# GLOBAL RADIATION AND ALBEDO IN THE RADIATION SYSTEM OF LAKE BALATON

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#### Abstract

The values of global radiation and albedo measured above Lake Balaton between July 2007 and August 2008 were examined and that was compared with the global radiation measured above the standard meteorological station of Keszthely. Different intensity of global radiation was found above the shore and above the water at the same elevation of the Sun. At low elevations the global radiation is higher above the shore while at solar elevation beyond 40° it is higher above the water. In 90 % of the examined period the values of albedo measured in the morning differed from that measured in the afternoon at the same elevations. The difference increases with the decrease of solar elevation. In summertime until the middle of September the albedo measured in the morning is regularly higher than that measured in the afternoon. From

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the middle of October the values measured in the afternoon are higher. The mean albedo of the Lake Balaton is the smallest in June: 3-5%, thereafter it rises gradually. The mean albedo was 6.8% at Keszthely Bay, in the course of our investigation.

**Key-words:** albedo, Balaton, global radiation, reflected radiation, Keszthely Bay

## Összefoglalás

A Balaton hőháztartásának legfontosabb komponense vízfelület által abszorbeált elektromágneses sugárzás. Jelen tanulmányban a tó felett mért globálsugárzás és albedó értékeket vizsgáltuk 2007 júliusától 2008 augusztusáig, illetve a víz felett és a standard meteorológiai állomáson mért globálsugárzást hasonlítottuk össze. Vizsgálataink technikai alapját képezi, hogy 2007-ben a Balatoni Integrációs Kht. két hidrometeorológiai paramétereket rögzítő mérőoszlopot telepített a Balaton parttól távolabbi tó-testébe. Az egyiket Siófok közelébe, a másikat pedig a Keszthelyi-öbölbe. Jelen tanulmányhoz felhasznált adatok mérése a Keszthelyi öbölben, a parttól 1000 méterre történt a vízfelület felett 3 m magasságban. A szárazföld feletti sugárzásadatok a keszthelyi Agrometeorológiai Kutatóállomás bocsátotta rendelkezésünkre.

A parton és a víz felett ugyanolyan napmagasság mellett különböző intenzitású globálsugárzást figyeltünk meg. Alacsony napállásnál a parton, 40°-nál magasabb napállásnál a víz felett erősebb a globálsugárzás. Szeptembertől, amikor a nap delelési magassága nem éri el a 45°-ot, egész nap a szárazföld fölött mértük a nagyobb értékeket. A napi összes globálsugárzásban a nyári hónapokban nem mutattunk ki szignifikáns eltérést, mivel a víz felett magas napállásnál mért nagyobb értékeket kiegyenlíti az alacsony napállásnál mért ellenkező előjelű különbség. Ezzel szemben az őszi időszakban, amikor egész nap a szárazföld felett mért értékek a magasabbak, a napi összes sugárzásban szignifikáns különbség lép fel.

Az albedó napi menetére vonatkozó vizsgálataink igazolták, hogy 30°-40°-nál magasabb napállás esetén az albedó nem függ a napmagasságtól. Alacsonyabb napállásnál az albedó napi menetében aszimmetriát figyeltünk meg. A vizsgált napok 90%-ában az ugyanolyan napmagasság mellett délelőtt és délután mért albedóértékek eltérnek, a különbség a napmagasság csökkenésével fokozódik. Nyári hónapokban délelőtt, októberben délután mértünk magasabb albedóértékeket, szeptemberben nem volt egyértelműen kimutatható tendecia.

Vizsgálataink szerint a Balaton átlagos albedója júniusban a legkisebb: 3-5%, ezt követően fokozatosan emelkedik.

#### 1. Introduction

The accurate knowledge of the heat balance of the Lake Balaton is essential for the discussion of flora and fauna of the lake. Lake Balaton acts as the habitat of a number of species of animals and plants. Climatic attributes of the habitat is greatly influenced by radiative energy absorbed, reflected and transmitted by the water surface.

The most important constituent of heat balance is the electromagnetic radiation absorbed by water surface. One component of it is the direct radiation coming through the atmosphere without being scattered. The other component is the diffuse radiation which has been scattered by the gas molecules of the air and the aerosol particles in the atmosphere. The sum of the diffuse radiation and the vertical component of direct radiation is called global radiation. The ratio of the two components strongly depends on the solar elevation and the meteorological conditions even at the same place. By annual mean the scattered radiation composes 40-50 % of global radiation in Hungary (Weingartner 1964). Global radiaton incident to the water surface is partly absorbed and partly reflected. The amount of energy absorbed and relevant to the heat balance can not be measured directly, however it can be calculated as the difference of incident and reflected radiation. This is why the subjects of our investigation are the amount of radiation energy reflected by the surface and the incoming radiation as well as the albedo defined as the ratio of those.

Last measurements related to radiation properties of Lake Balaton were carried out in the early seventies (Weingartner 1964, Dávid 1973). According to Weingartner's results, the albedo of water surface does not depend on the solar elevation when it is higher than 30°- 40°. In his publication the mean albedo varies between 6-8% in summer (Weingartner, 1964). 10 years later Dávid detected mean albedo between 8-10% in the same season (Dávid, 1972). The lowest value was measured in July (Dávid, 1972), though the albedo of Lake Fertő was minimal in June (Dávid, 1970). The values of radiation and albedo were not examined as a function of exact values of solar elevation but as a function of time. Because of the ecliptic movement of the Sun, the results of different days are intricate to compare on this way.

Major (1982) examined the Fresnel reflection of ideal water surface separately in the case of direct and diffuse radiation. The albedo referred to the former decreases exponentially as a function of the solar elevation while the albedo referred to the latter is nearly constant. The albedo referred to the global radiation including both of them in different ratio is the weighted average of their albedos. The ratio of the direct and diffuse radiation varies as a function of solar elevation. In consequence the albedo referred to the global radiation has a peak at solar elevation of 5 degree. This is a theoretical value deduced from the Fresnel equations. Previously the measurements of albedo concerning Lake Balaton were carried out with sensors mounted on columns based on the shore. The columns settled by the Balatoni Integrációs Kht are the first ones which are able to record the real radiation conditions above the lake, filtering out all interfering effects completely due to being settled 1000 m from the shore. We applied two of these columns sited to Keszthely Bay and near Siófok. The continuous observation is also a new feature of current investigation.

In last decades the technological progress has made much more exact measurements possible, furthermore the change of climatic attributes has made them necessary. We intended to accomplish this purpose by this paper.

#### 2. Materials and methods

#### 2.1. Radiation data

The technical bases of our investigation are two hydrometeorological columns settled to Lake Balaton into the lake-body situated far from the shore in 2007 as part of the Life Project of Lake Balaton Development Coordination Agency (Balatoni Integrációs Kht). One of them is settled near Siófok (latitude: 46°58,28'; longitude: 18°03,67'), the other one is in the Keszthely Bay, a few hundred meters from the mouth of the River Zala (latitude: 46°47,01'; longitude: 17°16,63'). Both of them are equipped with sensors measuring global radiation, reflex radiation, air temperature, relative humidity, wind direction and wind speed. The values measured by the sensors has been averaged on five minutes basis and they are sent to the central computer of the measuring circuit. The global radiation and the reflex radiation are measured with outstandingly precise Kipp & Zonen CMP3 pyranometer. The spectral range detected by this device is between 310 nm and 2800 nm. The series of data necessary to our investigation were made available for us by the Balatoni Integrációs Kht. This paper analyses the measurements related to the Keszthely Bay.

The measurements began on 3rd July, 2007. The columns are taken out of the water in winter to prevent ice damage. Accordingly data series are available from periods between 3 July 2007 and 21 November 2007, 7 May 2008 and 6 November 2008 as well as 26 May 2009 and 21 September 2009.

The Agrometeorological Station of Keszthely made their data series of global radiation available for us as well. These data has been measured on ten minutes basis. The database enlarged on this way makes it possible to carry out the comparative analysis of values measured above the water with those measured in the standard meteorological station of Keszthely. The station of Keszthely is the vital part of the measuring network of the Hungarian National Meteorological Service.

As we have no exact figures of cloudiness we classified the days of the period under survey as clear, partly clouded and fully clouded by using the measured radiation data. The cloudiness influences both the intensity of global radiation and the spectral combination of it. Therefore it is necessary to examine the mentioned classes separately. At first the fully cloudless days were examined. Those days were ranked into this group on which the course of global radiation had regular shape. There were sixty days like this in our database. The results presented hereafter were made on the basis of the cloudless days.

#### 2.2 Preparation of data series

The values measured were forwarded by sensors with SI unit of measurement, time was measured in Unix time. Its epoch is 01.01.1972. 00:00:00. Time value given in Unix time indicates how many seconds

had passed since midnight of 1 January 1972 until the given time excluding leap-seconds. Unix time passes evenly while the standard time needs to be corrected from time to time because of the irregular rotation of the Earth. 23 leap-seconds were inserted between 1 January 1972 and the beginning of the measuring in 2007, the twenty-fourth was inserted at midnight on 31 December 2008. It was taken into account at converting of time values.

Solar elevation and azimuth of the Sun were calculated from exact time values, latitude and longitude. Beside the astronomical formulae, the apparent displacement caused by refraction in the atmosphere was taken into account. So the solar coordinates represent the apparent position of the Sun in our database.

#### 2.3. Time correction

Time values of data series measured in 2008 on the column placed in the Keszthely Bay were incorrect. Daily maximum of global radiation should be at the highest solar elevation in clear days. According to measurements this maximum occurred before culmination. The difference was the same every day, its exact value was calculated by regression analysis. A third-degree polynomial was fitted on values of global radiation measured at solar elevation higher than 30 degree. The exact time of maximum was determined from its derivative. Time of culmination was determined from the date and the geographical coordinates. Difference of this values was calculated for each day, then they were averaged. In compliance with it, time values of data series measured in 2008 on the column placed near Keszhely were increased with 921 seconds (15 minutes 21 seconds).

#### 3. Results

# **3.1.** Comparison of global radiation measured above the water and in the meteorological station

As the distance between the Agrometeorological Station of Keszthely and the sensors mounted on the column placed in the Keszthely Bay is not more than 4500m, significant difference in global radiation was not expected. Unexpectedly the next results have been found. Daily course of global radiation values measured in the two different places differ significantly (Fig. 1a and b). At low solar elevation the values measured above the shore were larger while at higher solar elevation they were smaller. Global radiation values measured in the two different places are nearly equal at solar elevation of 40 degree. In summer between 10 a.m. and 15 p.m. higher global radiation can be expected above the water than predicted from values measured above the shore (Fig. 1a). From early autumn when angle of culmination is under 45° the higher values are measured above the shore (Fig. 1b).



Figure 1: Global radiation measured above the water and above the shore as a function of time (UTC + 2h)



Figure 2. Global radiation measured above the water and above the shore as a function of elevation

Time values of the Fig. 1 are presented in standard time and daylightsaving time thus they are equal to UTC+2h. Values of global radiation are shown as a function of solar elevation in the Fig. 2. The chart of July (Fig. 2a) points out that global radiation values measured in the two different places are equal at 38 degree. Difference of daily peaks changes continuously, its trend is shown in Table 1, where exact values are listed from May to the beginning of November.

Peak of global radiation measured above the water and on the shore				
	Peak above the	Peak in the	Difference	
	water [W/m <sup>2</sup> ]	shore [W/m <sup>2</sup> ]	$[W/m^2]$	
27 May 2008.	883	854	29	
24 Jun 2008.	901	876	25	
15 Jul 2007.	908	883	25	
06 Aug 2008.	872	849	23	
19 Aug 2008.	818	810	8	
21 Sept 2007.	683	715	-32	
15 Oct 2007.	540	592	-52	
1 Nov 2007.	436	487	-51	

Table 1.Peak of global radiation measured above the water and on the shore

From May to July peak values measured above the land are 2-4 percent (20-30 W/m<sup>2</sup>) lower than those measured above the water. This relatively small difference is balanced by reverse difference measured at low solar elevation. Therefore significant difference is not found in the daily radiation sum calculated by integrating global radiation by time.

#### Table 2.

	Total radiation above water [MJ/m <sup>2</sup> ]	Total radiation above shore [MJ/m <sup>2</sup> ]	Difference [MJ/m <sup>2</sup> ]
27 May 2008.	27.4	27.8	-0.4
24 Jun 2008.	27.6	27.7	-0.1
15 Jul 2007.	28.1	27.9	0.2
06 Aug 2008.	24.5	24.5	0
19 Aug 2008.	23.4	24.1	-0.7
21 Sept 2007.	17.0	18.3	-1.3
15 Oct 2007.	12.2	13.7	-1.5
1 Nov 2007.	8.5	9.3	-0.8

Daily total global radiation on clear days from May to November

On the contrary, autumn peaks measured above the land are 5-12 percents  $(30-50 \text{ W/m}^2)$  higher than those measured above the water. As the global radiation measured in the station is higher all day, there is a significant difference in the daily total radiation (Fig. 3, Table 2).





Theoretically the peak of total global radiation is expected to be on 22nd of July and formerly and thereafter it is expected to decrease almost symmetrically on the basis of insolation time (period between sunrise and sunset) and the angle of incidence of solar radiation. According to our investigation, the peak occurred in early July in 2007 and 2008, later than expected. It can be explained by features of the atmosphere. In the examined years, the sunny but strongly vaporous days at the end of June were followed by quite dry, hot days at the beginnig of July. There is no way to consider this phenomenon general because of the shortness of the examined period.

#### 3.2. Daily course of albedo

Daily variations of albedo calculated as ratio of reflex radiation to global radiation measured above the water were investigated. A symmetrical chart as a function of time with a peak at solar elevation of 5 degrees was expected on the basis of both calculated and measured values of references. According to the measurements carried out by Weingartner (1964) above Lake Balaton the albedo decreases exponentially at lower solar elevation than  $30^{\circ}$ - 40 degree, on the other hand it does not depend on the solar elevation at higher position of the Sun (Weingartner, 1964). Latter statement is verified by our results. Deviation was found at low solar elevation. The chart of albedo was not symmetric on 90 percents of examined days, that is the values measured in the morning and in the afternoon at the same solar elevation were different (Fig. 4).



Figure 4. Difference of albedo at the same elevation in the morning and in the afternoon (1 Aug 2007)

The albedo measured in the afternoon was higher on 20 % of the days while on 70 % of them the albedo measured in the morning was higher. The difference of albedos measured in the morning and in the afternoon at the same solar elevation is a function of the solar elevation. It is the smallest above solar elevation of 35 degree, where it is between 0-0,5 %. When the position of the Sun is lower than that, it increases significantly with decrease of solar elevation. The differences are very different in this range.

In summertime until the middle of September the albedo measured in the morning is regularly higher than that measured in the afternoon (Table 3). However, from the middle of October the tendency is reverse, the values measured in the afternoon are higher.

#### Table 3.

Average of albedo values measured in the morning and in the after	r-
noon from June 2007 to August 2007.	

Solar elevation (°)	Albedo in the morning	Albedo in the afternoon
10	0.30	0.19
11	0.27	0.19
12	0.24	0.17
13	0.21	0.16
14	0.20	0.15
15	0.18	0.14
16	0.16	0.13
17	0.16	0.13
18	0.14	0.12
19	0.14	0.11
20	0.12	0.11

The peaks in the diurnal course of albedo were also examined. According to the references this peak is accepted at solar elevation about 5 degrees and its value is about 0,2 (Major, 1982). Albedo values measured below solar elevation of 5 degrees were not utilized because a tiny inaccuracy in the measurement may result in large relative error. In 50 percent of the examined days this peak was not found in the morning while in 15 percent of the days it was not found in the afternoon. This peak was probably below 5 degrees. It is notable that the peak in the morning was not detectable in summertime while the peak in the afternoon was not detectable in fall. It is in accordance with our former findings. The smaller ratio of diffuse radiation causes that the peak of albedo is at lower solar elevation. The series of peaks was only complete for summer afternoons. On the basis of these data the mean albedo peak in summer cloudless afternoons is 0,27 at solar elevation of 7,7 degrees on the average.

Mean albedo is defined as the ratio of daily sum of radiation reflected by the surface to the daily sum of incident global radiation. Mean albedo of Lake Balaton is 6-8 % according to the measurements of Weingartner (1964). Our results verify these values. Mean albedo of Lake Balaton in Keszthely Bay was 6.8% in the examined period.

#### 3.3. Annual course of albedo

Trend of albedo was investigated in each months. We had investigatable data from 2007 between July and October, and from 2008 between May and August. The values of solar elevation measured in degree were rounded to integer, the records were classified on the basis of it. Monthly average of the values of albedo of each class were calculated. This procedure was executed with the data from both Keszthely and Siófok (Fig. 5a, b, c, d).

According to the previous measurements carried out above the lakeshore (Dávid, 1973, 1976) the albedo of the surface is minimal in July then it increases continuously to set-in of the winter (Dávid 1970; Anda and Varga 2007). In 2007, the albedo of June was the lowest according to our investigation. Thereafter it increased continuously until autumn. Measurements on Lake Fertő (Dávid, 1971) pointed out results like this previously (Dávid, 1970). As both of the columns are several kilometres from the area used by bathers, the shift of minimum of albedo to earlier can not be explained with dreggy, less transparent water caused by more intense leisure time activities in the water. The most probable causes of this phenomenon are microorganisms, algaes and other plant species appearing in summer and influencing the colour and transparency of water.

Similarly to daily mean albedo the monthly mean albedo was calculated as the ratio of monthly total reflected radiation to monthly total global radiation (Table 4).

Moniniy mean albeaos for Reszinely bay and Slofok						
	May	June	July	August	September	October
2007 Keszthely			6.2	6.7	8.1	9.7
2007 Siófok			3.8	4.7	7.0	9.5
2008 Keszthely	6.1	3.3	5.5	6.3		
2008 Siófok	5.5	4.3	6.3	6.4		

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Table 4.

Striking difference can be observed in the albedos of Keszthely and Siófok during 2007. This difference was the highest in July (48%), later it decreased to 2%, the two kinds of values were nearly equal in October.





Figure 5 a,b: Monthly mean albedo as a function of elevation in 2007





Figure 5 c,d: Monthly mean albedo as a function of elevation in 2008

The higher albedo of Keszthely Bay in comparision to Siófok was observed by Anda and Varga (2007) too. The sensors at Keszthely are settled about 1000 m distance from the mouth of the River Zala. So the difference can be explained with the impact of organic and inorganic matters coming from the river to the lake (black coloured humin acid). The characteristics of Keszthely Bay were already pointed out by Anda and Varga (2004).

#### 4. Discussion

Significant difference is found between the global radiation measured above the water and in the shore at the same time. Previous measurements carried out above the Lake Balaton did not examine this aspect. The difference can not be explained with terrestrial long-wave radiation and the radiation of the atmosphere because their wavelength are larger than 3  $\mu$ m while the upper limit of the pyranometers' spectral range we used is 2,8  $\mu$ m. Difference found in daily peak of global radiation and alike in daily total of it results in difference in UV radiation. This difference could be taken into account making medical weather forecast. These predictions are calculated from values of global radiation measured above the shore. However, this investigation points out that in summer just in the solar noon involving the largest UV-risk the difference is considerable.

In our study the mean albedo of the Lake Balaton is 6-8 %, which completely accords with earlier findings. It proves from a new aspect that the amount of components influencing both albedo and water quality did not change considerably.

Deviation of daily course of albedo from that expected on the basis of previous measuring was found at low solar elevation. Decrease of albedo with decrease of solar elevation under solar elevation of 10° was established by Dávid (1976) and Dávid and Kozma (1976). In addition two further results were observed. In Keszthely Bay in summertime the albedo measured in the morning is regularly higher than that measured in the afternoon at the same solar elevation. However, from the middle of October the values measured in the afternoon are higher. These results may be interpreted with the change in diffuse-to-direct ratio. At low solar elevation the albedo of the direct radiation increases with decrease of solar elevation angle, while the albedo of diffuse radiation is nearly constant. For this reason the diffuse-to-direct ratio affects the albedo to a large extent. During the daytime the amount of the precipitable water in the atmosphere increases and it causes the ratio of the diffuse radiation increases as well. That is why the albedo is lower in the afternoon. In the autumn days this effect becomes weaker and it is replaced by a stronger effect, the morning mist formation. These small waterdrops scatter considerably the solar radiation. Thus, the ratio of the diffuse radiation is larger at the same solar elevation in the morning than in the afternoon and so does the albedo. To sum it up, in summer afternoon the Rayleigh scattering caused by the water molecules while

in autumn morning the Mie scattering caused by the water droplets result in lower albedo.

These effects result in the second finding, that is the higher the diffuse-to-direct ratio the higher solar elevation the peak of albedo is at. Asymmetry in the diurnal course of the surface albedo were found by Song (1998) and Mayor et al. (1997).

Investigating annual course of albedo significant difference was found between the values measured by Siófok and in the Keszthely Bay. Distance of the two columns is 62km, waterdepth is 3.3m at both of them and there was no significant difference in radiation attributes on examined days (same cloudiness). The albedo of the water surface was distinctly greater in Keszthely Ba10 y in 2007 and in May, 2008, while it was greater near Siófok in the summer of 2008. This period of two years is too brief to draw considerable conclusions from these data but points out that surface of Lake Balaton is not an uniform watersurface from the aspect of albedo.

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# EFFECTS OF HEAVY METALS ON THE WATER BALANCE OF CUCUMBER DETECTED BY MRI MEASUREMENT

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#### Abstract

The purpose of our study was to extend the potential applications of MRI (applied in human diagnostics) to plant-water relations, and to verify the previous knowledge about it. Effects of heavy metals on the water balance of the plants can be examined by MR measurements.

Three-week-old seedlings of cucumber (Cucumis sativus) were polluted with Pb-nitrate, Zn-sulfate, Cd-nitrate and Hg-chloride solutions at 10<sup>-5</sup> M concentration. The incubation time was 1 week. The plants were grown in nutrient solution in growing chambers. The effects of heavy metals on the water balance were measured by classical method and MRI technique. The MRI measurements were carried out at the Diagnostics and Oncoradiological Institute of the University of Kaposvár (Hungary), using a Siemens Avanto MRI equipment.

The MR measurements are made on the spin's system. The procedure is based on the interaction of the external magnetic field, electromagnetic waves and the hydrogen nucleus' in the substance. Namely the quantity and distribution of the protons are measured by MR. If we ask a question, where can we find relatively a lot of protons, our answer is in the water. So the MR doesn't measure the anatomic structure, but the quantity of protons. The anatomic structures are determined by the distribution and quantity of protons.

As classical method stomatal resistance was measured by AP4 porometer in conductance mode. Water content was determined by drying until weight consistence. Water content percentage is determined by the dry and fresh weight.

Significant differences can be detected between the different heavy metal treatments by MRI measurements, but the classical methods did not prove these deviations. In consequence the MRI measurements can provide more detail information about water content and transport. In addition MRI measurement is a non-destructive method, opposite to the classical techniques.

MRI measurement can increase our knowledge on the cycling and pathways of heavy metals in the plants.

Key words: MRI, heavy metals, water balance

## Összefoglalás

A vizsgálatunk célja a humándiagnosztikában alkalmazott MRI alkalmazási lehetőségének növény-víz kapcsolatra történő kiterjeszté-

se, valamint a korábbi ismeretek igazolása volt. A nehézfémek növényi vízháztartásra gyakorolt hatása vizsgálható-e MRI mérés felhasználásával.

Három hetes uborka palántákat szennyeztünk ólom nitrát-, cinkszulfát-, kadmium nitrát- és higany klorid oldattal, melyek koncentrációja 10<sup>-5</sup> M volt. A szennyezést egy héten keresztül végeztük. A növényeket tápoldaton, klímakamrában neveltük. A nehézfémek növényi vízháztartásra gyakorolt hatását klasszikus és MRI mérésekkel is vizsgáltuk. Az MRI méréseket a Kaposvári Egyetem Diagnosztikai és Onkoradiológiai Intézetében végeztük, egy Siemens Avanto típusú MR felhasználásával.

Az MR mérés elve nem más, mint a spinek rendszerén végzett mérések. A folyamat a külső mágneses tér, az elektromágneses hullámok, és az anyag hidrogén atomjainak kölcsönhatása révén valósul meg. Vagyis az MR a protonok mennyiségét és eloszlását méri. Ha feltesszük a kérdést, hogy hol található relatíve sok proton, akkor erre a kérdésre a válasz az, hogy ahol sok hidrogén van tehát elsősorban a vízben. Vagyis az MR nem az adott anatómiai struktúrát méri, hanem az adott anatómiai struktúrában levő víz mennyiségét és eloszlását.

A klasszikus mérések közül a sztóma ellenállást vizsgáltuk, AP4 porométer felhasználásával konduktancia üzemmódban. A növények víztartalom %-át a friss és a száraz tömegből határoztuk meg.

Szignifikáns különbséget tudtunk kimutatni az MRI mérés során a különböző nehézfém kezelések között, azonban a klasszikus mérésekkel ezt nem tudtuk kimutatni. Az MR mérés a víztartalom és vízszállítás folyamatáról egy sokkal részletesebb elemzést tesz lehetővé. Mellette nem destruktív mérési eljárás, ellentétben a klasszikus elemzésekkel.

Az MR mérések növelhetik ismeretanyagunkat a nehézfémek körforgásáról és áramlásáról a növényekben.

#### Introduction

Environmental pollution by heavy metals is a global problem (*Xiangyang et al.* 2009). Heavy metal contamination also occurs in industrial zones, where the sources include heavy vehicular traffic, refuse dumps and sewage sludge (*Larcher 2003*). The trace metals are not essential elements for plants take up from soil and atmosphere and accumulate them in their edible parts in various concentrations. Increasing heavy metal uptake causes stress by modifying the water transport in plant.

An everyday problem in the classical measurements of the components of plant water balance is the way in which a component of the plant – as the "random taken" component of the soil-plant-atmosphere system – will respond to interventions by the study and the parameter to be measured is affected, and to what extent, by the measurement procedure itself. Another basic problem of measurements applied in studying classical plant-water relations is their destructive nature; the opportunity of repeating the measurement is entirely eliminated for a given test specimen. Of the deficiencies of traditional approaches, MRI is capable of eliminating the second potential error – it is a non-destructive method, thus a particular sample of live and functional plant can be tested even several times in succession.

Studies of plant physiological aspects often involve an inaccuracy of measuring the components of plant water balance (*Pearcy et al.* 1991). The duty of researchers is made fairly difficult by the sensitivity of living organisms responding immediately to any minor or major changes, external or internal ones. The sensitivity of a plant often manifests itself in the broad scattering of the parameter being measured; this may often be due to the measurement itself – covering the actual relations as well as the comparisons. MRI appears to be an extremely efficient means of eliminating that potential error. The procedure involves a measurement of spin systems in the live plant; it will not intervene harshly into the system under test. The spins only have weak interactions with macroscopic parameters of the biological system under test that will affect its behavior from biological and chemical aspects. Magnetic properties play a fairly irrelevant role in biochemical processes at levels of cells (*Berényi et al.* 1997). On the other hand, biochemical parameters will affect the behavior of spin systems in a readily measurable manner – i.e. conclusions may be drawn from measurements of spin systems on behavior of the biological system under test (*Berényi et al.* 1997, *Földes et al.* 2003).

A few decades ago the plant-water relations were studied primarily in their components, as fractional processes. They are the water absorption of the root, its transmission to the evaporating surface, and the transpiration through the leaf. This subject has a literature too broad to be listed (Ketelapper 1963, Kanemasu 1969, Lange et al. 1976, Lange et al. 1976, Jarvis and Mansfield 1981, Monteith 1973, 1976, Monteith et al. 1990, etc.) – occasionally discussing in detail the potential errors of measurement (Johnson 1981, Norman et al. 1981, Meyer et al. 1985, McDermitt 1990, etc.). It must have been the error of measurement that led to the need of studying the complex behavior of the soil - plant atmosphere system. That need may not be referred to as having been focused recently (Shawcroft et al. 1974, Norman 1979, Goudriaan and van Laar 1974, Bouman et al. 1996) -although its real renaissance coincides with the energetic-based approaches, the spreading of simulation models. Spreading of the systems outlook has given rise to the need of viewing the living beings in conjunction with their environments, holding in mind all possible and negative consequences of removing them from the environment (Brisson et al. 2003, Pronk et al. 2007). Although the importance of systems' outlook is recognized, most of the analyses

of plant – water relations focuses on two end points of the system even today – on water intake from the soil (e.g. *Jackson et al.* 2000, *Novak et al.* 2005) or delivery of water, transpiration (e.g. *Langensiepen et al.* 2009). The transport of water inside the plant the changes occurring in the stem are reviewed less frequently. This may be due to the fact that, since the exact determination of transport of water inside the plant – definition of water potential – it is a familiar fact that such a process requires no extra energy input by the plant; the process is maintained by the water potential difference between the soil and the atmosphere (*Sutcliffe* 1984). (*Jakusch et al.* 2010).

Our analysis has focused on the effects of heavy metals on the water balance of the plants can be examined by MR measurements.

#### Materials and methods

Our test plant was cucumber (Cucumis sativus) (*Fig 1*), which was grown in nutrient solution in growing chamber. The seeds were germinated in culture dish, on filter paper moistened with distilled water for two days in darkness at 30 °C. The seedlings with 15-30 mm-long primary roots were transferred to  $CaSO_4$  solution at 5\*10<sup>-4</sup> M concentration for 24 hours in darkness. The radicles of cucumbers were gained by the  $CaSO_4$ . After the  $CaSO_4$  incubation the plants were put in the nutrient solution and the growing chamber. The lighting was given fluorescent and metal-halogen lamp. The light intensity was 100-140 µmol photon\*s<sup>-</sup> <sup>1\*</sup>m<sup>-2</sup>, and the period of the light intensity was 14/10 hour (light/dark). The temperature of the room was 22/26 °C. The cucumbers were grown at fourfold dilution Hoagland-solution (*Table 1*). Fe-citrate was the Fe source. The cucumbers were polluted with Pb-nitrate (PbNO<sub>3</sub>), Zn-sulfate (ZnSO<sub>4</sub>), Cd-nitrate (CdNO<sub>3</sub>) and Hg-chloride (HgCl<sub>2</sub>) solutions at 10<sup>-5</sup> M concentration (*Fig 2*). The treatments held 7 days.



*Fig. 1. MRI record of cucumber* 

Compound	Concentration (mol/dm3)
KNO <sub>3</sub>	1.25*10-3
$Ca(NO_3)_2$	1.25*10-3
MgSO <sub>4</sub>	0.5*10-3
KH <sub>2</sub> PO <sub>4</sub>	0.25*10-3
H <sub>3</sub> BO <sub>3</sub>	1.156*10-5
MnCl <sub>2</sub> *4H <sub>2</sub> O	4.6*10-6
ZnSO <sub>4</sub> *7H <sub>2</sub> O	1.9*10-7
Na <sub>2</sub> MoO <sub>4</sub> *2H <sub>2</sub> O	1.2*10-7
CuSO <sub>4</sub> *5H <sub>2</sub> O	8*10-8
Fe-citrate	1.0*10-5/2.0*10-5

Table 1Components of Hougland-solution



# Fig. 2. Polluted cucumber plants (from the left: control, lead-, zinc-, cadmium-, mercury treatment)

The MR measurements are made on the spin's system. The procedure is based on the interaction of the external magnetic field, electromagnetic waves and the hydrogen nucleus' in the substance. Namely the quantity and distribution of the protons are measured by MR. When we ask the question, where we can find relatively a lot of protons, our answer is: in the water. So the MR doesn't measure the anatomic structure, but the quantity of protons. The anatomic structures are determined by the distribution and quantity of protons.

The site of studies was the Diagnostics and Oncoradiological Institute of the University of Kaposvár (Hungary). The studies were carried out by using a Siemens MR apparatus Type Avanto (*Fig 3*) capable of generating a magnetic field of 1.5 T. The repetition and echo times applied in the measurements were 5.27 and 2.38 sec, respectively. The study made by us involved a pixel spacing of 0.78 mm and a slice thickness of 0.7 mm; according to this the resolution referred to a pixel slab/ block of 0.43 mm<sup>3</sup> (*Jakusch et al.* 2010).



Fig. 3. Siemens Avanto MRI

The stomatal resistance was measured by AP4 porometer in conductance mode in the growing chamber. The data were collected in mmol  $H_2O m^{-2} s^{-1}$  in conductance mode.

After all measurement, the water content measurement was followed. Water content was determined by drying until weight consistence. Water content percentage is determined by the dry and fresh weight. Being a non-normal distribution involved, the distribution of data has been examined by the chi<sup>2</sup> test. The findings have been evaluated by the application of correlation analysis in studying the relation between the signal intensity and water content in the stem.

#### Results

#### **Classical measurements**

Several experiments confirm that the stomatal resistance, the water content of the root and the shoot are diminished by trace metals (*Hernandez et al.* 1997, Lozano-Rodriguez et al. 1997).

The stomatal resistance was detracted by all heavy metals however the Pb and Zn pollution did not prove significant differences. The strongest inhibition was effected by the Cd and Hg contamination. Significant differences can be detected between these heavy metal treatments and the control cucumber (*Fig 4*).



Fig. 4. Stomatal resistance

Heavy metals' effects on plants' development usually decrease their water content (*Seregin et al.* 2004). Some percent of water content deviation can cause remarkable changes in plants. The water content of the root, hypocotyl and epicotyl of the 6-week-old cucumber plants treated by lead and zinc did not differ significantly from the control (*Fig 5, 6, 7*).

In case of the cadmium and mercury treatment significant changes could be detected. The water content of the cucumber plant treated by cadmium declined by 1% compared to the control, and mercury treatment caused more than 2% of decrease in it (*Fig 5, 6, 7*). Differences between the control and these two treatments are statistically proved.

Regarding the first and the second leaf of the plants significant modification cannot be found in any treatment (*Fig 8, 9*). In case of the third and fourth leaves the water content was equal to the control for lead and zinc treatment, but mercury and cadmium treatment both caused 1% of decline in it (*Fig 10, 11*).





Fig 11 Water content of the fourth leaf

#### Findings by MRI measurements

During the research heavy metal treatments were applied when the test plants developed their third leaf, and in consequence heavy metals affected these leaves. According to the previous findings of *Sárvári et al.* (1999) heavy metals are transported to the youngest, actually developing part of the plant. Our measurements are shown on *Fig 12*.



Fig. 12. Intensity of water in the xylem of cucumber

Signal intensity detected by MRI was high in the hypocotyl and epicotyl of the control cucumber plant. Regarding the hole plant the distribution of water was balanced. Examining the hypocotyl slight increase can be detected towards the top of the shoot. This can be explaned with the decline of root pressure. The signal intensity increased by more than 60 units in the epicotyl above the first node compared to the upper part of the hypocotyl. In the epicotyl above the second node the signal intensity decrased. Above the third node increase of 50 unit can be observed. These modifications in signal intensity can be explained in more ways. According to the theory of cohesion the continious fluid column is moving towards the leaves in the elements of the xylem. In this case deviations in the signal intensity are caused by anatomic properties (*Jakusch et al.* 2010). An other solution can be that the decrease and increase of signal intesity in the epicotyl is caused by different speed of transpiration.

The intensity of the plant's shoot towards its tip also decreased under Pb treatment. The bottom side of the hypocotyl showed 25 units less intensity than the control. The reason is that the Pb accumulated and started blocking transpiration (*Fig 4*) at the location of the intake, the root. This affected the bottom of the hypocotyl as well. The increase in intensity at the center area of the hypocotyl, and the intensity noticed at the top of the hypocotyl was equal to the control cucumber, presumably because of the transpiration blocking. All of the intensities in the epicotyl over the first node were equal to the control plant's intensity, because of the Pb was blocking transpiration, however the water uptake was slightly modified. Significant differences were noticeable during the tests between the Pb treated and the control plant (Chi<sup>2</sup>: 6972; pvalue: 0.2389).

The intensity grew from the plant's (treated with Zn) hipocotyl towards its epicotyl over the first node. The Zn is essential to plant's growth, and it fastens the process until a certain level. However higher doses are toxic to plant and can easily be transported to the shoot. The intensity decreased in the epicotyl over the first node in comparison to the control plant. It was due to Zn infection. At the end of the treatment the leaves turned clorotic (light green with spots), and turned necrotic. This process was followed during the tests. Significant differences were noticeable during the tests between the Zn treated and the control plant (Chi<sup>2</sup>: 6972; p-value: 0.2389).

The intensity of the Cd treated plant's hypocotyl's bottom side was 150 units lower than the control plant's. This was because the Cd blocks the root's growth, water uptake, and affects the hypocotyl's bottom side. However in the hypocotyl towards the tip of the shoot until reaching the epicotyl over the first node the intensity decreases. The Cd blocked transpiration, so the leaves were filled with water. In the epicotyl over the second node the intensity decreased along with the water content (*Fig 7-11*). Significant differences were noticeable during the tests between the Cd treated and the control plant (Chi<sup>2</sup>: 6972; p-value: 0.2389).

The intensity of the Hg treated plant's hypocotyl's bottom side was 100 units lower than the control plant's, this maximum is due to the blocking of the root's aquaporins. In the hypocotyl towards the tip of the shoot the intensity increased, and in the upper part of the hypocotyl it even exceeds the control cucumber's results, because the Hg blocked not just the water uptake, but the transpiration as well. The intensity in the treated plant's epicotyl decreased without exception, in comparison to the control and all the other subjects. This was noticeable in the decreased water content too. Significant differences were noticeable during the tests between the Hg treated and the control plant (Chi<sup>2</sup>: 6972; p-value: 0.2389).

### Discussion

MRI may offer a novel procedure of studying the water flows of plants. The measurement accuracy of the system surpasses by far the errors of traditional plant and water studying procedures. This nondestructive technique can be carried out on live plants offering an unlimited number of repetitions.

Significant differences can be detected between the different heavy metal treatments by MRI measurements, but the classical meth-

ods did not prove these deviations. MRI measurement can increase our knowledge on the cycling and pathways of heavy metals in the plants.

MRI as a technique is bound to perform important roles in science and education as well. Its applications are expectable particularly among the research workers and teachers.

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# COMPARATIVE STUDY WITH A SET OF PESTI-CIDES TO ESTABLISH EYE IRRITANCY WITH HET-CAM AND DRAIZE TEST

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#### Abstract

The chorioallantoic membrane (CAM) of chick embryo has been used extensively for many years in various fields of biological research, including virology, bacteriology and toxicology. The CAM is a complete tissue that responds to injury with a complete inflammatory reaction, this process similar to that induced by chemicals in the conjunctival tissue of the rabbit eye.

A possible model for assessing the irritation potential of a chemical or product to such a vascularized tissue is the chorioallantoic membrane of the embryonated hen's egg, as this is a highly vascular, thin membrane with relatively easy access for both treatment and assessment.

In recent years various *in vitro* methods have been developed to replace the heavily criticized Draize rabbit eye test for irritation testing.

One of the most studied alternative methods is the Hen's Egg Test - Chorioallantoic Membrane (HET-CAM).

In our studies a comparative screening was done with a set of pesticides to establish paralel data on *in vitro* (HET-CAM) and *in vivo* (Draize) results.

In most cases good correlation was found between the HET-CAM assessment and results from the Draize rabbit eye test. The actual form of the HET-CAM test is a valuable prescreen for predicting ocular irritation potential of chemicals, and can be used to reduce the number of experimental animals. The HET-CAM test is useful as a part of a battery of tests to replace the Draize rabbit eye test.

Keywords: chorioallantoic membrane, in vitro, rabbit, Draize

# Összefoglalás

A tyúktojás chorioallantois membránját évek óta hasznáják különböző tudományok biológiai kutatásaiban, úgymint a virológiában, bakteriológiában és toxikológiában. A membrán a nyúl szeméhez hasonlóan reagál a különböző vegyi anyagok okozta káros irritációs hatásokra.

Alkalmas modellnek ígérkezik az embrionálódott tyúktojás chorioallantois membránja az irritációs potenciálok meghatározásában, mivel rendkívül könnyen kezelhető és értékelhető.

Az elmúlt években számos alternatív módszert dolgoztak ki a sokat bírált Draize-féle primer szemirritációs vizsgálat helyettesítésére. Ezen alternatív technikák közül az egyik legtöbbet tanulmányozott, a tyúktojás chorioallantois membránját felhasználó (HET-CAM) teszt.

Vizsgálataink néhány peszticid *in vitro* és *in vivo* eredményeinek összehasonlítására irányultak.

A legtöbb esetben jó korrelációt találtunk a két teszt értékei között, azonban jelenlegi állapotában még nem jelenthető ki, hogy a HET-CAM teszt a Draize-féle primer szemirritációs vizsgálat kiváltására alkalmas lenne. Jelenleg, mint elővizsgálati módszer alkalmazható a kísérleti állatok számának csökkentésére.

### Introduction

Pesticides must undergo numerous toxicological tests before registration. To determine the ocular irritation, only the Draize rabbit eye test is accepted now, which is one of the most criticized methods because of the pain inflicted on the test animals. Several *in vitro* methods have been used to investigate the toxicology of potential eye irritants with a view to replacing *in vivo* eye irritation testing. Now most of these methods are in process of validation.

The HET-CAM test, using the chorioallantoic membrane (CAM) of the chicken egg, is one of the possible suggested alternatives (Walum et al., 1992). In the HET-CAM test the chemicals are placed in direct contact with chorioallantoic membrane of the hen's egg. The occurrence of vascular injury or coagulation in response to a compound is the basis for employing this technique as an indication of the likelihood that a chemical would damage mucous membranes (especially the eye) *in vivo*. The CAM is a complete tissue including arteries, capillaries and veins, and is technically easy to study (Leighton et al., 1985). It responds to injury with a complete inflammatory reaction, similar to the tissue of the rabbit eye.

In our studies comparative screening was performed with a set of pesticides to establish paralell data on *in vitro* (HET-CAM) and *in vivo* (Draize) results. In our experiment nine pesticides were evaluated by the HET-CAM test. The results were compared with *in vivo* data from Draize eye irritation test.

### Materials and methods

### **Test materials**

The test preparations were included Total (480 g/l glyphosate), Orius 20 EW (200 g/l tebuconazole), Systhane Duplo (240 g/l myclobutanil), Domark 10 EC (100 g/l tetraconazole), Bumper 25 EC (250 g/l propiconazole), Megatox 40 EC (400 g/l chlorpyrifos-methyl), Glyphogan 480 SL (480 g/l glyphosate), Vertimec 1.8 EC (1.8 % abamectin), Glialka Star (441 g/l glyphosate). Pesticides were tested in all cases at 100% concentrations.

### Methods

### **HET-CAM Test**

Shaver Rusticbrow chicken eggs were used. Before testing eggs were candled and discarded any which were defective. The eggs were incubated in a Ragus incubator. The temperature was 37.8°C and relative humidity was 70%. Eggs were rotated for 8 days to prevent the attachment of the embryo to one side of the egg. They were again candled on the 9<sup>th</sup> day and discarded any non-viable. The eggs were replaced into the incubator with the large end upwards but did not rotate, this was ensuring accessibility to the chorioallantoic membrane. On the 10<sup>th</sup> day they were prepared for assaying. The air cell was marked and the section of shell was removed with scissors. The membrane was moist-ened carefully with 0.9% NaCl solution and eggs were replaced in the incubator until ready for assaying.

Standards and test materials were prepared directly before each assay. Standards: 2 eggs with 1% Sodium dodecil sulphate and 0.1 M NaOH. Controls: 2 eggs with 0.9% NaCl.

Test: 6 eggs/group were treated with pesticide.

All of test solutions were run on 4 separated replicates.

The membrane was removed carefully with tapered forceps. A volume of 0.3 ml of test pesticide was added to the chorioallantoic membrane and the effect was observed over a period of 5 minutes. Haemorrhage, vascular lysis or coagulation can be seen on the chorioallantoic membrane. The starting time of reactions were recorded in seconds. A computer software was used to evaluate data (Invittox Protocol No. 47.).

The computer software uses the following algorithm:



Where H = haemorrhage, L = vascular lysis, C = coagulation, RI = irritation index, and

sec = start second.

The classification categories based on irritation index are presented in Table 1.

Table 1. Classification categories of HET-CAM test

Irritation index	Irritation category
0-0.9	no irritation
1-4.9	weak irritation
5-8.9	moderate irritation
9-21	severe irritation

### **Draize Rabbit Eye Test**

In our experiment 3 New-Zealand albino rabbits were used in each assay. Based on the international guidelines (OECD Guidelines for Testing of Chemicals, Number 405, 2002), a separate control group was not necessary, the untreated eye serves as control. Rabbits were kept in individual cages of a climatic animal room. The temperature was 22-25 °C and relative humidity was 50-70%. Laboratory rabbit diet as food and tap water to drink were served *ad libitum*.

A volume of 0.1 ml of the pesticide was instilled into the conjunctival sac of each rabbit. Test solutions were prepared before each assay.

Ocular irritation was evaluated at 1 hour, 1, 2, 3, 4 and 7 days post instillation (Draize et al., 1944). Individual scores were recorded for each animal. The time interval with the highest mean score (Maximum Mean Total Score - MMTS) for all rabbits was used to classify the test substance.

The classification categories according to MMTS are shown in Table 2.

Table 2.	Classification	categories	of Draize	rabbit eye	irritation tes	t

MMTS	Irritation classification	
0-19	no irritation	
20-49	moderate irritation	
50-79	severe irritation	
80-110	super irritation	

### Results

### **Results of the HET-CAM Test**

The numerical data are summarised in Table 3.

After the treatment with Total first vascular lysis was occurred between 9 and 26 sec, followed by haemorrhage that was observed from 11 to 200 sec. Total was a severely irritative pesticide with irritation index of 10.29.

After the treatment with Orius 20 EW vascular lysis was recorded from 20 to 37 sec and the haemorrhage was noted from 28 to 50 sec. On the base of irritation index (10.79) Orius 20 EW was severely irritative.

After instillation of Systhane Duplo vascular lysis was observed between 12 and 20 sec, and followed by haemorrhage from 40 to 80 sec. The test material has severe irritative potential according to the irritation index (10.76).

After the treatment with Domark 10 EC vascular lysis was started in 20<sup>th</sup> sec and haemorrhage was occurred in 90<sup>th</sup> sec. The irritation index showed that Domark 10 EC was severe irritant (9.62).

The treatment of eggs with Bumper 25 EC resulted in vascular lysis between 50 and 60 sec, followed by mild haemorrhage that lasted from 75 to 130 sec. Bumper 25 EC was a severely irritative pesticide with irritation index of 9.18.

After instillation of Megatox 40 EC vascular lysis was recorded from 20 to 37 sec, and the haemorrhage was observed from 45 to 140 sec. The test material had severe irritative potential according to the irritation index (10.4).

When Glyphogan 480 SL was used, vascular lysis was experienced between 15 and 25 sec. On the base of irritation index (6.55) Glyphogan 480 SL was moderately irritative.

The Vertimec 1.8 EC caused vascular lysis from 10 to 40 sec and haemorrhage was occurred from 60 to 70 sec in some eggs. The test material resulted in moderately irritative potential according to the irritation index (6.45).

After instillation of Glialka Star vascular lysis was observed from 9 to 35 sec. The irritation index showed that Glialka Star was moderately irritant (6.67).

Test materials	Irritation index
Total	10.29
Orius 20 EW	10.79
Systhane Duplo	10.76
Domark 10 EC	9.62
Bumper 25 EC	9.18
Megatox 40 EC	10.4
Glyphogan 480 SL	6.55
Vertimec 1.8 EC	6.45
Glialka Star	6.67

Table 3. Irritation indices from HET-CAM test

### **Results of the Draize Rabbit Eye Test**

The data are presented in Table 4.

After the instillation of Total followed by positive conjunctival responses with severe redness, severe chemosis and strong discharge up to day 2 after treatment. Responses turned slight from day 3 after instillation. There was no corneal reaction and iritis. On the base of irritation index (22) Total was a moderately irritative in rabbits.

Severe redness, severe chemosis and strong discharge were noted for 3 days post instillation of Orius 20 EW. The observations were reduced to the 4<sup>th</sup> day. On the cornea moderate opacity was noted from 1<sup>st</sup> day, which was observed during the whole observation period. Iritis was occurred from the 1<sup>st</sup> day and it was permanent during the whole observation period. Orius 20 EW was a severely irritative pesticide with irritation index of 66 in rabbits.

Severe redness, severe chemosis and strong discharge were observed for 3 days post instillation of Systhane Duplo. Responses turned slight from day 4 after instillation. Moderately corneal opacity and slight iritis were noted in two rabbits. Slight corneal opacity and severely iritis were observed in one rabbit. The test material had severely irritative potential according to the irritation index (52) in rabbits.

The instillation of Domark 10 EC caused positive conjunctival responses with severe redness, severe chemosis and strong discharge up to day 4 after treatment in two rabbits. In one rabbit severe conjunctival responses were observed that lasted for day 1. On the cornea moderate opacity was noted from the 1<sup>st</sup> day until the end of the observation period in two rabbits. Iritis was noted on the 1<sup>st</sup> day that did not return to normal in the case of two rabbits. On the base of irritation index (48) Domark 10 EC was a moderately irritative in rabbits.

The treatment with Bumper 25 EC resulted in slight redness, slight chemosis and slight discharge to the 2<sup>nd</sup> day. Iritis was not recorded. On the cornea no opacity was noted during the whole observation period. Bumper 25 EC was not irritative pesticide with irritation index of 11 in rabbits.

Severe redness, severe chemosis and strong discharge were observed for 4 days post instillation of Megatox 40 EC in one rabbit. Slight redness, chemosis and discharge were noted up to day 3 after treatment in two rabbits. The observed symptoms were returned to normal by day 4. Iritis was not seen. On the cornea opacity was observed in one rabbit. On the base of irritation index (22) Megatox 40 EC was a moderately irritative in rabbits.

The instillation of Glyphogan 480 SL induced positive conjunctival responses with moderate redness, severe chemosis and strong discharge up to day 4 after treatment. Iritis was not observed. On the cornea moderate opacity was noted from the 1<sup>st</sup> day and strong opacity from the 4<sup>th</sup> day that lasted to the end of observation period. On the base of irritation index (65) Glyphogan 480 SL was a severely irritative in rabbits. The treatment with Vertimec 1.8 EC resulted in moderate redness, chemosis and discharge to the 1<sup>st</sup> day in one rabbit. In other rabbits these symptoms were observed to the 4<sup>th</sup> day. Iritis was occurred only in 1 hour. Weak corneal opacity was noted from the 1<sup>st</sup> day until the end of the observation period, but this alteration was moderate in one rabbit from the 3<sup>rd</sup> day. Vertimec 1.8 EC was moderately irritative pesticide with irritation index of 46 in rabbits.

After the treatment with Glialka Star moderate conjunctival responses were recorded to the 2<sup>nd</sup> day. The iris and the cornea did not change. The eyes returned to normal in 3<sup>rd</sup> day. The test material had moderately irritative potential according to the irritation index (21) in rabbits.

## Table 4.

# Draize irritation indices of test materials corresponding irritation categories

Test materials	Irritation index
Total	22
Orius 20 EW	66
Systhane Duplo	52
Domark 10 EC	48
Bumper 25 EC	11
Megatox 40 EC	22
Glyphogan 480 SL	65
Vertimec 1.8 EC	46
Glialka Star	21

## Discussion

The HET-CAM test has the upper edge on *in vivo* methods. It is faster, cheaper and simpler than the Draize rabbit eye test. Several substances and formulations have been tested by HET-CAM and a good correlation has been found between the HET-CAM results and data based on the Draize eye test (Leighton et al., 1985; Luepke and Wallat, 1987; Sina et al., 1995; Spielmann et al., 1996; Budai and Várnagy, 2000; Tavaszi and Budai, 2006; Tavaszi et al., 2008; Budai et al., 2010.).

In our study, a good correlation was seen between the data from *in vitro* and *in vivo* methods (Table 5). However, based on the results, the HET-CAM test gave the same irritancy category for four pesticides (Orius 20 EW, Systhane Duplo, Vertimec 1.8 EC, Glialka Star). Based upon the comparison of *in vitro* and *in vivo* tests, the HET-CAM test showed a lower irritation potential for one pesticide (Glyphogan 480 SL) and a more severe irritation potential for four products (Total, Domark 10 EC, Bumper 25 EC, Megatox 40 EC). The drawbacks are: it is subjectivity, it can not be used in case of colored materials because they hide the membrane and the testing of solid materials is circumstantial. The results of the investigated agricultural materials with HET-CAM test achieved similar results to the Draize rabbit eye test.

Table	5
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irritation categories by Draize rabbit eye test			
Test materials	Category from	Category from	
	<b>HET-CAM</b> test	Draize rabbit eye test	
Total	severe irritation	moderate irritation	
Orius 20 EW	severe irritation	severe irritation	
Systhane Duplo	severe irritation	severe irritation	
Domark 10 EC	severe irritation	moderate irritation	
Bumper 25 EC	severe irritation	no irritation	
Megatox 40 EC	severe irritation	moderate irritation	
Glyphogan 480 SL	moderate irritation	severe irritation	
Vertimec 1.8 EC	moderate irritation	moderate irritation	
Glialka Star	moderate irritation	moderate irritation	

# Irritation categories by HET-CAM test in comparison to irritation categories by Draize rabbit eye test

Today there is still insufficient comparative data available using methods based on the chorioallantoic membrane of hen's egg to be an alternative to Draize rabbit eye test. As a result we need to involve further agricultural chemicals and keep on testing.

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# KEY QUESTIONS OF SAMPLING FREQUENCY ESTIMATION DURING SYSTEM CALIBRATION, ON THE EXAMPLE OF THE KIS-BALATON WATER PROTECTION SYSTEM'S DATA SERIES

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#### Abstract

In this study the practice of sampling frequency estimation is described on data series from the Kis-Balaton Water Protection System.

The main aim was to point out the milestones, and common problems of sampling frequency estimation using variograms. Firstly, the importance of sampling frequency estimation during the calibration of the system is emphasized, embedding it in the idea of sustainable development. The applied method itself, the variogram is then discussed. This is a function used in geostatistics; basically, it is the expected squared increment of the values between locations x and y (Wackernagel, 2003).

The results of the variogram analysis pointed to a series of problems and solutions which must be faced when an attempt is made to prepare the data sets for analysis. These problems mainly concern the removal of periodicity as a "special trend".

The final results explicitly show a 7 day (or less) sampling frequency is needed in the case of the total phosphorous parameter in order to permit long term assumptions and interventions to be based on it. The method described in the article can be used in the analysis of many other parameters complying with the needs of the variogram analysis, so it may prove to be highly useful to any scientist working with data received from sampling.

**Keywords:** sampling frequency estimation, monitoring system calibration, variogram, Kis-Balaton Water Protection System,

# Összefoglalás

Alábbiakban leírt kutatásban a mintavételezési gyakoriság becslése kerül bemutatásra a Kis-Balaton Vízvédelmi Rendszer (KBVR) adatsorán.

A kutatás elsődleges célja, hogy rávilágítson a variogramok segítségével történő mintavételezési gyakoriság becslés menetének problémáira. Első lépésként a mintavételezési gyakoriság becslésének rendszer kalibrálásban betöltött lényeges szerepe kerül kiemelésre a fenntartható fejlődés szemszögéből, majd maga a becslési módszer kerül bemutatásra. A mintavételezési gyakoriság becslését a geostatisztikában több függvénnyel is el lehet végezni, jelen esetben az empirikus félvariogram került alkalmazásra. A variogram vizsgálatok alatt számos problémába merülhet fel, elsősorban az adatok előkészítése során, amikor a periódus, mint egy "speciális trend" kerül eltávolításra. Ezen problémák és lehetséges megoldásai is bemutatásra kerülnek.

A KBVR összes foszfor paraméterének variogram vizsgálatának eredménye kimutatta, hogy 7 napos vagy annál kisebb mintavételezési gyakoriság szükséges ahhoz, hogy a paraméter adatsoraiból visszaállíthatóak legyenek a vizsgált területen zajló folyamatok. (Ennek a követelménynek jelenleg is eleget tesz a Kis-Balaton Üzemmérnökség Laboratóriuma).

A leírt módszer a tudományok minden területén alkalmazható ahol mintavételezésből származó adatsorok állnak rendelkezésre és lényegi kérdés a pontos mintavételezési gyakoriság meghatározása, hogy a kutatásokból reprezentatív eredmények és szakmailag megalapozott döntések születhessenek.

### 1. Introduction

"Sustainable development is a pattern of resource use that aims to meet human needs while preserving the environment so that these needs can be met not only in the present, but also for generations to come (Anonymous, 1987)". This was one of the concepts that urged scientists to make environmental monitoring a basic element of every case study. Therefore, during the past few decades a great "mass of data" has been produced describing the environments we live in.

The question is whether or not the data produced in diverse fields of science and by different sampling methods conforms to standards so it would produce a representative data set. This is a highly important problem because these data define and limit the mathematical methods that can be applied to them, and, in this way, the conclusions and interventions as well.

Following the outline described above, the sample itself has to be defined from a statistical perspective. The statistical sample is a random variable X with distribution F, a random sample of length n = 1, 2, 3, ...is a set of *n* independent, identically distributed random variables with distribution F (Samuel, 1962). The data in fields, such as meteorology, hydrology, and limnology are not always independent. For example, an annual one pH sample from 10 consecutive years should be independent; however, when the data follow each other at short intervals (daily sampling) they are not independent because they are too close to each other in time. Examining the results of such daily sampling results in a time series is received where each datum cannot be interchanged with another. In many cases, because of environmental and man-made impacts, the samples cannot be described with the same distribution. In conclusion, the sampling of a certain parameter should be as frequent as needed for the sample to include each important property of the aggregate. If all of these requirements are fulfilled the data set could allow the estimation of the expected value.

The final question is, what should the sampling frequency of a certain process be, so that the aims of the study could be achieved and estimates could be given regarding future events?

Viewed from this perspective, it is obvious that higher the parameter's variability within h (time or space) distance, the more frequent the sampling should be. Many functions are known that are able to describe the variability of a parameter in h (time of space) distance. In this case, the basic function of spatial statistics, the variogram was used, in determining the optimal sampling frequency (Füst, 1997; Márkus et al., 1999; Dryden, 2004) during system calibration (Füst and Geiger, 2010, 2011).

### 2. Materials and methods

# 2.1 Data-series acquired from the Kis-Balaton Water Protection System (KBWPS)

In the course of this research, the Total phosphorous (mg l<sup>-1</sup>) parameter of the KBWPS's Z11 (Balatonhídvég) sampling location was examined for the time interval 01.01.1988-31.12.2006. The data was received from the laboratory of the West Transdanubian Water Authority's Kis-Balaton Department, where daily sampling was conducted following the water authority's national code of practice, and analyzed in the same laboratory during the investigated time period (Kovács et al., 2010).

## 2.2 The variogram

Three functions are used in geostatistics for describing the spatial or the temporal correlation of observations: these are the correlogram, the covariance and the semivariogram. The variogram and the semivariogram originated from the variogram can be described mathematically as follows (Füst, 2004; Molnár and Füst, 2002; Molnár et al., 2010). Let Z(x) and Z(x+h) be the values of two sampled parameters in *h* distance from each other. Distance *h* can be measured in time or space. The variance of the difference of values Z(x) and Z(x+h) is  $D^2[Z(x)-Z(x+h)]=D^2[Z(x)]+D^2[Z(x+h)]-2COV[Z(x),Z(x+h)]$ . In case of samples taken from the same population we could assume that  $D^2[Z(x)]=D^2[Z(x+h)]$ so  $D^2[Z(x)-Z(x+h)]=2D^2[Z(x)]-2COV[Z(x),Z(x+h)]=2\gamma(h)$ . Function  $2\gamma(h)$  is called the parameter's variogram, while  $\gamma(h)$  is its semivariogram. If we introduce the simplified notation  $D^2[Z(x)]=D^2(x)$ , then  $\gamma(h)=D^2(x)-g(h)$ . If the sample size is N in case of discrete samples from a nominally

distributed population, the semivariogram could be calculated with the

Matheron algorithm (Matheron, 1965):  $\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z(x_i) - Z(x_{i+h})]^2$ 

In the case of non-nominal distribution, there are different transformations that can ensure it. Many publications in the geostatistical literature however refer to this as an unimportant distribution type (Clark, 1979., Cressie, 1993).

In practice  $Z(x_i) \ge 0$  (i = 1, 2, ..., n)  $\sigma^2[Z(x)] \ge g(h) \ge 0$ , so theoretically the semivariogram can only take values from the  $0 \le \gamma(h) \le \sigma^2[Z(x)]$  range. The most important properties of the function are as follows (*Fig. 1*):





Properties of the variogram, with the curve (with the circles) indicating the empiric and the other the theoretical semivariogram (based on Füst, 1997)

Continuity can be seen from the  $\gamma^{(h)}$  function's accession. If the function does not start from the origin of the coordinates, there must have been drastic changes in the parameters' processes. This is called the

**nugget effect**. It is the height of the jump  $(C_0 \ge 0)$  of the variogram at the discontinuity at the origin.

If the semivariogram does not have an uprising part, the empirical semivariogram's points (the circles on the graph) will align along an h line parallel to the abscissa. If this occurs, the continuity has fully ceased.

Sill  $(C + C_0)$  is the limit of the variogram tending to infinity lag distances. However *C* itself is the reduced sill.

**Range** is the distance in which the difference of the semivariogram from the sill becomes negligible. In models with a fixed sill, it is the distance at which this is first reached; for models with an asymptotic sill, it is conventionally taken to be the distance when the semi variance first reaches 95% of the sill.

If the semivariogram stabilizes at  $h \to \infty$  after a fast ascent, then the parameter is stationary. However if  $\gamma^{(h)}$  is an increasing function (if  $h \to \infty$  then  $\gamma^{(h)} \to \infty$ ), the parameter is non-stationary.

The empirical semivariograms can be approximated with many theoretical functions. However, discussing these is not an aim of the study (Füst, 2004; Molnár és Füst, 2002; Molnár et al., 2010).

Our estimation of sampling frequency is based on the fact, that samples outside the range (let it be temporal or spatial) are -in practice- independent. In other words, the samples taken outside the range (temporal or spatial) can only describe the vicinity of their environment, and in this way cannot provide scientists with information regarding the processes' deeper structure.

## 3. Example of range estimation in practice on the KBWPS' data series

As described previously the daily sampled total phosphorous (mg  $l^{-1}$ ; TP) parameter of the KBWPS was analyzed using variograms. A part of the original data series can be seen in *Fig.2*, while the empiric semi-variogram of the total time interval on *Fig.3*.





A short period of the original total phosphorous (mg l<sup>-1</sup>) data series, showing the periodical structure of the signal.



*Fig. 3 Empiric (dotted line) and theoretical semivariograms of the total investigated time period (01.01.1988-31.12.2006) of the total phosphorous parameter (mg l<sup>-1</sup>), indicating a 182 day range.* 

The fitted semivariogram is spherical. In this case, the nugget effect is zero. This is important because the nugget effect is the characteristic that describes the margin of error caused by the sampling method or instrument, the parameters' accuracy, and the parameter's change in time or space. The estimated range in *Fig. 3* is 182 days; this concurs with the facts stated in the Nyquist–Shannon sampling theorem for processes describable with annual periodicity (Shannon, 1998). If we evaluate the results from a sampling perspective, it becomes clear that a sampling frequency of 182 days can only give us information about annual processes. (Here we must state that in most cases of the KBWPS's parameters the 182 day range was found, which indicates annual periodicity). If there is a need to observe processes describable only with periods smaller than one year, the sampling frequency must be adjusted accordingly to them. Hence, the smallest range acquired from the empiric semivariogram should be used.

If we look at the TP's semivariogram for an interval of 40 days *(Fig.4)* no range can be seen; it is covered by the "special trend" caused by periodicity seen in Fig. 3.



*Fig.4. The total phosphorous' empiric semivariogram for an interval* of 40 lags (days), where no range can be seen.

So the periodicity -as a special trend- must be removed, because we are interested in the more detailed processes within the residual. The most obvious method would be to remove a repeated average function, (generated from the average of the same day of every year (*Fig. 5*) from the realization of the parameter's time series. The variogram fitted on the residuals can be seen in *Fig. 6*.



Annual period generated from the average of the same day of every



The empiric (dotted line) and theoretical semivariograms fitted on the total phosphorous residuals, after the removal of the generated average annual period

According to *Fig. 6* a long period can still be found in the signal. In other words the trend removal was unsuccessful. We must ask ourselves the question why?

The answer can be found on *Fig.* 7, which describes the time elapsed between the peaks of the periods.



### Fig. 7.

Time elapsed (days) between the peaks of the periods.

It is true that the average of these values is 365, but the smallest is 292, and the highest is 422 days. The conclusion is that the period's length is not constant, so the removal of the average period (*Fig. 5*) from the whole time series can only be successful if all of the years consist of 365 day periods. If not, than we are the ones implanting the periodic process into the signal. This is known as the Slutsky effect (Slutsky, 1937).

Taking these facts into consideration, the range (time) examination of a more than one year interval (01.06.1996.-19.08.1997.) seemed the most appropriate. In contrast to the previously discussed trend removal, in this case a polynomial trend was estimated, and because the interval was longer than one year, instead of a quadratic, a polynomial (n=5) trend was removed (*Fig. 8*).



Fig. 8. The x-y scatter plot of the examined period (01.06.1996.-19.08.1997.) with the fitted polynomial (n=5) regression line.

The variogram fitted to the residual can be seen on *Fig. 9*. The method was repeated for many other time periods and similar results were produced. The principal range was to be found at 8-10 day lags. These results were acceptable. The slight distortions are caused by the range's characteristic that it is an estimated aleatory variable, because all of its estimated values depend on the examined interval's data, as in a sample realization.



Fig. 9. Empiric and theoretical (dotted line) semivariograms fitted to the total phosphorous residuals indicating an 8-10 day range after the removal of the polynomial (n=5) trend.

To ensure reliability in future sampling, the employment of a lower frequency, than the one in the results is advised. In regard to the TP on sampling location Z11 a frequency of 7 days is suggested. A minor break can be seen in *Fig. 9* at 3 day lag. We assumed that there are significant changes in the parameters processes after three days, and that a second 3 day range may be determined as well. However further investigations (using more effective trend removal methods) should be carried out to answer this question.

### 4. Summary

With the method described above we were able to determine exactly the most appropriate sampling frequency for the TP parameter of the KBWPS's Z11 sampling location. Fortunately this suggested sampling frequency is equal to the one already applied (7 days).

Knowledge of the ideal sampling frequency is a key question in monitoring practice. If it is not chosen wisely, the data obtained is useless, or can only be used for certain analyses. This is what science nowadays cannot afford, because first of all data is valuable whether expressed in terms of goodwill or money, and secondly data not representing the area it was received from can lead to unprofessional interventions and, as a result, further damage to the environment.

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# EFFECT OF DIFFERENT HARVESTING METHODS ON THE GERMINATION AND VIGOUR OF HYBRID MAIZE (ZEA MAYS L.) SEEDS

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#### Summary

The germination ability and vigour of the seeds of eight Pioneer hybrid maize varieties were examined in 2009. On the experimental plot the combine shelled the harvested ears, while on the control plot the ears were harvested intact. After preliminary cleaning, the seeds from the shelled and control groups were germinated. Some of the seeds from each group were divided into four fractions and analysed separately. The germination was carried out according to the International Seed Testing Association (ISTA) Rules (2009), in four replications of 100 seeds, between moist filter papers in controlled climate chambers in four seed testing laboratories. The germination percentage satisfied the standard criterion (above 90 percent) in both the shelled and control groups, but the germination rate was lower for the shelled group, while the number of abnormal seedlings was greater than in the control group. The effect of harvesting intact ears on the seed quality was evident for two hybrids (PR35Y65,  $LSD_{0.1\%}$ ; PR39R20,  $LSD_{5\%}$ ). In the case of the various seed fractions, the lowest germination percentage was obtained for the medium-sized round fraction and the highest for the large flat fraction. The superiority of the control (intact ears) group was not manifested in all the fractions and no significant difference was found between the two treatments. The results obtained for the biological value of the seed were surprising. For one hybrid ears harvested intact exhibited the same superiority as recorded for the germination percentage, while for three hybrids the vigour proved to be better in the shelled group. For two hybrids little or no difference in vigour was observed between the shelled and control groups.

Keywords: hybrid maize, harvesting, germination, vigour, seed testing,

# Összefoglalás

2009. évben összesen 8 Pioneer fajta hibridkukorica vetőmag csírázóképességét és életerejét vizsgáltunk. A vetőmag szaporító táblákon kísérleti táblarészeket jelöltünk ki. A táblarész egyik felét szemesen, a másik részét pedig csövesen takarítottuk be. A hibridek morzsolt és kontroll csoportjait előtisztítva, frakcionálatlanul csíráztattuk, emellett két, véletlenszerűen kiválasztott hibridet 4 frakcióra bontottunk, és frakciónként is vizsgáltunk. A vizsgálatot 4 vetőmagvizsgáló laboratóriumban, ISTA szabvány szerint, klimatizált kamrákban végeztük. Négyszeri ismétlésben, nedves szűrőpapír között, 100-100 szemet, csíráztattunk. A morzsolt és csöves betakarítású vetőmagok csíráztatása során megállapítottuk, hogy összességében valamennyi csírázóképességi eredmény szabványos (90% feletti) volt. A morzsolt betakarítású vetőmag csíraeredményei nem érték el a csövesen betakarítottét, az abnormális csíranövényeknek a számában viszont a kísérleti (szemes) csoport meghaladta a kontrollét (csöves). Két hibrid esetében (PR35Y65 SzD 0,1%; PR39R20 SzD 5%) a csöves betakarítás vetőmag-minőségre gyakorolt pozitív hatása statisztikailag igazolható volt. A frakcionált vetőmagtételek csíráztatása során a legkisebb csírázóképességi mutatót a közepes gömbölyű, a legnagyobbat a nagy lapos frakció érte el. A csöves csoport fölénye nem jelent meg minden frakcióban, a két kezelés között nem találtunk szignifikáns különbséget. A vigorvizsgálati értékek között meglepő eredményeket kaptunk. Az egyik hibridnél a csöves betakarítású vetőmag hozta a csíráztatásban tapasztalható fölényét, míg három hibridnél a morzsolt csoport életképessége bizonyult jobbnak a csöves csoporttal szemben. Két hibrid esetében nem, vagy minimális különbséget tapasztaltunk a kísérleti és a kontroll csoport biológiai értéke között.

### Introduction

Hybrid maize plants are exposed to numerous stress factors in the course of seed production. The best known of these are the effect of unfavourable soil conditions on germination, drought or heat stress during various phases of the vegetation period, and mechanical damage. In addition to quantitative seed losses, there may also be a deterioration in quality due to human error (e.g. grain moisture content chosen incorrectly at harvest, unsuitable drying temperature).

Many authors have investigated or modelled the effects of chilling stress during germination (Loeffler et al., 1985; Nijenstein, 1985; Bruggink et al., 1991; Hope and Maamari, 1994). Odiemah (1991) studied the effects of various environmental factors on the seed of hybrid maize.

Weather conditions do not always make it possible to harvest whole ears from seed production fields rapidly, on a single occasion. If the ears are harvested at low moisture content, seed shedding may result in losses of 5–25% during processing, according to data from Pioneer Hi-Bred "Zártkörűen működő részvénytársaság (Zrt)" (Nagy, 2009), which could be avoided by shelling the ears during harvesting. Mounsey et al. (2002) also reported higher seed shedding losses when harvesting at low moisture content, and recommended combine (shelled) harvesting as an alternative solution, besides the traditional harvesting of whole ears. If ear harvesting is protracted due to wet weather or the lack of machine capacity, the seeds may germinate on the ear, or *Fusarium* species may cause ear and grain rot.

In order to eliminate these stress factors, maize breeders and variety owners have suggested the application of combine harvesting on seed production fields. Investigations on the feasibility of this proposal are now underway with the support of the Central Agricultural Office and the Hungarian Seed Association, with the cooperation of the variety owners.

The aim of the present research was to determine whether the proposed harvesting method caused a deterioration in the biological value of the seed. Quality deterioration was measured in terms of germination ability and vigour. It should be emphasised that the first year of the investigations involved only six hybrids belonging to a single variety owner. It is planned to expand the research to include a larger number of hybrids with a broader genetic background.

The work was aimed at determining whether the germination ability of combine harvested seed reached the level laid down in the official standards and whether it was poorer than that of seed from traditional ear harvesting. The investigations also covered the response of hybrid maize seed from combine harvesting to various stress factors in the course of germination. The effect of stress factors on seed was previously studied by a large number of scientists (Barla-Szabó and Berzy, 1989; Gáspár, 1980; Van de Venter, 1988).
The biological value of seed harvested in optimum condition in the field may be compromised by an incorrect choice of drying conditions during seed processing (Burris, 1975; Loeffler, 1985), depending on the initial grain moisture content (Gáspár, 1980) and the genotype.

In the course of processing in seed plants, the seed is divided into fractions, depending on the hybrid. The biological value of the seed fractions may vary for each genotype and year (Thielebein, 1958; Pásztor, 1962; Germ, 1966; Fiala, 1973; Eisele, 1981; Berzy et al., 1996).

Based on the data reported by Shieh and McDonald (1982), it was aimed to discover whether there was any significant difference between the two treatments in the biological value of the two randomly selected hybrid seed fractions, especially as regards germination ability, which is considered to be the most significant trait.

When estimating germination ability it is important to ensure satisfactory, uniform water supplies in order to avoid hypoxia, a factor that inhibits germination. During evaluation, it must not be forgotten that the results were obtained under optimum conditions in the laboratory, which means, however, that they can be reproduced at any time (Ertseyné, 2004).

To determine how the seed lot performs under unfavourable environmental conditions, it is also necessary to carry out vigour tests. Sub-optimum environmental conditions were imitated using the complex stressing vigour test, a special technique elaborated for the rapid determination of vigour in maize seeds (Barla-Szabó and Berzy, 1989).

After several years of experimentation it may prove necessary to elaborate a new system of field quality control criteria, allowing combine harvesting as well as ear harvesting in the case of seed production.

#### Materials and methods

Seed production fields on which half the maize was to be harvested as whole ears and the other half shelled were designated in September 2009. The experiment was set up on a field scale to facilitate the application of the results in practice. All the fields were planted with Pioneer hybrids. The fields were chosen to ensure an approximately equal quantity of seed from both treatments. Within varieties, harvesting was begun at the same time, at the same grain moisture content, which was below 20% in all cases for both whole ear and combine harvesting. The combine harvesting was carried out using a John Deere 98.80 STS axial flow combine, while the whole ears were harvested with OXBO 8430XP and 8420 XP machinery. The shelled seeds were transported straight to the drying chambers, where they were stacked to a height of 80-90 cm. Composite samples of approx. 20-25 kg per variety were taken using an automatic sampler when the lots were removed from the dryers. Samples (approx. 40 kg) of the maize harvested as whole ears were taken from the loading hoppers, dehusked manually and put into jute bags. These were then placed on the conveyor belt taking the dehusked ears into the drying chambers. Drying and processing were carried out in the Pioneer Hi-Bred ZRt seed plant in Szarvas.

The shelled seeds were dried for 2–6 hours and the whole ears for 3–12 hours. In both cases, moisture extraction involved preliminary drying at 38°C, followed by further drying at a maximum of 42°C. After drying, the whole ears were shelled and all the samples were passed through a 6.5–10.5 mm mesh.

The undressed seeds were then germinated in four seed testing laboratories in Szarvas (Pioneer Hi-Bred ZRt; ISO9001:2000 accredited), Budapest (Central Agricultural Office; ISTA, Hungarian Accreditation Board accredited), Székesfehérvár (Regional Agricultural Office: Hungarian Accreditation Board accredited) and Martonvásár (ARI HAS Seed Testing Laboratory).

It was deemed advisable to divide the seed of two randomly selected hybrids into four fractions (large flat: LF; large round: LR; medium flat: MF; medium round: MR) and to germinate each fraction separately. The germination of the seed fractions was examined in three laboratories (Székesfehérvár, Szarvas, Martonvásár).

All the germination tests were carried out according to the standard, method, with 100 seeds per lot, in four replications, thus ensuring a total of 16 (4 labs) or 12 (3 labs) replications. In all cases the seeds were rolled in three layers of crepe filter paper (Between Paper - Roll), moistened with 1.4–1.7 cm<sup>3</sup>/g water. Illumination was provided for at least 8 hours, the temperature was 30-20°C day/night or a constant 25°C (ISTA method), and the relative humidity was 70%. The seedlings were evaluated on the 6<sup>th</sup>–7<sup>th</sup> day, depending on their state of development, and classified as normal, abnormal or dead. The germination tests will be repeated after 6 and 12 months on undressed seed samples stored under the conditions normal in the seed plant in Szarvas.

In addition to the germination tests, seed vigour (Complex Stressing Vigour Test) was also analysed at ARI HAS, Martonvásár. The germination medium was the same as that used in the germination tests. In the first step 200 seeds were soaked in 0.15% chlorogen at 25°C for 48 h, followed by a further 48 h at 5°C. Both low temperature and hypoxia cause severe stress. Finally, eight lots of 25 seeds were germinated as described above at 25°C with constant illumination for 96 h. The percentage of normal, abnormal and low vigour seedlings and of dead seeds was then calculated. The shoot and root length of the five most vigorous seedlings from each roll were recorded and averaged, and the shoot and root weight of all the seedlings in each roll were determined.

#### Results

The germination percentage of both the shelled and whole ear samples exceeded the minimum (90%) laid down in the standard. For the eight hybrids tested, the seed did not suffer any substantial loss of germination ability after the harvesting and drying methods outlined above. It could be seen from the results of laboratory analysis that although the germination percentage of seed from combine harvesting was slightly lower than that for ear harvesting, the difference was not significant (Table 1.). On the basis of biometric analysis, significant differences in germination ability were found for two (PR35Y65, PR39R20) hybrids. The results suggested that some hybrids have better tolerance of combine harvesting than others. The mean germination percentages for  $4 \times 100$  seeds (not divided into fractions) for each laboratory are presented in Table 1. For two of the hybrids (presented in italics) samples from combine harvesting were only germinated in one laboratory, while kernels from ear harvesting were tested in all four.

Table 1. Effect of combine and ear harvesting on the germination abil-ity of seed from Pioneer maize hybrids (Szarvas, Martonvásár, Székes-fehérvár, Budapest, 2010)

Hybrid	Harvesting method	Székes- fehérvár	Marton- vásár	Szar- vas	Buda- pest	Lab Mean	Signifi- cance
PR39F58	Shelled	95.25	92.00	98.30	94.00	94.89	
PR39F58	Ear	98.00	96.00	97.80	95.50	96.83	NS
PR39R86	Shelled	95.00	94.50	94.90	93.25	94.41	
PR39R86	Ear	98.00	94.00	95.10	98.25	96.34	NS
PR39G83	Shelled	97.25	98.75	97.60	97.00	97.65	
PR39G83	Ear	97.75	97.00	99.50	98.75	98.25	NS
PR38H67	Shelled	93.75	93.00	95.70	93.75	94.05	

PR38H67	Ear	94.50	96.75	97.25	95.00	95.88	NS
PR35Y65	Shelled	95.25	96.25	97.30	92.00	95.20	
PR35Y65	Ear	98.50	100.00	98.75	98.50	98.94	***
PR39R20	Shelled	95.00	96.00	95.80	95.50	95.58	
PR39R20	Ear	97.00	97.25	98.50	97.75	97.63	*
Anasta SV	Shelled			96.80		96.80	
Anasta SV	Ear	95.50	98.25	98.50	97.25	97.38	NS
PR39H32	Shelled			96.20		96.20	
PR39H32	Ear	97.50	97.25	97.50	96.25	97.13	NS

Szfv: Székesfehérvár; Mv: Martonvásár; Bp: Budapest; \*:  $LSD_{5\%} = 2.01$ ; \*\*\*:  $LSD_{0.1\%} = 3.56$ ; NS: non-significant.

The results of germination tests on different seed fractions are shown in Table 2. Studies on the seed fractions of two randomly chosen hybrids indicated that the LF fraction had the greatest germination vigour after both combine and ear harvesting. The germination ability of the LR fraction was only slightly poorer. For one hybrid the control gave considerably better results, while for the other, seeds from combine harvesting had slightly better germination. In the case of the MF fraction the difference between the treatments was not significant for the PR39F58 hybrid, while for PR39R86 no difference was observed. Germination was poorest (though still above the minimum laid down in the standard) for the MR fraction, with better results for both hybrids when whole ears were harvested, so if field observations reveal a large proportion of medium round seeds, the wisdom of combine harvesting is questionable.

In the course of biometric calculations, no significant differences were found for any of the fractions. It should be noted that for these two varieties there was no significant difference between the treatments for unfractionated seed.

Table 2. Effect of fractionation on the germination of seeds from Pioneer hybrids harvested using different methods (Szarvas, Martonvásár, Székesfehérvár, 2010)

Hybrid	Fraction	Harvesting method	Székes- fehérvár	Marton- vásár	Szar- vas	Lab Mean	Signif- icance
PR39F58	MF	Shelled	90.25	93.50	98.75	94.17	
PR39F58	MF	Ear	95.25	95.00	97.00	95.75	NS
PR39F58	MR	Shelled	93.75	86.00	96.50	92.08	
PR39F58	MR	Ear	93.75	95.00	98.00	95.58	NS
PR39F58	LR	Shelled	97.25	93.75	99.00	96.67	
PR39F58	LR	Ear	97.25	93.75	98.00	96.33	NS
PR39F58	LF	Shelled	97.25	95.00	98.25	96.83	
PR39F58	LF	Ear	97.25	94.00	98.00	96.42	NS
PR39R86	MF	Shelled	94.00	92.00	96.25	94.25	
PR39R86	MF	Ear	95.00	95.25	92.50	94.25	NS
PR39R86	MR	Shelled	93.25	87.50	93.75	91.50	
PR39R86	MR	Ear	93.50	91.50	96.00	93.67	NS
PR39R86	LR	Shelled	94.25	93.00	93.50	93.58	
PR39R86	LR	Ear	98.25	98.00	96.25	97.50	NS
PR39R86	LF	Shelled	97.25	95.50	93.00	95.25	
PR39R86	LF	Ear	99.25	99.00	97.50	98.58	NS

Szfv: Székesfehérvár; Mv: Martonvásár;

 $LSD_{5\%} = 4.25$ ; NS = non-significant

In all the seedling examinations, a larger number of abnormal seedlings were found in lots from combine harvesting, though the difference was only significant in a few cases. This suggests that a slightly larger number of kernels were damaged during mechanical shelling at harvest. It is also worth noting that the proportion of abnormal seed-lings was higher for the round fractions and lower for the flat fractions (Table 3).

*Table 3.* Effect of combine or ear harvesting on the abnormal seedlings of the seed of Pioneer maize hybrids (Szarvas, Martonvásár, Székesfehérvár, Budapest, 2010)

	Abnormal seedlings (%)				
II-douid	Shelled	Ear			
Hybrid	Lab mean	Lab mean			
PR39F58	2.64	1.34			
PR39R86	3.26	2.31			
PR39H32	3.00	1.63			
PR39G83	1.94	1.44			
PR38H67	3.55	2.94			
PR35Y65	3.44***	0.75			
PR39R20	2.66	1.31			
Anasta SV	2.40	1.25			
PR39F58 MF	2.00	1.17			
PR39F58 MR	4.42**	1.58			
PR39F58 LR	2.17	2.00			
PR39F58 LF	1.75	1.67			
PR39R86 MF	3.00	2.00			
PR39R86 MR	4.25	3.25			
PR39R86 LR	4.83**	2.33			
PR39R86 LF	2.92*	0.92			

Unfractionated: \*\*\*LSD<sub>0.1%</sub> = 2.43. Fractionated: \*\*\*LSD<sub>0.1%</sub> = 2.88; \*\*LSD<sub>1%</sub> = 2.2; \*LSD<sub>5%</sub> = 1.65.

Surprising results were obtained in the vigour tests (Table 4). In the case of the PR39F58 hybrid, the superiority exhibited for germination ability by seed from ear harvesting was confirmed, and for two fractions the vigour was significantly better in terms of both root and shoot weight. For the LR fraction, however, seed from combine harvesting exhibited greater vigour, though the difference was not significant. In the case of the PR39R86 hybrid better vigour and higher root and shoot weights were recorded after combine harvesting for three of the fractions (LF, MF, MR), for two of which the differences were significant. For hybrids where the seed was not fractionated, combine harvesting resulted in seeds with better vigour compared with the whole ear control for PR38H67 and PR39G83, while seed from ear harvesting was slightly more vigorous in the case of PR35Y65. No difference was observed for PR39R20.

### Table 4.

Effect of combine or ear harvesting on shoot weight (SW) and root weight (RW) of the seed of Pioneer maize hybrids (Martonvásár, 2010)

Hybrid	Fraction	Harvesting method	SW g/25 plants	RW g/25 plants
PR39F58	LF	Shelled	4.38	3.01
PR39F58	LF	Ear	6.3**	4.8***
PR39F58	MF	Shelled	3.76	2.51
PR39F58	MF	Ear	lg	lg
PR39F58	MR	Shelled	3.17	2.35
PR39F58	MR	Ear	4.82**	4.65***
PR39F58	LR	Shelled	6.37	4.5
PR39F58	LR	Ear	6.58	5.1
PR39R86	LF	Shelled	5.61*	2.9*
PR39R86	LF	Ear	4.35	2.28
PR39R86	MF	Shelled	3.9	2.25
PR39R86	MF	Ear	3.46	1.57
PR39R86	MR	Shelled	3.37**	2.31**
PR39R86	MR	Ear	2.27	1.34
PR39R86	LR	Shelled	5.76	3.06
PR39R86	LR	Ear	6.06	4.06**
			*SzD5%=1.21 **SzD1%=1.44 lg= lack of grain in the fraction	**SzD5%=0.62 **SzD1%=0.83 ***SzD0,1%=1,37 lg= lack of grain in the fraction



Effect of different harvesting methods on the germination and vigour of hybrid maize (Zea mays L.) seeds

\*SzD5%=8,32 (PR39F58 MR Combine, PR39R86 LF Combine) \*\*\*SzD0.1%=13.96 (PR39R86 MR Combine)

# Fig. 1. Effect of combine or ear harvesting on the vigour of the seed of Pioneer maize hybrids (Martonvásár, 2010)

These contradictory data suggest that the genotypes may respond differently to harvesting methods and to the artificial stress factors exerted during processing. In the near future the present work will be expanded to include a larger number of hybrids, and the seed lots will be tested in field performance trials.

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# EXAMINATION OF CONCENTRATED ENVIRONMENTAL ENDURANCES DURING THE CLEANING OF PLANT PROTECTION SPRAYERS

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#### Abstract

Unsatisfactory technical condition or insufficient adjusting of plant protection machines may cause considerable environmental pollution. During their maintenance and cleaning (mechanical errors) more or less concentrated plant protecting chemicals may get into living water and from these even into drink water. Naturally, during spraying the wind may drift the chemicals into natural waters. In 1999 the International Standardization Organization has established a working group to elaborate rules to decrease environment pollution by efficient cleaning of sprayers. As a result of their work, in March 2004 the ISO/DIS 22368 standard plan came into force, which describes in three chapters the inner cleaning of sprayers as well as the investigation of effectiveness of cleaning devices for outer and inner side of pesticide tank of sprayers. Our aim was to investigate the effectiveness of the cleaning devices and to make proposals to improve directions of the standard. **Key words:** plant protection, maintenance and cleaning, international standards, measuring methods, effectiveness.

# KONCENTRÁLT KÖRNYEZETI TERHELÉSEK ÉS ELJÁRÁSTECHNIKAI ÖSSZEFÜGGÉSEK VIZSGÁLATA NÖVÉNYVÉDŐGÉPEK TISZTÍTÁSA SORÁN Sándor Tamás, Pályi Béla, Németh Kornél

## Összefoglaló

A növényvédelmi gépek rossz műszaki állapota vagy helytelen beállítása jelentős környezeti terhelés forrása lehet. Karbantartás és tisztítás során (mechanikai hibák) a vegyszerek nagy koncentrációban, közvetlenül juthatnak az élővizekbe és így az ivóvízkészletekbe egyaránt. Természetesen permetezéskor a szél hatására (elsodródás) szintén eljuthat a permetszer az élővizekbe. Ezen károk csökkentése érdekében a Nemzetközi Szabványügyi Szervezet (ISO) 1999-ben egy munkacsoportot állított fel. Munkájuk eredményeképp 2004 márciusában megjelent a három fejezetből álló ISO/DIS 22368 szabvány tervezet, mely leírja a permetezőgép teljes belső tisztítását, a permetlétartály belső és a külső tisztítóberendezések hatékonyságának vizsgálatát. Kutatásunk e normák alapján vizsgálja, hogy mennyire hatékonyak e tisztítóberendezések, illetve ajánlásokat tesz ezen iránymutatások javítására.

**Kulcsszavak:** növényvédelem, karbantartás és tisztítás, nemzetközi szabvány, mérési módszerek, hatékonyság.

### Introduction

The plant protection is an indispensable part of the agricultural production, since it influences the profitability (high prices of pesticides, damages by insects, fungi, microorganisms, weeds, etc.). Beside this we can often hear in the media news about vegetable- and fruit lots having chemical residues above the sanitary regulation levels. Higher residue levels may be due to unsatisfactory technical state or lack of maintenance and of thorough cleaning of the sprayers. Different types of pesticides are used in plant protection, like insecticides, fungicides and herbicides, which can be applied at different plant types and in many cases could not be mixed, consequently cleaning machines between sprayings is always necessary. Also the subsidies by the European Union are subjects to conditions of environmentally-friendly farming and proper practice, which means that farmers have to take care of cleaning (Sándor, 2007).

Everywhere in Europe efforts have been made to decrease pesticide residue problems. As a result of this the regulation DIN/EN 12761 (2001) came into force, which oblige sprayer producers to provide the new sprayers with inner cleaning devices, rinsing tank and to create possibilities of attaching outer cleaning unit, by which quick and water saving cleaning can be made (Csizmazia, 2006). Beside this the European Plant Protection Association created the TOPPS-program (2005) to which the "Life" program of the European Committee joined. They organize demonstrations, create and distribute brochures for further training of farmers to avoid point source pollutions (EU Commission, 2008). To achieve these goals, the International Organization for Standardization (ISO) has established a working group in 1999 with the leading of Institute Julius Kühn, Application-technics in Plant Protection, to elaborate measuring methods for the evaluation of efficiency of cleaning devices. Experts of Belgium, Denmark, England, France, Italy and Sweden are members of its working group. Their work resulted in the Standard ISO/DIS 22368, with has three chapters. The first part deals with the full inside cleaning of machines, the second with the outer cleaning, and the third with the inner rinsing of the liquid tank. Nowadays the group deals with the determination of output criteria (Herbst and Ganzelmeier, 2002; Wehmann, 2008).

Our department joined these research works in 2005, helping the measurements of the inner cleanings. Nowadays our research is focused on the third chapter of the theme, we investigate the efficiency of tank rinsing nozzles, and we make proposals to increase the efficiency of measuring methods, as well as to decrease cost and time demand of the cleaning.

## Materials and Methods

The measuring of effectiveness of cleaning nozzles has been made according to the mentioned ISO/DIS 22368 regulation of the third chapter. There can only be used sprayers, which do not have direct liquid injection systems.

We investigated in Hungary available cleaning nozzles (Table 1, Photo 1) with the described pressures.

8						
Pagallar	Manufac-	Part number /	Mounting	Capacity (litre/min)		
Kesellei	turer	Туре	connection	2 bar	3 bar	
Farmcenter Kft.	Teejet	(B)27500E-8TEF	1/2	26.00	32.00	
AXIÁL Kft.	Lechler	500.191	1/2	20.00	24.00	
Farmgép Kft.	Polmac	63408399	1/2	20.10	24.00	
	ARAG	510.120	1/2	47.00	57.00	

Table 1. The investigated cleaning nozzles



*Photo 1 The nozzles used for the measuring (Teejet, Lechler, Polmac, ARAG)* 

For test liquid is a 1 % solution diluted copper-oxy-chloride product (Funguran, OB21, Cupravit) in use, containing 45 % copper. Since in Hungary no product of this concentration is available, we made an appropriate dilution from a product (ASTRA) containing 50 % copper. (We measured its sedimentation and its concentration to be the same quality as for the testing described product). This is a rather instable suspension, sediments quickly; therefore care had to be taken during the stirring and sampling. The measuring started, when the sprayer tank was filled with the comparison liquid up to its maximum capacity. During this the mixer was in action (Photo 2).



Photo 2 Testing liquid in the tank



Photo 3 Simulation of spraying

Then, as a simulation of spraying the tank was depleted including the technical rests in the sprayer (Photo 3). Following this, all part of the sprayer was washed out except for the tank and the mixer device.





Photo 4 Sedimented pesticide

Fig. 1 Location of the nozzle in the tank

This was followed by a 24 h waiting. During this the pesticide sedimented, dried out, it became easier to measure the effectiveness of the different cleaning devices and nozzles (Photo 4). After drying the cleaning process was performed according to the manual of the machine and nozzles. It has to be stressed out, that nozzles were located in the same height in the tank (Fig.1). The rinsing water was collected and samplings were taken (Photo 5). At the end a high pressure washer was used to remove the sediment rests, its water amount was also measured and samplings were taken also from this.



Photo 5 Collection of rinsing water



Photo 6 The VARIAN SpectrAA 300

The copper concentration of the two rinsing water were measured by a VARIAN SpectrAA 300 atomic spectrophotometer (Photo 6), for which the samples were treated with Titriplex III (Selecton B2) reagent, which made the copper content measurable.

The efficiency of cleaning nozzles was counted by the equation given in the standard plan as follows:

$$M = \frac{C_{AM} \times V_A}{C_{AM} \times V_A + C_{BM} \times V_B} \times 100$$
(1)

where

- M amount of copper washed out by the cleaning device in percentage of the full amount of copper remained in the tank, in %.
- $C_{AM}$  average copper concentration of rinsing water washed out by the cleaning device, in mg/litre.
- $V_A$  water amount used by the cleaning device in litre.
- $C_{BM}$  average copper concentration of cleaning water used by the high pressure washer in mg/litre.
- $V_{\rm B}$  water amount used by the washer in litre.

(ISO/DIS 22368-3:2004)

## Results

The results are summarized in Figure 2. It can be seen, that in case of three nozzle types the higher pressure caused 15 - 18 % decrease in the cleaning efficiency.





Cleaning efficiency of nozzles in relation to the operating pressure

This can be explained by the fact, that the higher pressure changed the angle of the water jet (Photo 7), therefore the water was directed more to the sides of the tank, instead of its bottom, where the copper easier sediments.



Photo 7 ARAG nozzle during operation



Photo 8 Polmac nozzle during operation

One of the nozzles however showed 15 % increase at the higher pressure. This is because at the higher pressure the bore-holes and the rotating part of the nozzle break the water jet better (Photo 8), therefore the water arrives in the same angle to the tank sides and to the bottom.

We have also investigated the improvement possibility of the standard plan, and possibility of its cost reduction. The cost reduction has two ways: reduction of water amount and of pesticide amount during the investigation. We could achieve cost reduction by the way, that we reused the testing liquid with repeated adjusting its pesticide concentration to be the same as it was in the first measuring. By this we could reduce water amount during the 8 measuring by 77 %, and of pesticide amount by 37 % (Table 2).

8	F	5
Denomination	Water usage (litre)	Pesticide usage (kg)
Without reuse of testing liquid	9 137.5	72
With reuse	2 137.5	45
Spared	7000	27
Saved in %	76.61	37.50
Prices (in HUF/m <sup>3</sup> ; HUF/kg)	948	2 000
Costs without reuse (HUF)	8 662	144 000
Costs with reuse (HUF)	2 026	90 000
Summary saved (HUF)	6 636	54 000

Table 2

Summarizing table of water and pesticide usage and their costs

Considerable sum of money could be spared with reusing of testing liquid, especially on the pesticide side, which takes 54 000 HUF. This way of measuring is better also regarding the environmental pollution, since much less liquid remains after testing, which later on has to be destroyed. This liquid may not be sprayed onto plant cultures or taken onto the fields, it is considered as dangerous waste, and has to be transported to special destruction.

#### Conclusion

The ISO/DIS standard plan gives good directions to the farmers and sprayer producers in all respect of sprayer maintenance and cleaning to achieve a cleaner environment and to get residue-free, healthier foods.

The examination of nozzle efficacy showed, that in the case of three different nozzles the increased water pressure caused 15 - 18 % efficiency decrease. Our aim was the comparison by the same settings (operating pressure, nozzle height determination in the tank), so these reductions can be improved with appropriate height adjustment.

Our methodological changes in the investigations (reusing of the test liquid) contribute to a better, quicker and cheaper measurements, which are also friendlier to the environment.

Our investigations will be continued to improve the standard plan and new investigations are planned in the field of the second chapter of the standard to determine outer pollution of the sprayer with pesticides and of its cleaning.

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# 'HERITAGE AS A RESOURCE' LEADER+ RESULTS OF GÖCSEJ-MIDDLE ZALA LAG

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#### Abstract

The European Union's LEADER Programme is held to be one of the most successful community initiatives, it integrates agriculture and rural development. In the program period 2004 – 2006 in Hungary, LEADER was a measure of the National Development Plan's Operative Program for Agriculture and Rural Development. The selected 70 LAGs could claim for EUR 340.000 or 377.000 subsidy. The Göcsej area's LAG is exceptionally important for its development program called 'Heritage as a resource for the welfare of Göcsej-Middle-Zala'. In this paper we are researching the implementation of the program in the Göcsej area, focusing on the involvement of the sectors and results of the supported measures. According to amount of support the program achieved its main objective, the preservation of the area's heritage proved to be an emphatic measure. The different results of the sectors and the lack of real local product development highlights the pilot- character of the program. The extension of the trade-mark system to local products is the future challenge of the next program-period.

Keywords: Leader, rural development, Göcsej, project results

## Összefoglalás

Az Európai Unió által kezdeményezett programok, ún. közösségi kezdeményezések közé tartozó LEADER, a helyi szinten működő aktív partnerség által kidolgozott integrált vidékfejlesztési programokat támogatja. Magyarországon az AVOP LEADER+ intézkedés keretében a helyi akciócsoportok 340.000 vagy 377.000 euró támogatásra pályázhattak a helyi vidékfejlesztési tervük megvalósítása érdekében. A pályázaton nyertes 70 akciócsoport közül a Göcsej-Közép Zala Helyi Akciócsoport kiemelkedik az örökségét, mint értéket megőrző programjával. Jelen publikációban a Göcsej térségi program megvalósulását az egyes szektorok bevonásának mértéke és a támogatott intézkedések eredményei alapján vizsgáljuk. A program elérte fő célját, hiszen a térség örökségvédelme nagy hangsúlyt kapott a ráfordított források tekintetében. Az egyes szektorok eltérő eredményei, a térség kitörési pontját jelentő helyi termék fejlesztés kihasználatlansága azonban a program kísérleti jellegét mutatja. A védjegyrendszer kiterjesztése a helyi termékek széles körére a következő programozási időszak nagy kihívása.

Kulcsszavak: Leader, vidékfejlesztés, Göcsej, pályázati eredmények

### Introduction

In the late eighties and early nineties, the diversity of rural areas and landscape, rich local identities and a quality protected environment were acknowledged to be the major assets of a 'European agricultural and rural model'. Inspite of this background, the LEADER Initiative proved to be an ideal instrument for testing the new opportunities that opened up for the countryside.

The European Union's Common Agricultural Policy focuses on the rural development instead of market regulation. Its reason is the fact that the technological development of the agricultural production has caused a lower employment in farming and animal husbandry. The policy prefers environmental management, local marketing of local products and village tourism as employment possibilities in rural areas. (*Ministry of Agriculture and Rural Development, 2004.*)

In accordance with forming the European agricultural model, the main goal of the Leader Community Initiative is to inspire and facilitate people living in rural areas to figure out the long- term possibilities of their own areas in community, and to develop their area in a sustainable way through integrated and innovative strategies. The key areas are: expansion of natural and cultural facilities, strengthening the economical environment, strengthening the self-organising ability of communities and motivating new partnerships. (*Dingenen, 2004.*)

The European Union's LEADER Program is held to be one of the most successful community initiatives. It integrates agriculture and rural development. (*Ray C., 2000*) The substance of the program is the following a Local Action Group (LAG) with local members prepares a rural development strategy for its region. This strategy will be subvented by the European Union, but the Local Action Group is responsible for the implementation and decides on the beneficiaries. The partnership network is very wide because members of the Local action Group come from the sectors of self-governments, agricultural enterprises, micro-enterprises, civil organisations and individuals. The decision-making process is delegated to local organisations and all local beneficiary-projects have to promote the process of reaching the LAG's strategic aim, have to be innovative and bring positive effects to the area. *(European Commission, 2006.)* 

In the programme period 2004–2006 in Hungary, LEADER was a measure of the National Development Plan's Operative Program for Agriculture and Rural Development. The Operative Program's financial frame allocated 4,5% – EUR 18,434 million – for the Leader Initiative. (*Ministry of Agriculture and Rural Development, 2006.*)

In the West-Pannon Region of Hungary, 10 LAGs obtained EUR 3,472 million subsidy from the LEADER+ 's fund. *(Ministry of Agriculture and Rural Development, 2008.)* One of them, the Göcsej area's LAG is exceptionally important for its programme called 'Heritage as a resource for the welfare of Göcsej-Middle-Zala'. We did research in the Göcsej area focusing on the involvement of the sectors and results of the supported measures.

#### Materials and methods

As rural development manager in the Göcsej-area one of the authors took part in the program-writing supported by the Ministry of Agriculture and Rural Development. We prepared a complex documentary (situation analysis, strategic and operative plan) of the area focusing on the possibilities of the countryside in agriculture and the main complementary activities. We collected the main issues and development ideas, strategic plans for this program on many forums such as villagesessions, local conferences, and by visiting local entrepreneurs, local authorities and civil organisations. We took part in the launch of the Leader program in Hungary as an employee of the Ministry of Agriculture and Rural Development's West-Pannon Regional Rural Development Office in 2002. This office had the responsibility to manage the Ministry's Leader pilot program in the West-Pannon Region. 14 Local Action Groups had the possibility in Hungary to prepare a local rural development plan, according to Leader principles, which was supported by the Ministry. We organised trainings for the members of the Local Action Groups to become familiar with the Leader-methodology and principles. During the implementation of the pilot program, the members shared their experiences continually and we prepared innovative local action plans according to the new methodology of the European Union's Leader Program.

Besides the personal experience in planning we applied first of all the empirical method, resource analysis and interviews as part of the research through the following elements in detail:

- Overview and critical analysis of literature, summary of problems, searching for impacts, results and deficits, summarizing and preparing a logical system of discussions and experiences
- Analysis of the European Union's and Hungary's rural development and Leader documentary, especially the implementation opportunities of the Hungarian Leader program, the so called 'laboratory role' of the Hungarian Leader pilot project, and the preparation of an integrated, innovative and sustainable development controlled method and process
- Consultation with Hungarian experts and participation in foreign study tours and attendance at the union's conferences
- Getting familiar with local experiences, evaluation of the experiences of Göcsej area's Leader LAG
- Data registration in the Göcsej-area, personal interview and consultation with the Local Managing Authority of Göcsej LAG on LEADER+

## Results

## 'Heritage as a resource for the welfare of Göcsej-Middle-Zala'

The Göcsej area as a beneficiary region of the Ministry's pilot project had already succeeded in implementing a Leader–preparatory process. In 2006, the Middle-Zala area joined the Göcsej area and their LAG obtained EUR 340.000 for its LEADER+ program called 'Heritage as a resource for the welfare of Göcsej-Middle-Zala'. The National Development Plan's Operative Program for Agriculture and Rural Development supported 70 LAGs in Hungary altogether.On the map of Figure 1 the green line shows the boundaries of Göcsej-Middle Zala LAG's area.



Figure 1. Göcsej-Middle Zala LAG's area (Göcsej-Middle Zala LAG, 2005)

The LAG was established in 2005, involving 48 settlements. Its local managing authority is the Becsvölgye-based Göcsej Regional Association and its leading organisation is the Gellénháza-based Rural Development Association of Gellénháza and its Region. 71 members joined the LAG: 48 local governments, 4 regional associations, 6 civil organisations, 4 individuals and 9 enterprises.

The measures of the local rural development plan are the following:

- 1. Traditional local product-based, integrated and ecosocial market economy
- 2. Development of the touristic system of Göcsej Middle-Zala
- 3. Renewable energy and technologies, planning, model programs
- 4. Programmes for environment and nature protection
- 5. Development of human resources, implementation of regional employment projects
- 6. Innovative community initiatives, strengthening the identity of the population
- 7. Regional communication and information-network
- 8. Improvement of life-quality and living-conditions in rural areas, keeping traditions alive *(Göcsej-Middle Zala LAG*, 2005)

## **Programme results**

The total cost of the development projects financed by the local rural development plan was EUR 634.000, of which the Leader+ support was EUR 340.000 claimed by the LAG. The LAG has used EUR 51.000 for its operational and administrative work, so the beneficiaries can claim for EUR 289.000.

Altogether 86 project proposals were handed in for the project call of the LAG claiming for a support of EUR 398.000. The project proposals were submitted to the Decision-making Comittee after the completion of documents and formal supervision of the local managing authority. The decision was the following: 22 proposals were rejected and 64 proposals won a support of EUR 290.000. During the implementation period, another project was rejected, so EUR 603 were not used from the amount of EUR 340.000. *(Interview with the Leader of the Local Managing Authority, 2007.)* 

## **Implementation of Leader+ measures**

If the amount of the claims was lower than the budget of a measure, it was possible to rearrange the budget of single measures with the approval of the Managing Authority of the Leader Program. Table 1 shows the rearrangements of Göcsej- Middle-Zala LAG.

Nr. of mea- sure	Name of measure	Number of bene- ficiaries (pcs)	Budget (EUR)	Paid support (EUR)	Sum of residue (EUR)	Rearrange- ments for the mea- sure (EUR)
1.	Local products	3	67 925	15 840	52 085	-
2.	Touristic devel- opment	9	62 264	46 704	15 560	-
3.	Renewable en- ergy	1	9 434	9 434	-	-
4.	Protection of nature and envi- ronment	5	26 415	21 793	4 622	-
5.	Development of human resources	6	11 321	16 792	-	5 471
6.	Community ini- tiatives	12	28 302	29 560	-	1 258
7.	Regional infor- mation-network	2	7 547	7 467	80	-
8.	Traditions, life- quality	26	75 472	142 452	-	66 980
	Total	64	288 680	290 042	72 347	73 709

Table 1. Budget and rearrangement of Leader+ measures of Göcsej-Middle Zala LAG, 2007

The difference between the funds of budget and the paid support in the Table 1 is EUR 1.362, which was rearranged from the operational costs of the local managing authority for the beneficiaries. The same difference for the same reason is indicated between the last two columns.

The rearrangements are significant in the case of 3 popular measures, which were additionaly financed from the measures 'local products', 'touristic development', 'environment-protection' and 'information-network'. The largest amount – 91% of all rearrangements – was used for the measure 'Development of life quality'. In this measure, most of the financial sources were appropriated for the renovation of common buildings of the village community and regional traditional programs of villages. Most of the project proposals also claimed for support this objective. 7% of the total residue was used for the measure 'Development of Human Resources', and 2% for the measure 'Community Initiatives'. The pie- chart on Figure 2 shows the the estimated share of the measures of the Göcsej-Middle Zala LAG's Leader Program.



#### Estimated share of the measures in Leader+ support

Figure 2. Estimated share of the measures, 2007

Figure 2 shows that the LAG has equally preferred tourism, the improvement of life quality and local products in the planning phase. Figure 3 indicates that the actual claim of the project proposals varies from the estimated share of the measures. The actual share of measures indicates that there was a reallocation of sources for the measure 'Tradition, life-quality' which shares 49% in the amount of subsidy.



#### Actual share of the measures in Leader+ support

Figure 3. Actual share of the measures, 2007

'Tourism' shares 4% lower than estimated in the planning-phase, but the biggest loser of the measures is 'Local products', which shares only 5% from the amount of subsidies, which is 19% lower than the estimated 24%. Actually, the most significant changes are the decreased support for local products and the increased support for village improvement.

The 19% decrease is not a succesful part of the program because the traditional local products have outstanding role in the Göcsej-region. Many studies, for example Kulcsár L. (1998), highlight the locally produced products as a key-source of income and breakout-point of the Göcsej-region. Even so, only 3 project proposals were supported by the LAG with this objective. One of them - as a primary producer - got support for building a beekeeper-house, and two projects resulted in the development of local products indirectly with facilitating the marketing and trade-mark system of local products. Based on our research at the Local Managing Authority, we explored that no project-support claim with this objective was rejected by the Decision-making Committee. That means that no more than only 3 project proposals arrived altogether from local enterpreneurs and primary producers to the Managing Authority for developing local products.

### **Involvement of different sectors**

It is also interesting to examine the share of the beneficiaries of Leader+ program in the Göcsej-area according to sectors. Table 2 shows the number of beneficiaries per sector.

Sector	Pieces
Civil organisation	25
Enterprise	5
Association	10
Institute	1
Local government	23
Total	64

Table 2. Number of beneficiary projects according to sectors, 2007

In our opinion, to see a clear result, it is important to group the sectors according to owners and final beneficiaries of the projects. The members of the Associations on the final-beneficiary list are exclusively local governments, in addition the financial operators and decision –makers of the Institutes on the list are also local governments. Consequently the final beneficiaries of both categories are local governments. Table 3 demontrates the number of final beneficiary- projects in the proper grouping of sectors.

Table 3. The number of final beneficiary- projects in proper	r grouping
of sectors, 2007	

Sector	Number of projects (pcs)
Civil organisation	25
Enterprise	5
Local government	34
Total	64

The percentage division indicates more visible results. Figure 4 demonstrates the fact that the local governmental sector won 53% of the projects, but enterprises won only 8 %.

Share of Leader+ beneficiary-projects according to sectors



Figure 4. Share of beneficiary projects according to sectors, 2007

The share of financial support of beneficiaries according to sectors shows the results in Table 4.

Table 4. The share of financial support of beneficiaries according to sectors. 2007

Sectors	Amount of Leader+ support (EUR)			
Civil organisation	103 816			
Enterprise	22 670			
Local government	163 555			
Total	290 041			
The percentage division of financial support of beneficiaries according to sectors is presented in Figure 5. It shows that 56 % of all financial support is obtained by the local governmental sector but enterprises managed to acquire only 8 % of it.

## Figure 5. Share of support of beneficiaries according to sectors, 2007.



The data collected indicate the following results:

1. The local governmental sector is the biggest 'winner' of the Leader+ program in the Göcsej-Middle Zala LAG's area. 53% of all beneficiary projects and 56% of all financial supports are allocated to this sector.

2. Civil organisations are very active members of the area. 39% of all beneficiary projects and 36% of financial support was attracted to this sector.

3. The sector of enterprises achieved lower level of participation and activity than estimated in the planning phase. No more than 8% of all beneficiary projects and financial support is invested into this sector.

4. There was a reallocation of sources for the measure 'tradition, lifequality', which had a share of 49% in the total amount of support. It means that the preservation of heritage and traditions is a very important development measure for the Göcsej-region. The area's development plan reached its main objective.

### Local product development

The Göcsej area is one of the well-known regions of Hungary, it is famous for its beautiful landscape, environment and its heritage of architecture. This marketing position is worth being used as a breakout– point with the help of the Göcsej trade–mark system. A very important project of the Foundation for Development of Enterprises of County Zala was supported by Leader+. The trade-mark system is owned by the Göcsej Regional Association and can help the development of local products. The system can differentiate the producers of local products in Göcsej from other parts of Hungary. As a marketing tool, it can build image and reputation for the area. The involvement of primary producers into the trade mark system and indirectly to the Leader program has already started in the Göcsej area. The popularity and success of local products in different fairs and programs is motivating the primary producers for a sufficient development.

### Discussion

A highly effective instrument of the rural development is the Leader program, which is

- based on a multisectoral, integrated and area-based strategy
- tries to utilize the entire local economic potencial
- prefers bottom-up approach
- activates the local population involving the entire community into the development-process
- · creates co-operation and common projects among rural areas
- supports innovation
- creates and strengthens local partnerships.

The Leader+ supports helped the Göcsej-Middle Zala LAG to realise the area's goals, the total amount of the LAG's support were used by the beneficiaries. 64 different kinds of development ideas, projects were realised from the EUR 340.000 funds of Leader+.

From the 8 measures of the program, the measure called 'Development of life-quality and living-conditions in rural areas, keeping traditions' was extremely popular among the beneficiaries: 49% of all the LAG's support was used for this goal. Unfortunately, these projects could not really develop the local economy, they rather helped the work of local governments or community initiatives. Nevertheless, the objectives of 'Keeping traditions and preservation of heritage' were fully completed by the program, so the Göcsej –area managed to accomplish its strategic goal.

The measure 'Traditional local product-based, integrated and ecosocial market economy' – called 'break-out point' of the area by the experts - had no more than 5% of the support, so eventualy the economy of Göcsej–area could not significantly develop in this Leader period. For this reason, the area has launched a special trade–mark system from the Leader+ support, which can result a real investment in local products in the next planning phase.

92% of all project proposals were handed in by the sectors of local governments and civil organisations, and only 8% of projects by enterprises, therefore the activity of the members of the LAG is more significant in the local governmental and civil sectors.

We investigated the reasons for the unactivity of enterprises, participants of local economy and we also interviewed the Local Managing Authority. The main reasons revealed are the following:

- 1. the project documentary was too complicated, so the enterprises could not prepare project proposals
- 2. it is difficult to pre-finance the project until the project-payment is fulfilled

- 3. deficit of the necessary private financial resources
- 4. the share of support was not high enough, so they preferred second-hand instruments and materials instead of new ones obtained from support

All in all, the Leader program was a breakthrough for the local governmental and civil sector, but could not attract and facilitate the private sector, the local enterprises and local producers to the project initiative system. The establishment of a Göcsej trade-mark system has been started, and with adequate financial support between 2007-2013, it can result in a real breakthrought for the area's economy as well.

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