of land use and soil contamination. The area chosen as an example is the disposal and utilization of sewage sludge, which is a growing task for today and for the future. Protection of soil requires the evaluation and rethinking of the effectiveness and efficiency of soil conservation policies in agriculture. There is a little understanding of how policy measures should be designed to encourage farmers to adopt soil conservation practices. We would like to contribute to these objectives with our research by integrating the relevant EU and Hungarian regulations into a transparent system. To improve and clarify the agro-environmental regulation this dissertation tries to formulate general aspects for the development of soil protection regulations based on the research.

Keywords: soil contamination, soil protection, land use policy, soil conservation programmes, agricultural law

Összefoglalás

A kutatás célja a talajvédelem intézményrendszerének, szabályozásának az elemzése különös tekintettel az agrár-földhasználat eredményeképp bekövetkező talajszennyezésre. Az összefüggés bemutatására a szervestrágya és szennyvíziszap szántóföldi elhelyezésének talajvédelmi aspektusainak probléma körét választottuk. A talaj védelme megköveteli a környezetvédelmi szabályozás időről időre történő értékelését és újra gondolását, ez indokolja, hogy a talajok minőségét veszélyeztető hatásokkal foglalkozzunk, a káros hatások lehetőség szerinti megelőzése és a talajok jó minőségének megőrzése érdekében. A kutatás eredményeként kísérletet teszünk átfogó szempontok megfogalmazására a talajvédelmi szabályozás fejlesztése számára és egy táblázatban foglaljuk össze a földhasználó kötelezettségeit EU-s és hazai szinten.

Kulcsszavak: talajszennyezés, talajvédelem, földhasználat, földvédelmi eljárás, agrárjog

Introduction

This publication discusses the agronomic and environmental regulations for the disposal of wastewater, sludge, compost and manure on arable land and develops a proposal for the further evaluation of soil protection regulations. In order to improve the Hungarian regulations, it is expedient to review the extent to which the current regulations cover the requirements of the general prevention strategy, and where the regulations need to be clarified or supplemented.

There is a lack of knowledge about the effectiveness and efficiency of soil conservation policies in agriculture and there is a little understanding of how policy measures should be designed to encourage farmers to adopt soil conservation practices. Despite the general legal obligations of nature protection - analysed inter alia by Bánhegyi (2015) Horváth (2013), Tóth (2017) and Olajos and Gyurán (2012) - , the soil is constantly degrading (Németh et al., 2016), 75 billion tons of arable land disappear annually as a result of soil degradation processes (Kertész, 2014), the extent of which depends significantly on land use patterns (Tóth et al., 2016). Improper land use and agrotechnics, monoculture farming leads to soil compaction caused by heavy machinery used in large-scale agriculture, and changes in soil structure associated with excessive use of pesticides and fertilizers lead to erosion, soil destruction or soil pollution (Ángyán et al., 1988). Neither the EU (Peine, 2007) nor Hungary has a land codex which address land use and soil protection together. Nevertheless, in the classical sense, we cannot speak about the legal regulation specifically related to soil. The Hungarian agricultural law literature and related legislation treat this issue as the environmental aspect of land protection (Fodor, 2007; 2008). There is a lack of synergy due to the different soil concepts at the intersection of environmental law and the regulation of the arable land in Hungary. It results that the current legal definition of land and soil is incomplete and not in line with the agro-ecological concept of soil (Kurucz, 2017). There are two sides of the Hungarian regulation: the quantitative land protection and qualitative soil protection aspect. From the environmental protection point of view, the former seeks to curb the waste (utilization) of natural resources, the latter controls the pollution and deterioration (load) of the environment (Kurucz, 2017). The state performs its soil protection tasks primarily through the maintenance and operation of the system of official institutions but the land user and the landowner are directly responsible for the soil protection measures. All land uses have an impact on the environment and within it to

the soil, these complex effects differ depending on their types and in their intensity (Vermes, 2007). Thus, it is important to explore and define the environmental impacts on the soil of different land uses as accurately as possible, because only with this knowledge can we decide the protection measures that are needed against adverse harmful effects. Worldwide the regulation of contaminated sites especially concerning the question 'who is obliged to clean up the contamination', has become the difficult focus of the legal system governing soil contamination (Wang, 2018). In this study, the EU and domestic soil protection obligations of land users related to soil contamination phenomena are comprehensively presented. The aim of the research is to point out the stress points of soil protection regulation that can contribute to soil contamination processes.

Materials and Methods

This paper surveys the normative obligations of land user in case of soil contamination processes. Literature and legal document analyses were complemented on EU and Hungarian level in order to generate qualitative data. We cannot deal with all land use methods here, so we explore features that can be used to develop regulations with the examples related to the agricultural utilization of sewage, sludge, compost, and manure. To illustrate the results of the policy survey analyses we designed a simple schematic table (Table 1). EU policies (EUR-LEX database) use a range of different types of instruments, the analysed policy documents can be divided into two major groups: 1. for the protection of soils which are not considered to be contaminated, and 2. regulations for remediation of already contaminated soils.

Results and Discussion

Impacts of land use on soil quality can be expressed in terms of their consequences by changing the physical, chemical and biological quality of the soil (Kádár, 2011). Depending on the extent of these modifications, the functionality of the soil changes, which decisively determines the soil fertility thus its type of use in the future (El-Swaify, 1994). The soil can therefore be loaded up to the limit until this undesirable change occurs, and this is considered in the regulation as a load level (still an acceptable level of load) (Várallyay, 2016). In the case of soils, this so-called The "A" value is the reference value for good quality, "clean" soil. Soil contamination occurs when substances are introduced into the soil which, alter it in a way that is detrimental to life and human use. In the following sections, the main EU and Hungarian policies and legal sources are presented that mainly affect soils contamination issues by sewage sludge, compost and manure from the perspective of the land user.

Regards the EU legislation on soil contamination we can state that although in 2006 the European Council adopted the Commission's Thematic Strategy for Soil Protection, which sets out the threats to soil resources and the tasks to prevent soil pollution in Europe, subsequently, a proposal for a framework directive existed (COM (2006) 232), but no final strategy has been decided since then. At the same time, the EU, primarily in the context of its environmental and agricultural policy, is increasingly paying attention to soil protection aspects, regulating it directly and indirectly. It resulted in that the EU's environmental directives are diverse however not complete protection of the soil. The polluter pays principle is known from international and EU documents, according to which the polluter is responsible for restoring the original state. In the EU environmental action programs the first version of the National Environmental Program (NEP-I) under the banner of the fundamental questions of Planning-Regulation-Financing can

be found the “Polluter pays” principle. The “Polluter pays” principle can be applied in a limited way only if the regulation disciplines any breach of law, because the principle is not based on the actionable conduct. The precise legal application of the principle prevails in Hungary in the Act XLIII of 2000 on the waste management, whereas the polluter shall be responsible for the abatement of environmental pollution caused by the waste, for the restoration of the state of the environment and the reimbursement of damages including costs of restoration (Bobvos et al., 2006). Agricultural use of sewage sludge falls within the scope of a separate directives. The contamination of soil and groundwater and heavy metal concentration of sewage sludge can greatly reduce soil biodiversity and thus fertility. To understand the Community legal framework for the sewage sludge utilization management we have to go back to the mid1970s until the first Waste Framework Directive was established. As a result of significant changes over the decades, Directive 2008/98/EC has become the central element of current regulation, which defines the general rules for waste management which includes sewage sludge. The Directive contains basic provisions and limit values (heavy metals: Cd, Cr, Cu, Hg, Ni, Pb, Zn) for the use and treatment of sewage sludge, which farmers cannot ignore. The directive requires the Member States to take all necessary measures to ensure that the disposal and recovery of waste is carried out without endangering human health and the environment. In Article 13, the importance of preserving soil is emphasized in addition to water, air, plants and animals, the disposal and recovery operations referred to in Annexes I-II. Council Directive 86/278/EEC (Sewage Sludge Utilization Directive) regulate the agricultural use of sewage sludge so that it does not have a detrimental effect on land, flora, fauna and humans. The Sewage Sludge Utilization Directive primarily defines the protection of soils against heavy metal pollution in agricultural soils and the permissible concentration in the sludge to be recovered. The detailed test requirements for the characterization of soil and sludge are defined by the Member States.

Member States should determine the maximum amount of sludge that can be applied to soil per unit area and per year. Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources (Official Journal, 1991) lays down the criteria for the designation of waters affected by nitrate pollution and a framework for Good Agricultural Practice measures to be taken to protect of vulnerable zones. The so-called Nitrates Directive is aimed at protecting human health and aquatic ecosystems against pollution caused by nitrates mainly from agricultural sources. This is required to achieve mainly by limiting the input of inorganic N fertilizers and manure on farmland. It requires that manure and slurry should not be stored in a way that endangers or damages the environment, especially groundwater and surface water. Member States are expected to identify and designate so-called Nitrate Vulnerable Zones (Tóth, 2017) and to draw up action programmes to reduce nitrate pollution. Requirements of Good Agricultural Practice contains the amount of active substance nitrogen applied to nitrate-sensitive agricultural areas per year. It is forbidden to apply manure from 15 October to 15 February, except for the top-dressing of autumn cereals. During fertilization, nutrients must not enter the surface waters directly or indirectly through infiltration or erosion. When determining the amount of nutrients to be applied in the field, the nutrient supply of the soil and the nutrient requirements of the cultivated plant adapted to the conditions of the production area must be taken into account. If the groundwater level can be reached within 5 meters on the irrigated area the quality of the groundwater must be checked every 5 years.

In Hungary Act LIII of 1995 on the general rules for the protection of the environment establishes a harmonious relationship between man and the environment, to protect the elements and processes of the environment, and to ensure the environmental conditions for sustainable development. The quality and quantity of arable land is protected by the Ministry

of Agriculture, however currently there is no effective legislation on contaminated soils in the Hungarian legal system that includes soil quality criteria and procedural rules too. Soil Protections Authorities may carry out control measurements on the basis of the authorization of the Environmental Protection Act, and may intervene if necessary in case of suspicion of contamination of arable land. 15. § of Environmental Protection Act stipulates that only such activities may be carried out both on the ground and in the ground, and substances may be placed there that do not endanger, pollute or damage its quantity or quality. Section 41 of Act CXXIX of 2007 on the protection of arable land (Arable Land Protection Act) stands that the land user is obliged to protect the arable land from soil contaminants that degrade its quality. Section 42 states that in the course of land use, environmentally friendly nutrient management shall be carried out, taking into account the nutrient supply of the soil and the nutrient requirements of the cultivated plants. According to the Arable Land Protection Act the purpose of arable land is agricultural utilization, which, if done properly, results in the maintenance or renewal of soil quality. According to the concept of the Arable Land Protection Act soil is a conditionally renewable natural resource and its most important feature is its fertility. The first level of quantitative land protection is the land use obligation and the regulation of its forms (Kurucz, 2018). It means that lands registered in the real estate register as vineyard or orchard have to be cultivated in accordance with their special needs for cultivation, but regarding the other types of lands it is enough to prevent the growing of weeds besides complying with the rules of soil protection (Csirszki, 2018). The Arable Land Protection Act distinguishes between the humus layer, which is the top layer of soil, and makes it the responsibility of the land user to preserve it, but it does not differentiate between living (edaphon) and dead organic matter in the soil, thus it cannot not provide adequate protection for the soil as a whole and its individual components. According to the Arable Land Protection Act, a soil protection plan is required for

the decision-making during certain procedures of the soil protection authority. The substantive requirements of soil protection plans are regulated by the Decree no. 90/2008. (VII. 18.) FVM on detailed provisions of the making of soil protection plan. The purpose of the soil protection plan is to explore from soil samples the soil characteristics of the area designated for the utilization of wastewater, sludge, compost and manure during which it can be determined whether the designated area is suitable for the application. According to Annex 2 2. an "average sample" of 20 subsamples shall be taken from the cultivated layer along different diagonals of the designated plot per different soil patch, but at least on every 5 hectares. Soil samples should be collected from depths of 0–30, 30–60, 60–90 cm. The following soil tests should be made on samples in each case: pH, KA, humus%, total salt%, CaCO3%, and nitrate-N content. The degree of contamination is judged on the basis of the concentration of the substance, compared to the contamination lists and limit values. The Dutch list, the Berlin list and the limit of the Hungarian sectoral technical directive "Disposal of sewage and sewage sludge" (MI-081735/1990) is used. Government Decree 50/2001 on the rules of agricultural use and treatment of municipal wastewater and sewage sludge and compost lays down that when and which crops special fertilizer is permitted and the characteristics of the soil on the angle of inclination the use of sewage sludge compost is prohibited. This regulation is in line with European directives, although it differs in that Annex 5 provides only a single value for the permissible limit for each parameter in sludge, while the EU limit apply intervals, which allows a more appropriate consideration of local conditions. Regulation 27/2006 deals with the rules for the designation of nitrate vulnerable zones. The designation of nitrate sensitive areas should take into account the characteristics and environmental properties of the water and soil, the behaviour of nitrogen compounds in the environment, and the agricultural activities that may affect surface and groundwater. The appointments shall be reviewed every 4 years. Regulation

59/2008 lays down detailed rules for an action program for protection against nitrate pollution, mandatory requirements for good agricultural practice and the provision of data. Decision 59/2008 of the Ministry of Agriculture and Rural Development contains the detailed rules of the action program necessary for the protection of waters against nitrate pollution of agricultural origin. Decree 59/2008. of the Ministry of Agriculture and Rural Development regulates that the amount of nitrogen active substance applied to nitrate-sensitive agricultural land with organic manure per year may not exceed the prescribed value, and also determines the time of application. The amount of nitrogen that can be applied with sewage sludge, compost in nitrate-sensitive areas must not exceed 170 kg/ha, and in non-nitrate-sensitive areas 220 kg/ha. In the case of soil contamination, various sanctions can be applied, e.g. soil protection fine is to be paid in case of a serious violation of the soil protection obligation and requirements. Contamination limit for pollutants and test methods are given in Decree 6/2009. KvVM-EüMFVM. Unfortunately, no specific standards have been developed since the first Environmental Protection Act of 1976, so the method has been developed to impose a sewage fine even in the case of soil pollution if the pollution endangers groundwater. Fines may be re-imposed, no payment may be granted and 30 days from the date of entry into force shall be payable. The Ombudsman pointed out in his resolution (2016) that, rather than fining, companies that cause or take responsibility for pollution should be obliged to carry out remediation at the expense of the state, since in the absence of a liable actor the state must intervene.

Table 1. Legislation of soil contamination in the EU and Hungary

Mandatory policy measures		
EU legislation	**National legislation**	**Priority obligations**
1386/2013/ Environment Action Programme	Act LIII of 1995 on the general rules for the protection of the environment.	"Polluter Pays" principle
Nitrates Directive (91/676/EEC)	Decree 27/2006. of Agricultural Ministry on the protection of waters against nitrate pollution of agricultural origin	Average content values of fertilizers at formation and application
	Decree 59/2008. of the Ministry of Agriculture and Rural Development on the detailed rules of the action program necessary for the protection of waters against nitrate pollution of agricultural origin, as well as on the order of data provision and registration	Good Agricultural Practice The amount of nitrogen that can be applied with sewage sludge, compost in nitrate-sensitive areas must not exceed 170 kg/ha, and in non-nitrate-sensitive areas 220 kg/ha.
Sewage Sludge Directive (86/278/EEC)	Government Decree 50/2001 on the rules for the agricultural use and treatment of municipal sewage and sewage sludge, sewage sludge compost	Necessary tests, requirements for representative sampling and permissible concentrations of harmful substances and toxic elements in the waste water, sewage sludge and soil
	KvVM-EüM-FVM joint decree on 6/2009. the limit values necessary for the protection of the geological medium and groundwater against pollution and on the measurement of pollution	
	Decree of the Ministry of Agriculture 36/2006 on the authorization, storage, distribution and use of the yield enhancing substances	

Conclusion

In the absence of effective EU action, soil contamination continues and, more than ten years after the adoption of the Thematic Strategy on Soil Protection, no systematic European control and protection of soil quality has been achieved. There is currently no effective legislation on

contaminated soils in the Hungarian legal system that includes soil quality criteria. The exact concept of the clean and the contaminated soil is not defined. The "polluter pays" principle cannot be enforced until the establishment of the cost of actual damage and the restoration the original condition. In terms of effective legal protection, the fining procedure needs fixed points for judgment, but such fixed points do not exist, as toxicity, load capacity, uptake depend on soil features and the local, natural and farming conditions are also modifying factors. The high degree of uncertainty regarding the contamination limits creates the need of the involvement of independent environmental protection authorities in the law enforcement system. There is a need to design policies that target the existing soil contamination threats in the light of agricultural management.

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SUSTAINABILITY ASPECTS OF DISASTER MANAGEMENT

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Abstract

The professional disaster management organization is the law enforcement body responsible for the safety of Hungary. When examining the basic objectives of disaster management, it becomes abundantly clear that applying, establishing and maintaining tools, equipment and operational conditions that may have harmful impacts on the environment are essential in accomplishing these objectives.

In this paper, the author introduces the sustainability aspects considered by the National Directorate General for Disaster Management of the Ministry of the Interior (NDGDM) in order to ensure sustainable development. The author also examines how these aspects have been applied, what activities and measures have been taken.

Keywords: Sustainable development, disaster management, sustainability program, environmental and sustainability agent

Összefoglalás

A hivatásos katasztrófavédelmi szervezet Magyarország biztonságáért felelős rendvédelmi szerv. Alaprendeltetését figyelembe véve, szükségesek olyan eszközök, felszerelések, működési feltételek alkalmazása, kialakítása, fenntartása, melyeknek vannak környezetet terhelő hatásai.

A szerző jelen publikációjában kísérletet tesz annak bemutatására, hogy a fenntartható fejlődés érdekében a BM Országos Katasztrófavédelmi Főigazgatóság (BM OKF) milyen fenntarthatósági szempontokat vesz figyelembe, illetve azokat hogyan érvényesítette, milyen tevékenységeket, intézkedéseket hozott eddig.

Kulcsszavak: Fenntartható fejlődés, katasztrófavédelem, fenntarthatósági program, környezetvédelmi,- fenntarthatósági megbízott

Introduction

By the 21st century, efforts and measures to secure a safe environment have become an integral part of the basic demands in our society. These have grown to be an ever-increasing social demand from the population – despite the technological advances of the present day (Teknős, 2015). Security has become the second most important aspect after elementary physiological needs as displayed in the Maslow hierarchy of needs. Security, on a social level, stands for a self-sustaining need that focuses on community-level survival (Teknős, 2017).

Hungary builds its security on three principal pillars: on its *domestic national means*, on the Euro-Atlantic integration and on international co-operation (Szenes, 2014). When discussing

domestic national resources, in relation to securing the protection of the population and material goods, the notion of disaster management is clearly present. Especially in the light of the fact that Act CXXVIII of 2011 clearly states *"disaster management is a national matter. The unified management of the protection is a national duty"* (URL1). Therefore, protection activities as well as the eradication of the consequences of disasters must be provided for by the continuous and strict harmonization of organs created for this purpose, the local authorities and state organs; altogether: with the involvement as well as co-ordination of all those participating in disaster management. The *professional disaster management organization* has an important task in this system, since its basic purpose is the protection of life and property of the Hungarian population as well as the security of the safe operation of the Hungarian national economy and the elements of the critical infrastructure.

Present paper examines the measures taken for the sustainability of the professional disaster management organization from the perspective of the relationship between sustainable development and state participation. With respect to this, the author anticipates the fact that tasks undertaken to further the criteria of sustainable development increase the chances for a high quality performance of the requirements incorporated in the basic objectives.

Materials and methods

Data introducing the activities of the professional disaster management organization are published in the yearbooks of 2016, 2017 and 2018. Concerning missions, the statistical database of MI NDGDM, KAP-online, has also been used. In the various years, delivered tasks in relation to the aspects of sustainable development as well as the related data were provided

by the annual sustainability report of IM National Directorate General for Disaster Management.

Results and Discussion

The National Framework Strategy on Sustainable Development defines the notion of sustainable development as follows: „*Sustainable development is aimed at enhancing a happy and senseful human life and at expanding public well-being while containing human actions within the limits of Earth's carrying capacity, maintaining and developing the quality and quantity of expandable human, social and economic resources...*" (URL2). Based on the latter quote, we can state, that sustainable development is the mutual interrelation of all natural resources (human, social, environmental and economical) in a way that their qualitative and quantitative state, their character, status, accessibility-availability, employability do not negatively influence the natural and built environment of a given region, they do not exert harmful (irreversible) impacts, natural processes. Angéla Anda defines sustainability as follows: *"...if the economy serves the demands of the society in a way that it does not deteriorate the future generations' conditions for life. Included in this is the notion that we do not deplete natural resources and we do not, in any way, impede the natural regeneration of our environment*" (Anda et al., 2011). According to Sándor Kerekes "*sustainable development does not require the limitation of our demands, it merely motivates us to attempt to satisfy them by the consumption of less materials and energy and to minimize the polluting effects of production"* (Kerekes, 2017). Based on the above notions, we can establish that the purpose of sustainable development is the creation of a self-regulating system that enables the operation

of the environment by eliminating the "over-use" of the environment and it helps the maintenance of human quality of life (URL3).

According to Indrajit Pal et al., the Sendai Framework for Disaster Risk Reduction (SFDRR), the Paris Agreement, and the Sustainable Development Goals (SDGs) are crucial in promoting the effectiveness of (disaster) risk reduction mechanisms in governments, such as sustainable, equitable (impartial) economy, society, and environmental development (Pal et. al., 2020). Based on disaster research, data collection and the results of the statistical analysis, it is possible to make recommendations for the government and political decision-making (Papp 2019). Indrajit Pal et al. argue that Sendai Framework for Disaster Risk Reduction (SFDRR) contributes to sustainable development, as well as the sustainable development goals support disaster risk reduction. Sustainable development may indeed be a solution to the global challenges of the modern age, but, since we are talking about interconnected systems, personal participation, as well as the state's commitment on a national level determine global attitudes and the qualitative outcomes and results of the series of actions to be taken. Global problems require responsible global answers; where the principle issue is what a nation will do for their own, and for humankind's sustainable development, and how the central power co-ordinates, directs and manages these efforts.

The professional disaster management organization is the law enforcement body responsible for the safety of Hungary. To perform its basic objectives, it requires instruments (e.g. vehicles for missions, for performing administrative duties, etc.) that release harmful emissions. The disaster management's staff size of 12 thousand envisages the fact that there has to be harmful material emission, energy, water, fuel consumption, etc. associated with their operation. However, it can also be concluded, that the organization seeks an environmentally friendly operation in a way that it maintains the efficiency for the performance of its duties stemming

from its basic objectives. This limits the scope for "environmentally friendlier" operation, however it can be concluded that there are outstanding results from the point of view of the criteria of sustainable development.

Measures taken by the professional disaster management organization supporting sustainable development between 2012 and 2019:[1]

- The separate collection and monthly delivery of used printing toner cartridges for reuse.
- During the execution of the refurbishment project, energy efficient solutions were taken into account (e.g. fluorescent tubes, motion sensor operated lighting, etc.). Enhancing effectiveness in relation to energy and water consumption. Refurbishing of water facilities (e.g. showers and toilets), installation of motion sensors.
- The renovation of the garage building of 41 Mogyoródi str. belonging to MI NDGDM: façade thermal insulation, replacing copolyte glass to walling, roof renovation.
- The exchange of area lightning apparatuses belonging to MI NDGDM into LED devices in order to reduce specific energy consumption.
- The installation of modern inverter air conditioning devices.
- Introduced the selective collection of batteries.
- Preparation and closure of agreements relating to the recycling and shredding of office paper waste generated in the office premises of MI NDGDM.
- Set up regional health centers (Szeged, Csorna, Debrecen) in order to reduce commuting.
- Establishing bicycle storages to support employees in commuting via bicycle.
- The reconstruction of the sustainability management system. Appointment of environmental/sustainability officer. The elaboration of the sustainability policy of MI NDGDM, the preparation of internal regulator.

[1] Without being exhaustive.

- Assigned energy policy officers for all departments, who draws the attention of colleagues to energy efficiency issues in relation to energy and water consumption.
- In co-operation with BM HEROS Zrt., the development of a new type of fire engine with a significantly lower emission rate.
- The decommissioning, and taking out of the system as well as recycling of outdated (non-conform with current environmental regulations) personal vehicles from the pool of the disaster management.
- On 20 December 2018, in the framework of a tender, MI NDGDM handed over 54 electric personal vehicles for use by the disaster management organizational units undertaking authority and official inspection duties. Undertook the installment of 64 fast charge stations at 32 disaster management locations nationwide; 8 of these are to be established on MI NDGDM premises by 30 June 2019.
- New command center construction and refurbishment projects. The improvement of the accommodation of the professional firefighting staff, in order to secure a higher level of performance for their basic objectives.

Figure 1. Examples of more modern disaster management for a more environmentally friendly organizational operation

It is clear that many aspects have been elaborated, such as the increase of the energy efficiency of the buildings (the subsequent heat insulation for the external structures of the buildings, the exchange of external doors and windows); the improvement of the performance of main system elements and heat production devices; the application of a more modern vehicle pool; eco-conscious education; waste management; preventive health care-recreation; fire station building programs; options for the application of renewable energy (geothermal, solar) etc.; which, altogether, contribute towards a more sustainable law enforcement following the principles of environmental protection.

Conclusion

In line with the basic principles of the European Union and the National Framework Strategy on Sustainable Development, the official organizational units of the Ministry of Interior as well as organizational units supporting the official activities of the Ministry, the autonomous organizations under the direction of the Minister of Interior (such as the professional disaster management organization) place a great emphasis on the elaboration and maintenance of a more economic and more eco-friendly operation, and also contribute to improving the conditions in the society. They consider and enforce the criteria of sustainability in their operations, within their capabilities.

Accordingly, the following criteria are to be met: (URL4)

- Planning and provisions serving sustainable development
- Preference to products that are more beneficial from an environmental point of view
- The reduction of instrument, energy and water use

- Waste management: a strive towards the reduction of the entire amount of waste generated, and to increase the ratio of waste handed over for reuse.
- Procurements: eco-friendly, lower energy-consumption, re-cycled products, instruments, solutions should come to the foreground and receive priorities.
- Instead of vehicle use, the consideration of other means of transportation
- Increasing the general awareness and pool of knowledge on sustainability for the staff
- Health prevention
- The development of public administration: the active participation of the colleagues in the formulation of an environmentally responsible public administration that conforms with the principles of sustainable development

As the managing body of the MI NDGDM is the Ministry of Interior, the above criteria must appear among their objectives. The professional disaster management organization intends to support the principles of sustainable development, as well as its sustainability:

- The monitoring and observance of legislation in relation to environmental protection.
- The continuous analysis of the performance data of MI NDGDM from an environmental aspect.
- The preservation, improvement of the status of the environment. The application of ecologically friendly procedures and methodologies in the activities of the organization. The economical use of natural resources, conscious resource management. Striving towards effectiveness in renovations concerning energy and water consumption.
- Minimizing environmental pollution – modernizing the vehicular pool, improving its environmental features.
- Recycling of generated waste in an increasing ratio, the establishment of the conditions for selective waste collection while reducing the total amount of generated waste.

- In procurement, the requirements for fulfilling real demands and for a prolonged use must be satisfied, while domestic businesses with environment management systems in place to be preferred as vendors.
- The improvement of the accommodation of the staff – station modernization, renovation programs.
- Supporting the recreation of colleagues. Improvement of health prevention conditions at the workplaces (dental treatment, gym, sauna, weekly working hour premiums for sporting activities). The environmental-centric improvement of the conditions for getting to work (enhancement of bicycle storage capacity).
- The continuous improvement of the eco-friendly behaviors and attitudes of the staff: Familiarizing with and applying the possibilities of diligent treatment of natural resources (energy and water efficiency, reduction of waste, etc.) It is important that the environmental aspects are present and considered in the activities of people working in the various professional areas and fields in an integrated way.
- The dissemination of organizational information related to sustainability via the intranet. MI NDGDM makes electronic knowledge sharing possible for the staff. Its purpose is to introduce good examples practiced by colleagues in relation to the subject of sustainability, to share sustainability information, etc.
- In relation to event organization, MI NDGDM considers sustainability aspects. As much as possible, it organizes video and teleconferences with partners and prefers the application of online communication channels.

The basic objective for the organization is the protection of the population and material assets. In the framework of this, during the performance of the daily tasks, there are incurring effects and loads concerning the environment (documents, water use of missions, the operation

of buildings, use of vehicle pool). However, it can be clearly concluded, that, by means of director general-level measures, regulations, action plans, organizational units, a possibility has arisen for the securing of a more sustainable operation. Based on the activities introduced annually, it can be established, that the leaders of MI NDGDM ensure the compliance with those incorporated in the Sustainable Development Regulations, and ensure the endorsement and performance of the objectives set therein. The system of direct and indirect activities supports the preservation and improvement of the environment, a more conscious resource management, the minimizing of the environmental pollution within the means, the more efficient use of natural resources, the increase of environmental awareness. These altogether can favorably influence the perception and image of the organization, contributing toward the endorsement of the aspects of sustainable development.

In summary, it can be concluded that MI NDGDM follows the environmental, sustainability principles of the Ministry of Interior. It ensures the enforcement of the sustainability aspects by means of an organizational unit enforced by experts (Environmental and Sustainability Work Group).

The performance of its goals and objectives takes place over a range of fields, such as the fields of fire protection, civil protection, industrial security, integrated authority, as well as technical-logistical-economical fields. In 2018, the tasks performed in relation to the various fields can be summarized as follows. The number of response events in year 2018 was 44,583 (15,330 fire and 29,253 technical rescue incidents), which is a 19% reduction compared to 2017 but a 15% increase in comparison with 2016. The member data of voluntary rescue organizations shows a very significant increase as the figure was 1,400 in 2012, while by 2018 this number had reached 20,200. The application of all rescue organizations (as supplementary forces to the professional forces) manifested in a number of various forms of supporting

activities in 2018, the most outstanding ones are search for missing persons (87 occasions), technical rescue (58 occasions), flood control and flood rescue (47 occasions). On the field of fire prevention, 59,991 official inspections were conducted by the fire authority, and 55,040 official procedures were conducted (this number was 72,512 in 2017). In the field of water and water protection authority, altogether 27,475 official procedures were conducted.

When examining the tasks in relation to the objectives, it is clear that there is a close relationship in a number of professional fields, authority competences, with high incident numbers, case numbers, authority tasks and inspections (carrying out daily duties) etc. The performance of these tasks has an ecological impact. Nevertheless, the professional fields determining the system of objectives for the professional disaster management organization are suitable for the preservation of the values of sustainable development, for the adherence and maintenance of the related criteria.

Tasks undertaken to perform the sustainable development objectives clearly increase the high-quality performance of tasks enclosed within the basic objectives, as the procured modern vehicles, instruments, equipment, more modern command centers, management principles, more developed mission methodologies etc. altogether improve the professional character, capabilities of the professional disaster management.

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RESPONSE OF *TRITICUM AESTIVUM L.* TO EXOGENOUS APPLICATION OF PLANT GROWTH REGULATORS

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Abstract

This research was conducted to evaluate the effect of zinc, proline, and biostimulant foliar application on yield of wheat. The trial was established in the research farm of the Department of Agronomy, Bahauddin Zakariya University, Multan, Pakistan. A randomized complete block design was used with three replicates. All three treatments were sprayed on *Triticum aestivum*

cv. Galaxy at the booting stage and results of yield were compared with control. One way analysis of variance revealed that all treatments affected wheat yield significantly. Zinc foliar application (4 m*M* solution) produced tallest plants (104 cm), longest spikes (13 cm), maximum biological yield (2.76 kg m^{-2}), and heaviest 1000 grains (45.2 g). While the maximum number of grains per plant (72) and maximum grain (602 g m^{-2}) yield was observed for biostimulant sprayed at a rate of 1.2 l ha^{-1} on wheat. Pearson correlation also revealed a positive correlation among yield components except for plant height and grain yield that were negatively correlated. The results revealed that foliar application of zinc, proline, and biostimulant can be used to improve yield components of wheat particularly zinc application that produced better biological and economical yield.

Keywords: Wheat; yield enhancements; foliar application; biostimulant; proline; zinc

Összefoglalás

A munkánkban a cink, a prolin és egy biostimulátor levélen keresztüli alkalmazásának a tavaszi búza termésére gyakorolt hatását vizsgáltuk. A kísérletet a Bahauddin Zakariya Egyetem Növénytermesztési Tanszékén vizsgáltuk Multan városban, Pakisztánban. A kísérletet teljes véletlen blokk elrendezésben állítottuk be 3 ismétléssel. Mindhárom kezelést a Galaxy tavaszi búza (*Triticum aestivum L.*) fajtán végeztük el lombra permetezéssel kalászhányás kezdetén és a kontrolhoz hasonlítva vizsgáltuk a kezelések terméselemekre gyakorolt hatását. Az egytényezős varianciaanalízis eredménye mindhárom kezelés esetében szignifikáns hatást mutatott. A cink levélen keresztüli alkalmazása (4 m*M* oldat) eredményezte a legnagyobb növénymagasságot (104 cm), a legnagyobb kalászhosszúságot (13 cm), a maximális biomassza

termést (2.76 kg m^{-2}) és a legmagasabb ezerszem tömeget (45.2 g). Ezzel szemben a növényenkénti maximális szemszám (72) és a legmagasabb szemtermés (602 g m^{-2}) a biostimulátor 1.2 l/ha adagjának alkalmazása esetén volt megfigyelhető. Pearson korreláció a terméselemek között a növénymagasságot és a szemtermést kivéve negatív összefüggést mutatott. Az eredmények alátámasztják, hogy a cink a prolin és a biostimulátor alkalmazásával javíthatóak az őszi búza terméselemei, különösen a cink alkalmazásával, ami magasabb összbiomassza tömeget és szemtermést eredményezett.

Kulcsszavak: búza; termés növelés; lombtrágyázás; biostimulátor; prolin; cink

Introduction

Since 1960, the world population has jumped from 3.1 billion to 6.7 billion making it a challenge for agriculture to feed the population. Due to the green revolution, mechanical, and genetic advancements; cereal production grew faster than the population. Among cereals, wheat is one of the most important cereal being a staple food for a major portion of the population. The increase in wheat production recorded is 3.38% per year in the past but in recent years (after 1990) the wheat production increased by just 0.67% yearly. From 1960-1990 global wheat area increased by 0.43% per year but shrank down to 0.23% per year after 1990. Moreover, a 2.95% yield yearly increase was observed in the period 1960-1990 but later it also reduced to 0.90% per year. Therefore, wheat production has failed to match pace with the global population increase (Pardey, 2011).

Researchers are trying to achieve short term success in improving wheat yield. Several strategies including seed priming (Harris et al., 2002), nutrient foliar spray (Arif et al., 2006),

use of plant growth regulators (Griffin and Hollis, 2017), and biostimulant (Al Majathoub, 2004) have been reported to improve wheat yield. Therefore, this research was designed to determine the usefulness and impact of nutrient, plant growth regulator and commercial biostimulant spray on wheat to improve yield per unit area of wheat.

Material and methods

Experimental site and seed material

To evaluate the impact of exogenous foliar application on yield and yield components of wheat; a trial was established at the research farm of Bahauddin Zakariya University, Multan (30.2°N, 71.47°E) Pakistan. Seed material was obtained from the Regional Agriculture Research Institute, Bahawalpur, Pakistan.

Crop Husbandry

The field was irrigated before sowing and allowed the water to infiltrate. Seedbed was prepared by cultivating the field thrice followed by planking. Seeds of *Triticum aestivum L.* cv. *Galaxy* were sown on 15th November 2016 using man pulled drill. Rows were 25 cm apart from each other. Recommended fertilizer (150: 100: 75 Kg ha^{-1} NPK) was used. Diammonium phosphate (DAP) containing 46% P and 18% N was used as the main source of phosphorous. Remaining nitrogen was provided by urea that contains 46% N. While, muriate of potash (MOP) containing potassium chloride (KCL) was used as a source of potassium (K) which contains 60% K. Half of nitrogen and full potassium and phosphorous dose were applied as a basal application while remaining nitrogen was applied at first irrigation. The crop was irrigated at critical stages to

avoid moisture stress. Weeds were removed manually after every two weeks and crop was harvested on 10th April 2017.

Treatments and experiment layout

The effect of foliar application of proline, zinc, and a biostimulant on wheat yield and yield components was compared with control. Foliar application of 100 m*M* proline (Talat et al., 2013), 4 m*M* solution of zinc (Aslam et al., 2014), and 1.2 liters ha^{-1} commercial biostimulant3D (Innovative chemicals, Pakistan) was performed at booting stage of wheat. Each treatment was applied to an area of 20 m^2 (5m x 4m) and was replicated thrice. Randomized Complete Block Design was used.

Observations

At harvesting, plants were harvested manually from 2 random points in each plot using a 1 m^2 quadrate. Plant height was measured from ground level to the top of 10 plants from each plot using a measuring tape and was averaged. Spikes of 10 random plants were plucked and their length was also measured using a measuring tape. Grains from these 10 spikes were counted manually and averaged to obtain the number of grains per plant. Later, a thousand seeds from each plot were counted and weighed to get 1000 grain weight. Harvested plants from an area of 2 m^2 were weighed to calculate biological (total biomass) yield (kg m^{-2}) and economic (grain) yield from plants harvested from an area of 2 m^2 were measured to obtain grain yield (g m^{-2}).

Statistical analysis

The collected data was put in MS Excel 2007 to measure standard deviation and to make graphs. SPSS was used for the analysis of variance at 0.05 and 0.01 level of significance and to determine the differences among the means of treatments when compared at a 5% probability level using the LSD test. Pearson correlation among yield components of wheat was also analyzed using SPSS.

Results and discussion

Statistical analysis of treatments effect on yield components showed that the effect of exogenous foliar applications was highly significant (P<0.01) on all yield components except 1000 grain weight where the effect was only significant (P<0.05) (Table 1).

Table 1. Mean square values and significance of exogenous application on wheat yield components

SOV	df	Plant height (cm)	Spike length (cm)	No. Of grains per plant	Biological yield (g m^{-2})	Economic yield (g m^{-2})	1000 grain weight (g)
Replication	2	30.438	0.8750	22.240	82769	195.1	1.2399
Foliar Application	3	695.008**	13.4351**	332.101**	2030703**	61065.3**	39.3436*
Error	6	13.598	0.4088	11.511	104978	987.2	8.2316

** P< 0.01, * P< 0.05

Zinc foliar application significantly affected vegetative growth of wheat and produced tallest plants (104 cm) that were statistically at par with proline foliar application (101 cm) followed by biostimulant foliar application (78 cm) (Fig. 1). Similar results were observed for biological

yield that was significantly affected by zinc foliar application (2.76 kg m^{-2}) and proline foliar application (2.48 kg m^{-2}) followed by biostimulant foliar application (1.62 kg m^{-2}) (Fig. 2). Longest spikes were also observed for zinc foliar application (13 cm) that was statistically at par with proline foliar application (12 cm) followed by biostimulant foliar application (11 cm) (Fig. 3).

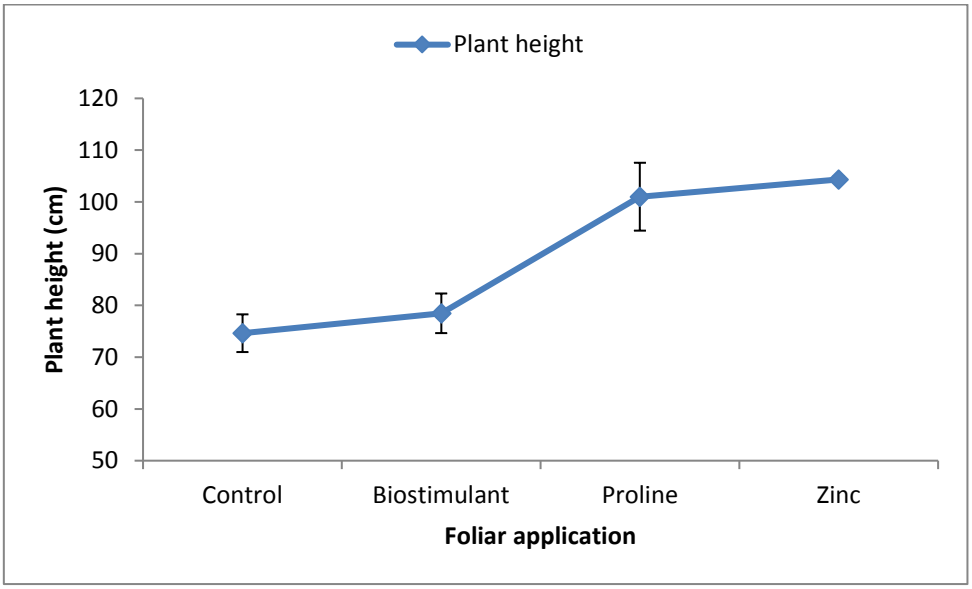

Figure 1. Effect of zinc, proline, and biostimulant foliar application on plant height of wheat

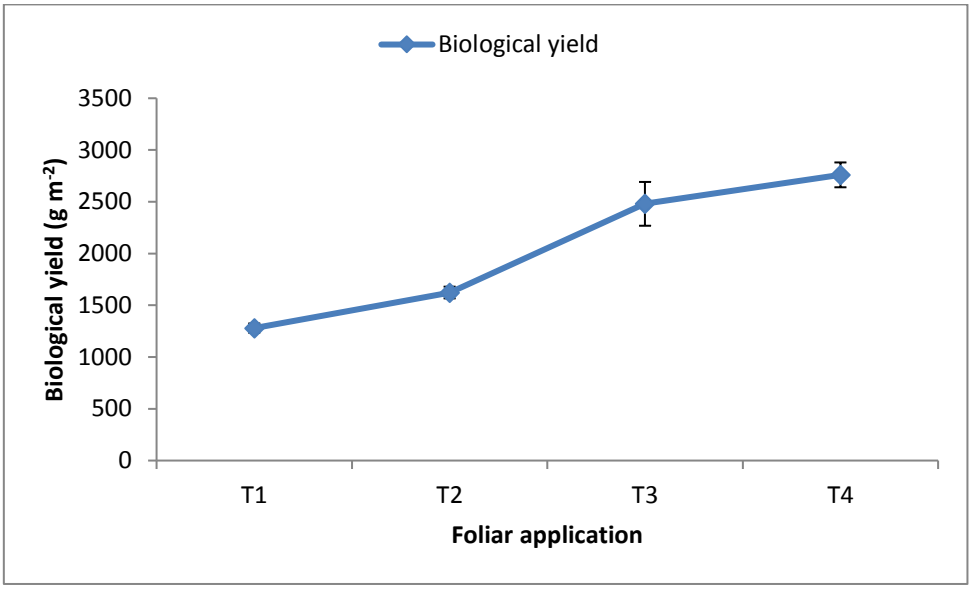

Figure 2. Effect of zinc, proline, and biostimulant foliar application on biological yield of wheat

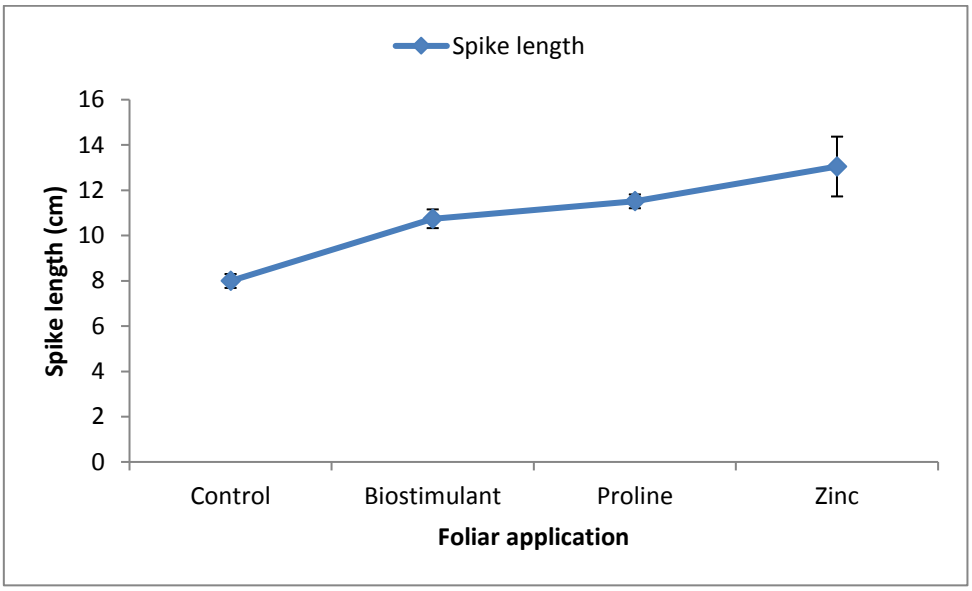

Figure 3. Effect of zinc, proline, and biostimulant foliar application on spike length of wheat

Maximum numbers of grains per plant (72) were observed for biostimulant sprayed plants followed by zinc foliar application (59) that was statistically at par with the other two treatments (Fig. 4). Maximum economic (grain) yield (602 g m^{-2}) was also observed for biostimulant foliar application followed by proline foliar application (424 g m^{-2}) (Fig. 5). Maximum 1000 grain

weight was measured for Zn foliar application (45.2 g) followed by biostimulant (42.7 g) that was statistically at par with proline foliar (39.4 g) (Fig. 6).

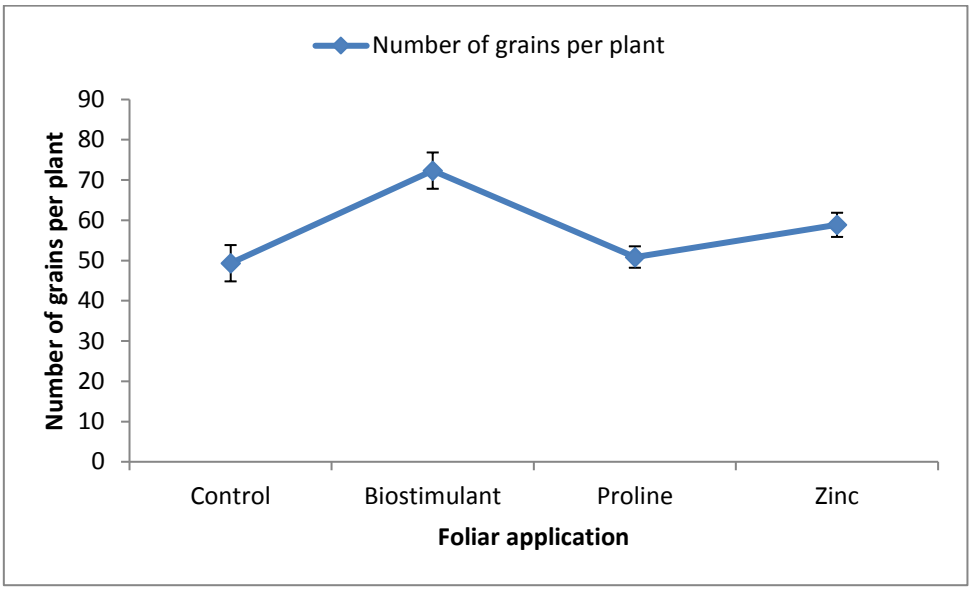

Figure 4. Effect of zinc, proline, and biostimulant foliar application on the number of grains per wheat plant

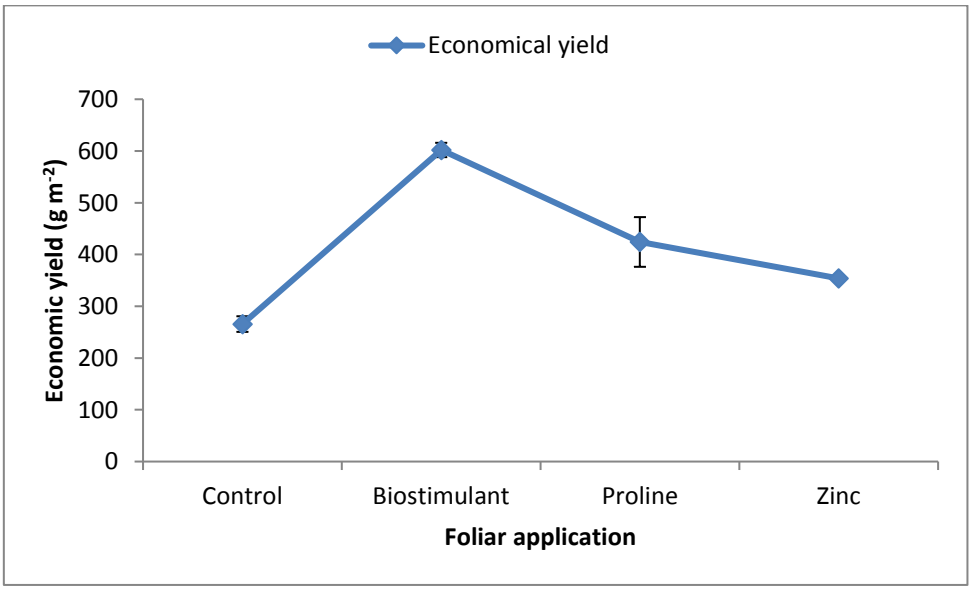

Figure 5. Effect of zinc, proline, and biostimulant foliar application on economic yield of wheat

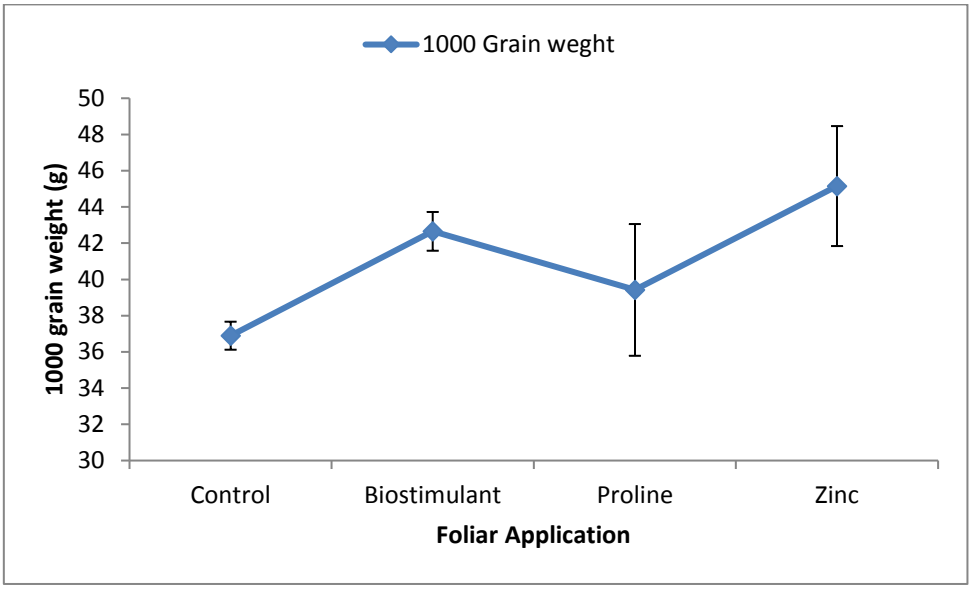

Figure 6. Effect of zinc, proline, and biostimulant foliar application on 1000 grain weight of wheat

The mean comparison of treatments revealed that all foliar applications significantly affected yield components of wheat. Zinc foliar application was found to be most effective as it significantly affected plant height, spike length, biological yield, and 1000 grain weight. Biostimulant foliar application significantly improved economic parts of wheat by producing the most number of grains per plant and maximum economic yield. Proline foliar application also affected plant height significantly and was statistically at par with zinc foliar application for spike length, biological yield, and 1000 grain weight (Table 2).

Table 2. Mean comparison of exogenous application on yield components of wheat

Treatment	Plant height (cm)	Spike length (cm)	No. of grains per plant	Biological yield (g m^{-2})	Economic yield (g m^{-2})	1000 grain weight (g)
Control	74.63b	7.993c	49.333b	1279.3c	265.67c	36.892b
Biostimulant	78.47b	10.740b	72.333a	1623.0bc	602.00a	42.652ab
Proline	101.00a	11.510ab	50.867b	2480.0ab	424.27b	39.420ab
Zinc	104.33a	13.047a	58.867b	3093.3a	353.67bc	45.153a

Mean having similar letters within a column do not differ statistically

Pearson correlation analysis showed that yield components are positively related to each other. Biological yield is significantly correlated to plant height and spike length at 0.001 levels and to 1000 grain weight at 0.05 levels. Plant height is positively correlated to spike length; however, it is negatively correlated to economic yield at 0.01 levels. The number of grains per spike is correlated to economic yield at 0.01 levels of significance (Table 3).

Table 3. Correlations among yield determining factors of wheat

Correlations

	Biological Yield	**Plant height**	**Spike length**	**Number of grains per plant**	**Economic yield**	**1000 grain weight**
Biological Yield	1					
Plant height	0.858**	1				
Spike length	0.750**	0.777**	1			
Number of grains per plant	-0.071	-0.226	0.344	1		
Economic yield	-0.506	-0.726**	-0.319	0.754**	1	
1000 grain weight	0.644*	0.407	0.578*	0.483	0.188	1

**. Correlation is significant at the 1% level, *. Correlation is significant at the 5% level

Discussion

Wheat being the staple crop of most developing countries demands to be produced on a larger scale to meet the requirements of the ever-increasing population. According to an estimate, the population has jumped from 3.1 billion to 6.7 billion while wheat production is increasing at a passive rate which is not enough to feed the population (Dixon et al., 2009). Therefore this research was established to assess the effect of exogenous foliar application on yield of wheat. Zinc foliar application improved wheat yield by significantly affecting wheat yield components. An increase of 8.3 g per 1000 grain weight due to zinc was observed and can be attributed to the fact that zinc is a vital part of enzymes and improves enzyme activity to deposit more photosynthates in grain leading to heavier grains (Hotz and Braun, 2004). Similarly, an increase in plant height, spike length, and biological yield due to zinc biofortification was also significant. These results are in line with the results observed by Paul et al., (2016) in which they reported an increase in plant height (4.49 cm), an increase in spike length (1.33 cm), and an increase in 1000 grain weight (3.95 g). Similar results have been reported by several researchers (Abdoli et al., 2014; Sultana et al., 2016; Esfandiari et al., 2016).

Effect of proline foliar application on plant height, spike length, biological yield, and 1000 grain weight was statistically equal or at par with zinc foliar application. Proline is an amino acid that regulates the activity and functioning of antioxidants to develop a better surviving response of plants to the environment (Öztürk and Demir, 2002). Proline at different concentration has been reported to improve growth and yield of *Urtica pilulifera* L. (Wahba et al., 2007), *Matricaria chamomilla* L. (El-Din and El-Wahed, 2005), *and Solanum lycopersicum* L. (Abd El-Latif, 1995; Ragab et al., 2001), *Zea mays* L. (Hamedand Al-Wakeel, 1994),

Nicotiana rustica L. (Darwish and Reda, 1975), and *Gossypium barbadense* (Heikal and Shaddad, 1982) due to nitrogen content of proline.

Biostimulant (3D) is a mixture of gibberellic acid and naphthalic acid that increased the number of grains per spike, economic yield, and 1000 grain weight. The increased number of grains per spike can be due to elevated transportation of sucrose (Shakirova et al., 2003). The increased 1000 grain weight has also been observed by Al Majathoub, (2004) who reported an influence of better translocation of assimilates leading towards heavier grains and eventually higher economic yield. Similar results were also reported by Naghashzadeh (2007), Wang et al., (1996) and Islam et al., (2014).

Conclusion

It is evident from the results that foliar application of nutrient or plant growth regulators or biostimulants is effective in improving wheat yield and yield parameters. The use of nutrient foliar spray proved to be the most effective treatment followed by plant growth regulator and biostimulant spray. Their use can be advised to farmers facing low yield problem in wheat. Moreover, the combined effect of these treatments can be studied in the future to determine whether they have positive interaction or negative interaction with each other.

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