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June 2014

Journal of the Ministry of Agriculture

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**HUNGARIAN AGRICULTURAL RESEARCH**

**June 2014**

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Published by
NAKVI National Agricultural Advisory, Educational and Rural Development Institute (NAERDI)
H-1223 Budapest, Park u. 2. Hungary
www.agrarlapok.hu I info@agrarlapok.hu

Owner
Vidékfejlesztési Minisztérium

Editorial Office
Faculty of Agricultural and Environmental Sciences
Szent István University, Gödöllő I H-2103 Gödöllő
Hungary

Subscription request should be placed with the Publisher (see above)
Subscription are HUF 3900 (only in Hungary) or $16 early plus $5 (p & p) outside Hungary

HU ISSN 1216-4526

# AFLATOXINS – INVISIBLE ENEMIES OF ANIMAL AND HUMAN HEALTH

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## ABSTRACT

Aflatoxins (AFs) are secondary metabolites of Aspergillus moulds which were considered as "storage moulds" in temperate climate because of their optimal conditions for growth. However, Europe's natural environment is affected by the climate change; therefore several Aspergillus moulds can be grown even in the field. Aflatoxin contamination can occur in feedstuffs, compound feeds and several human food products. The major forms of aflatoxins in feeds and foods are $B_1$, $B_2$, $G_1$, $G_2$; and $AFB_1$ is one of the most potent naturally occurring human carcinogens. Aflatoxins are rapidly absorbed from the gastrointestinal tract and metabolised in the liver to active or detoxified metabolites. Transformation of aflatoxin can occur also in the rumen, and it can be converted to less toxic aflatoxicol and/or to $AFM_1$, which is also a human carcinogen. In farm animals, intake of feeds contaminated with aflatoxins can cause liver damage, reduced milk yield, feed intake, and overall performance. The biological effects of aflatoxins include immunosuppression, mutagenicity, and teratogenicity, and impaired reproduction efficiency. Aflatoxin is secreted into animal products, mainly in cow's milk and hen's egg, but it is also accumulated in some edible tissues, such as liver. Due to the high intake of cows' milk by humans, especially infants and children, studies have been conducted measuring the carry-over of aflatoxin in the feed to the milk, and the generally accepted value is 2.2%, but it depends on the rate of milk production, and consequently dry matter intake. The control of aflatoxin contamination of feed and food commodities should be started before harvest and follow up is a must during the storage and processing. Mycotoxin binders, such as clays, added to the feed or food may reduce the absorption of aflatoxins. Biotransformation with safe strains of soil and rumen bacteria, rumen protozoa or yeasts would be a novel perspective of mycotoxin control.

**keywords: aflatoxin, AFBs, AFGs, feeds and human food products**

## OCCURRENCE OF AFLATOXINS IN THE FOOD CHAIN

Aflatoxins are the secondary metabolites of moulds, Aspergillus flavus and Aspergillus parasiticus, which are considered as "storage moulds" because temperature of 25 °C and water activity ($w_a$) of 0.83-0.95 are ideal conditions for growth and mycotoxin production[1]. Aspergillus flavus has capacity to synthesize AFBs, while A. parasiticus can produce both AFBs and AFGs[2]. Aflatoxin production is induced as stress response in moulds, for instance oxidative stress activates simultaneously gene clusters responsible for aflatoxin synthesis and antioxidant defence[3].

Contamination with aflatoxin can occur in feedstuffs, such as corn and corn products, including by-products from bio-ethanol industry (such as DDGS), oilseed cakes and meals (cotton, peanut, sunflower etc.), silages, compound feeds and several human food products such as cereals, dry fruits, legumes and others[4]. The contamination can occur on the field and during storage if environmental conditions are suitable for growth of the mould. The major forms of aflatoxins in feeds and foods are $B_1$, $B_2$, $G_1$, and $G_2$. Among them $AFB_1$ has been found as to be the one of the most potent naturally occurring carcinogens[5].

Binder et al.[6] have completed a global survey of the incidence of mycotoxins, including aflatoxin $B_1$, in animal feedstuffs, and they found significant regional differences, especially between tropical and temperate areas. However, climate change is one of the most critical issues for the future and Europe's annual temperature has warmed by almost 1 °C over the past century. Thus Europe's natural environment is predicted to be highly affected by climate change, consequently several Aspergillus moulds can grow even in the field as it has already shown in Hungary[7].

## EFFECT OF AFLATOXINS IN ANIMALS AND HUMANS

Historical perspectives on animal diseases related to feeds contaminated with aflatoxin were published[8]. Aflatoxin was first isolated[9] following the outbreak of Turkey X disease in the United Kingdom in 1960[10]. Aflatoxins, after intake and absorption from the gastrointestinal tract, are metabolised

primarily in the liver by the microsomal xenobiotic transforming system to active or detoxified metabolites[11,12]. Transformation of aflatoxin can also occur in the rumen of ruminants; it is converted to less toxic aflatoxicol and hydroxylated to some hydroxylated metabolites, including $AFM_1$[13]. However, transformation to aflatoxicol is a reversible process; therefore aflatoxicol is toxic as $AFB_1$. Due to the rate of metabolism and types of metabolites determine differences in susceptibility of different species to aflatoxin[14]. However, considerable individual differences can be also found within a species. In many animal species, in particular birds, males are more susceptible than females and young birds than adults[15]. The carcinogenicity of aflatoxin has been identified more than forty years ago[16] and $AFB_1$ has been demonstrated to be a major aetiological factor in hepatocellular carcinoma in human individuals infected with hepatitis B virus[17], therefore, International Agency for Research on Cancer classified $AFB_1$ as Group 1 human carcinogen[18].

The primary factor for carcinogenicity is one of the intermediate metabolite, $AFB_1$-exo-8,9 epoxide, which partly conjugates with glutathione to $AFB_1$-exo-8,9 epoxide-GSH, but it also covalently binds to DNA, where 8,9 dinhydro 8(N7guanyl)-9 hydroxy $AFB_1$ adduct is formed[19]. This consequentially disrupts the transcriptional and translational processes.

Carcinogenic, mutagenic and teratogenic effects of aflatoxin $B_1$ are of particular concern in human populations and are subjected to low level mycotoxin consumption over many years[20]. In respect of carcinogenicity, there is no upper safe limit for aflatoxins in human, for this purpose the so-called ALARA principle (As Low as Reasonable Achievable) should be used. Among farm animal species rainbow trout (Salmo gairdneri) has extreme susceptibility to hepatocellular carcinoma in aflatoxicosis[21].

The other, oxidised, hydroxilated or dealkylated metabolites of $AFB_1$, such as $AFP_1$, $AFQ_1$, $AFB_{2a}$ or $AFM_1$ have lower toxicity[22]. Glutathione-S-transferases have primary importance for detoxification of aflatoxins, for that reason farm animal species (like turkey), which has low glutathione-S-transferase activity show higher sensitivity to aflatoxicosis[23].

In farm animal practice, aflatoxin contamination levels in feedstuffs are usually not high enough to cause an overt disease, but may result in economical loss through clinically obscure changes in growth, production and increased susceptibility to infectious diseases or impaired response to vaccination[24]. Intake of feeds with high level aflatoxins can cause liver damage, reduced milk yield, feed intake, and overall performance. Detrimental effect of $AFB_1$ mainly is based on the impairment of liver function, which is caused by the effect of $AFB_1$ on hepatic gene expression, in particular on apoptosis genes[25]. The biological effects of aflatoxins also include immunosuppression, mutagenicity, and teratogenicity[26].

In human, besides hepatocellular carcinoma, aflatoxins have been implicated, , also in acute hepatitis, cirrhosis in malnourished children, Reye's syndrome and kwashiorkor[27,28].

Aflatoxins influence the reproduction efficiency indirectly through reduced feed intake and impairment of metabolic function, especially of the liver[29]. In a field outbreak of aflatoxicosis[30] in laying hens, egg production has dropped to 5%, and egg size decreased. In a feeding trial with layers[31] and broiler breeds[32], aflatoxin has been shown to cause decreased egg production. Marked reduction in hatchability has also been noted[32], which may be, at least in part, due to the deposition of aflatoxin into eggs[33]. There is also evidence of aflatoxin transfer in utero in mammals to the developing foetus both in pigs[34] and humans[35].

## AFLATOXINS IN ANIMAL PRODUCTS

Aflatoxin can be converted to other toxic metabolites in farm animals that are then secreted into animal products, mainly cow's milk and hen's egg, but it also can be accumulated in some edible tissues, such as liver. The principal metabolite of $AFB_1$ is, what was first termed as "milk toxin"[36], $AFM_1$. Conversion of $AFB_1$ to $AFM_1$ involves a hydroxylation reaction at the 9a position of the $AFB_1$ molecule. $AFM_1$ remains carcinogenic, but it is less biologically active than $AFB_1$[37]. Although, $AFM_1$ was originally classified by the International Agency for Research on Cancer as a Group 2B human carcinogen, there are subsequent evidences of its carcinogenic effects led to a new categorization of $AFM_1$ as Group 1 human carcinogen[18].

Due to the high intake of cows' milk by humans, especially infants and children, many studies have been conducted measuring the carry-over, or transfer, of aflatoxin

**TABLE 1: Calculated $AFM_1$ contamination in milk based on $AFB_1$ intake and milk production**

| $AFB_1$ content of TMR (µg/kg) | Milk production (litre/day) | TMR intake (kg dry matter/day) | $AFM_1$ content of milk (µg/litre) |
|---|---|---|---|
| 5.00 | 15 | 16 | 0.106 |
| 5.00 | 20 | 17 | 0.090 |
| 5.00 | 25 | 18 | 0.076 |
| 5.00 | 30 | 20 | 0.070 |
| 5.00 | 35 | 21 | 0.063 |
| 5.00 | 40 | 22 | 0.058 |

in the feed to the milk. Carry-over of $AFB_1$ to $AFM_1$ was investigated in many studies. A range of 0 to 4% was reported in an early review[38] showing the relationship between $AFB_1$ intake and $AFM_1$ secretion in the milk. Patterson et al.[39] proposed, that an average of 2.2% of the ingested $AFB_1$ was converted to $AFM_1$ and secreted in the milk of cows. Based on the "Patterson formula" (2.2% carry-over) and using the threshold value of $AFB_1$ (5 μg/kg with 12% moisture content) in TMR (total mixed ration) of dairy cows according to the EU regulation (574/2011/EU), the following $AFM_1$ contamination can occur in the milk at different milk production capacities, and consequently at different dry matter intake levels (Table 1).The result showed that even when the $AFB_1$ contamination of the TMR is within the EU limits, $AFM_1$ content of the milk produced is above the maximum EU levels (0.05 μg/l or 0.025 μg/l for baby formulas) according to the European Union Regulation No. 466/2001/EC.

## STRATEGIES FOR REDUCTION OF AFLATOXIN CONTAMINATION

The control of aflatoxin contamination starts already before harvest and continues during the storage of cereal grains and oilseeds[40]. Successful protection against Aspergillus infection requires proper agritechnical procedures[41]. During storage, the maximum moisture content ensuring safe storage is 13–14% for cereal grains and 7% for oilseeds[42]. The removal of damaged and mould-infected grains, glumes, and other contaminants before storage is an efficient prevention method that reduces further mycotoxin production[43]. Feed or food processing do not substantially decrease the aflatoxin content of feedstuffs or foods, and the efficiency of the recommended chemical and/or heat treatment procedures is also questionable as they are expensive and may reduce the nutrient content and/or digestibility[44].

To minimise the adverse effects of aflatoxins on animals and humans, the use of products capable of binding and biologically transforming mycotoxins is also recommended; however, such products have varying efficacy[45]. Mycotoxin binding agents belong to a new functional group of feed additives (286/2009/EC), which are added to the feed to reduce the absorption of mycotoxins in the gastrointestinal tract, thereby reducing the accumulation of mycotoxins into animal tissues and excretion with products. The mechanism of different mycotoxin binders has yet to be fully determined[46]. As adsorbent agents, clays are widely used against aflatoxins. Different types of clays, such as aluminas, silicates, phyllosilicates, zeolites and bentonites have different binding capacity because of their different chemical composition and secondary physical attributes[47], therefore, there are marked differences among them in the detoxification of aflatoxin as was shown in a comprehensive review[48]. It was proven that $AFM_1$ content of milk can be reduced with bentonite supplementation of dairy cow's diet[49]. Based on the present research, aflatoxin sequestering clays, in particular those proposed for humans, must have favourable characteristics of mycotoxin sorption, contain only tolerable levels of metals, dioxins/furans and other hazardous contaminants and have negligible interactions with vitamins, iron, zinc and other micronutrients[50].

Biotransformation is a novel perspective way of mycotoxin control, which is based on findings that mycotoxins can be detoxified by safe strains of soil and rumen bacteria, rumen protozoa or yeasts, or their purified enzymes[51]. However, there are very few data about the possibility of $AFB_1$ degradation by microorganisms[49] (Krifaton et al., 2011), for instance by several Rhodococcus strains[52].

Biocontrol of Aspergillus flavus and A. parasiticus and their aflatoxin production is also possible way to reduce aflatoxin contamination of feeds and foods using competitive exclusion method. The proposed biopesticides are atoxigenic strains of A. flavus and A. parasiticus, which were found effective in corn, cottonseed, peanunts, rice kernels and wheat seed[53,54].

## ACKNOWLEDGEMENTS

The publication is supported by the Research Centre of Excellence-17586-4/2013/TUDPOL project.

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# IN MEMORY OF ETHNOGRAPHER PROFESSOR SÁNDOR BÁLINT (1904-1980)

## SACRA AGRICULTURA HUNGARICA

### Documents on the religious aspects of the agriculture of Hungarian people

DEZSŐ **SURÁNYI**

Centre of National Agricultural Research Institute, Research Station of Fruit Research Institute, Cegléd

The appearance of Hungarian people and ethnic groups allied with it in the Carpathian Basin has changed the great historical face of the great region. As a result of the Pannonian Roman, Frankish and Slavic traditions, the nomadic population of the lowlands and the mountains as well as population from migrations, gradual changes of the natural landscape can be observed. The Neolithic Revolution that began in Jarmo (Zagros Mountains) – with its domesticated grass species (cereal species) – reached the Carpathian Basin around BC 4000, where the climatic circumstances have created a favorable geographical position and natural production potential.

One of the roots of the tangible culture of the Hungarian people was the above-mentioned set of natural and man-made features of the greater region that, especially after the centuries following the conquest of the Carpathian Basin, have set the geopolitical, economic and cultural role and situation of the country in Europe. But it would not have been sufficient to make the country a strong power through the country's cereals, wine, livestock and non-ferrous metals at the end of the reign of the Árpád dynasty. The genetic features of the constituents of the Hungarian people, their migration that can be measured in thousands of years

**Figure 1: Vincent Day caning (inoculation of wine bud fertility) (22nd January)**

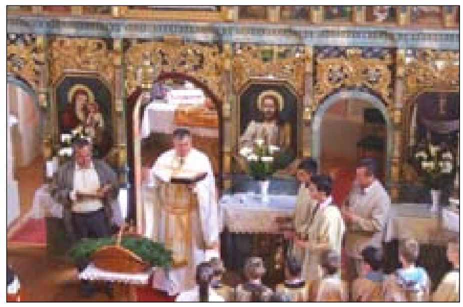

**Figure 2: Crucifix of Mikelaka (near Abony, next to the road) (Easter)**

**Figure 3: Consecrating of wheat seedling by a Greek Catholic priest (25th April)**

**Figure 4: Escaping geese on Saint Martin's Day (11th November)**

**Figure 6: Biblical crop altar decoration in Jánosháza (Mid of September)**

Figure 5: Indulgence of Saint Orban's Day in Csongrád (25th May)

Figure 7: Striped Maypole tree (1st May)

have accumulated a lot of knowledge on nature and using it. After natural religions (especially shamanism), learning about Judaism and Islam – and despite the presence of Eastern Christianity in the Carpathian Basin –, the nation's founder, Saint Stephen I chose Western orientation.

Of course there have been very tragic events in our past from civil discord to external wars (Tartar invasion, Turkish occupation of the country, and its break-up to

Figure 8: Taking noble grape

three parts, a partial loss of national sovereignty and territorial contraction at the end of World War I) as well as a string of natural disasters and economically adverse policy decisions. These interrupted the linear development of agriculture countless times, but the natural features of the Carpathian Basin always promoted growth again. The love of land and farming of people living here surely had and has a role in this, the birth of many plant and animal breeds point to that in our age, there is a near-optimal interaction between natural and cultivated vegetation in Hungary and the necessary attention of our people.

Perhaps the most important component of human resources is the religious factor. In particular, people living by the Catholic religion and preserving its traditions, the customs of the sacred calendar days - even in the absence of practicing religion - preserved, and what is more, renewed people.

As it is impossible to defend protected species without saving their food source, the protection of breeds that can be classified as Hungarians, keeping their cultivation ("on-farm") and crop and product diversity without the related sacred and non-sacred cultural knowledge can be hollowed out. The programme-setting paper wants to provide answers to why it is not sufficient to respect and love the old varieties in themselves as well as to maintain the diversity of gene bank collections. More than a thousand years in the Carpathian Basin is a huge period in the life of a nation, but it could mean more in the history of cultivated and bred varieties.

Idea-generating is based on the gallery with pictures that has been permeating the farming of Hungarian people. The pictures can be grouped thematically according to the following:

a./ yield prediction and fertility (Examples: Fig. 4 and Fig. 6)

b./ successful production activity (Examples: Fig. 3 and Fig. 8)

c./ requests and thanksgiving (Examples: Fig. 1 and Fig. 7)

d./ expression of religious respect (Example: Fig. 2 and Fig. 5)

***Note: He was the greatest figure of Hungarian sacral ethnography**

Photo selection from the work of
Ethnographer Professor Sándor Bálint









# EVALUATION OF ELUTION EXPERIMENTS ON SOIL-BIOCHAR-COMPOST-ARTIFICIAL FERTILIZER SYSTEMS TO DETERMINE THE MOBILIZATION OF NUTRIENTS AND POSSIBLE TOXIC ELEMENTS

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## ABSTRACT

In the last few decades, societies' food demand has been increasing continously, while our agricultural technologies are still not safe and sustainable enough with respect to the health of our environment. Biochar and compost products have advantageous properties in soils, such as increased nutrient retention capability, high organic matter content and capacity to adsorb toxic elements. To study these features, elution experiments were conducted. Our purpose was to determine, how the amendments impact the leaching properties of soil and the mobilization of nutrients and toxic components, such as heavy metals and PAHs. Two different types of soil, four kinds of soil amendments and three kinds of solvents of different pH values were used for elution. Mixtures of the soils and the amendments were filled into columns, each having a tap at the bottom. Each column, i.e. every mixture was eluted with one out of three different solvents. Soils, amendments as well as the eluates were analysed for the above-mentioned target components. Measurements were performed using GC-MS, ICP-MS, ICP-OES, AAS and IC instruments. Our results show that biochar and compost can improve soil structure and leaching properties significantly, but the feedstock they are made of and the type of soil to be treated have a significant impact on their effects.

**keywords: biochar, compost, soil, leaching, nutrient**

## INTRODUCTION

Approximately 40% of the land area of the 28 member states of the European Union is farmed and human pressure is increasing to convert natural landscapes to agricultural fields, while there is no doubt about the negative impact of the fertilizers and chemicals used. Furthermore, we also need to deal with other consequences of outdated agricultural technologies, such as loss of soil organic matter (SOM) and leaching of plant available nutrients. Under such conditions crop production can neither be safe and satisfy demands. To increase food production the key is to prevent the above-mentioned negative environmental effects of intensive agriculture. One efficient way to increase the SOM level, nutrient content, water retention and productivity of soil is to apply biochar and compost as fertilizer and soil improver. These materials are produced by recycling and reusing organic waste, food industrial by-products and organic farm residues via pyrolysis or composting. Biochar and compost have several supposed advantages and their application in soil can be a sustainable solution for reducing the use of agrochemicals, improve the quality of soil and increase crop production. As soil improvers, compost and biochar make soil more fertile and reduce the need for chemical input. Biochar retains nutrients and so leaching out and eutrophication can be reduced to a minimal level and water quality can also be improved. Moreover, pyrolysis and composting provide a solution for utilizing agricultural wastes, while its by-products can support bioenergy production. Because of these properties it is worthwhile to study the behaviour of biochars and composts in soil. It is very important to know their leaching properties, and to be able to tell to what extent nutrients and toxic components can be mobilized. Before agricultural use, we must ascertain environmental safety of biochars and composts, therefore, we have to find the answers to these questions.

In this article, an elution experiment is presented in order to investigate the mobilization properties of nutrients, heavy metals and organic pollutants in soil-biochar, soil-compost, soil-biochar-compost and soil-superphosphate fertilizer systems. The mixtures were filled into PVC columns and the elution was executed by 3 kind of elution solvents. All the soils, amendments and eluates were analyzed for nutrients, heavy metals and organic contaminants.

## Biochar

Biochar is produced by thermal degradation of organic materials under oxygen deficient conditions, by pyrolysis or gasification, resulting in three fractions: char, gas and oils. Depending on the parameters of the process technology (temperature, pressure, residence time, feedstock type), there can be significant differences in the quality and amount of the char. Biochar is the main product (30-50%) of the pyrolysis process under reductive (indirectly heated) thermal conditions. The temperature of pyrolysis is above 450°C. On the other hand in the case of gasification biochar is a by-product, while oils and gas can be used in bioenergy production. It occurs at 650°C-950°C and biochar yield is less than 10%.

Pyrolysis conditions have a significant impact on the characteristics and chemical composition of chars [1]. In general, as pyrolysis temperature increases, ash content, pH, electrical conductivity, the ratio of the stabile organic content and total C, N, P, K, Ca and Mg contents also increase, while biochar yield, total O, H and S contents, unstable forms of organic carbon decrease. The ratios of O/C, H/C, (O+N)/C and (O+N+S)/C tend to decrease with increasing temperature. Fourier transformation infrared spectroscopy indicates an increase in the aromaticity and a decrease in the polarity of biochar produced at high temperature. Thus, the chemical composition of biochar cannot be clearly defined. Instead, it is a class of compounds and thresholds are needed to be defined for materials which are claimed to be biochar. Recently, on the basis of the results of the analysis of about 100 biochar samples differing in feedstock and production conditions, the following elemental ratio thresholds were suggested for biochar: O/C <0.4 and H/C <0.6. The later is important, because the condensed aromatic compound content of biochar is responsible for its resistancy against microbial degradation, thus for its stability in the environment. Residence time in soils could be 1300-4000 years based on controlled biochar decomposition experiments [2].

Biochars have two main types, depending on the feedstock and the production technology. Plant based biochar (PBC) is produced from plant biomass materials between 450°C-550°C via reductive thermal processing. It has high ratio of stabile organic content and the structure is characterized by micro- and mesopores (1-50 nm), based on scanning electron microscope imaging analyses. PBC has a relatively high water holding and nutrient retention capacity, but hardly any fertilization effects. Consequently PBC can be applied as soil improver, but not as fertilizer. Animal bone biochar (ABC) is produced from animal bone meal between 500°C-650°C via reductive thermal processing. ABC is typically macroporous and composed primarily of carbon and hydroxyapatite. Its carbon content is low, smaller than 20%, but its calcium phosphate content is relatively high. Hydroxyapatite is chemically similar to the mineral 'apatite', which is the only mineral on Earth that contains high proportion of phosphate naturally. Therefore, animal bone can be recognised as an organic fertiliser with regards to its phosphate content.

## Compost

Compost is a product of biological decomposition of organic matter derived from plants, animals or human activites, through the action of microorganisms under aerobic conditions. Mature compost, a stable material with a high humus content free of pathogens, can be applied beneficially to soils as a soil amendment or as a medium to grow plants. Numerous publications [3, 4] provide evidence about the benefits of compost application to soils. The two most important impacts are the ability to increase microbiological activity (especially when applied in combination with biochar) and SOM content, both stable and labile. Hardly decomposable organic compounds have long-term effects on soil amelioration and conservation, while a labile pool of SOM provides easily accessible food for soil organisms and nutrients for plant growth.

Both biochar and compost have their own advantageous properties, but in combination their application is remarkably more efficient. The reason of that is the complementarity of their properties which makes them more efficient together [4]. Beside the addition of nutrients, compost ensures microbiological activity, while biochar provides a habitat for the microorganisms and also retention capability. On the other hand the recommended doses of ABC, PBC and compost are 300 kg/ha, 2500 kg/ha and 5000 kg/h, respectively. With such enormous amounts, one has to be careful to make sure that these products are environmentally friendly and safe for humans even though they may contain toxic elements, organic pollutants and heavy metals which can damage processes in the ecosystem seriously and can accumulate in organisms. Any such contamination may have its origins in the input material or can form during the treatment.

## MATERIAL AND METHODS

Two different types of soils, four kinds of soil amendments (ABC, PBC, compost and superphosphate fertilizer) and three kinds of elution solvents (distilled water, Olsen solution and ammonium lactate solution) were used. ABC was produced by Terra Humana Ltd., PBC was from Pyreg GmbH, compost was from Profikomp ZRt and superphosphate (SP) was produced by Florimo (Superphosphate 20.5% Florimo®). The analytical grade sand was from Supelco. Olsen solution (O, sodium hydrogen carbonate solution with a pH of 8.5 +/- 0.02) was prepared according to international standard

ISO 11263:1994 (E). Solid sodium hydrogen carbonate was purchased from Supelco. Ammonium lactate (AL) solution was prepared according to Hungarian standard MSZ 20135:1999. Its pH was 3.7 +/- 0.02. For the production of AL, lactic acid was purchased from Sigma-Aldrich, while ammonium acetate and acetic acid were from Merck. The third elution solvent was distilled water (DW).

The material of the columns in which elutions were carried out was PVC, the diameter was 6 cm, the height 80 cm, and there was an adjustable tap at the bottom.

Samples were prepared by mixing soils with additives and sand (inert dilution material to ensure solvent permeability because of the compact structure of the soils). In the case of blanks the soils were also diluted with sand. The doses of the additives applied are listed in Table 1. This way, for the two soils we obtained 20 samples. Thus, using three different solvents, 60 eluates were analyzed in all.

**TABLE 1: Amendments used and doses applied. kg/m³ values were calculated supposing 0.25 m depth for ploughing.**

| | Dose (kg/ha) | Dose (kg/m³) |
|---|---|---|
| Blank | - | - |
| ABC 1 | 300 | 0.12 |
| ABC 2 | 600 | 0.24 |
| PBC 1 | 2500 | 1 |
| PBC 2 | 5000 | 2 |
| Compost 1 | 5000 | 2 |
| Compost 2 | 10000 | 4 |
| ABC + Compost | 300 + 5000 | 0.12 + 2 |
| PBC + Compost | 2500 + 5000 | 1 + 2 |
| Superphosphate | 300 | 0.12 |

1.5 kg samples were filled in each column, so as the height of the mixture approximately equalled the depth of ploughing (0.2 – 0.3 m). On each column one of the eluents was applied with a leaching ratio of 1/2.5, which meant the addition of 2.5 l of solvent to 1 kg of soil (taking into consideration only soil, without additive and sand. Elution was considered complete when the tap at the bottom of the column was dry. After leaching the eluates were filtered and processed for analysis. Targets of the measurements were the nutrients, heavy metals and polyaromatic hydrocarbons (PAHs), so ICP-MS, ICP-OES, GC-MS, IC and AAS instruments were used for the analyses. Results of previous analyses of biochars and composts showed that the polychlorinated biphenyl (PCB) content of the samples was so low that they were not included in this study.

## Characterisation of the soils and amendments

Before the elution experiments, the soils used were analyzed to determine their chemical properties and elemental compositions (Table 2).

**TABLE 2: Main chemical parameters of the soils.**

| | Soil 1 | Soil 2 |
|---|---|---|
| Dry matter (%) | 84.9 | 85.96 |
| Bulk density (kg/m3) | 1090 | 1170 |
| pH | 7.96 | 5.52 |
| TOC[a] (%) | 1.69 | 1.64 |
| CEC[b] (meq/100g) | 28.2 | 8.8 |
| Phosphorus (mg/kg) | 893 | 688 |
| Potassium (mg/kg) | 15500 | 15000 |
| Calcium (mg/kg) | 22700 | 5660 |
| Magnesium (mg/kg) | 9910 | 1650 |
| Copper (mg/kg) | 27 | 9 |
| Zinc (mg/kg) | 65 | 46 |
| Manganese (mg/kg) | 828 | 322 |
| Strontium (mg/kg) | 105 | 104 |
| Cobalt (mg/kg) | 11 | 3 |
| Arsenic | 10 | <5 |
| Cadmium (mg/kg) | <0.3 | <1 |
| Chromium (mg/kg) | 67 | 21 |
| Lead (mg/kg) | 21 | 17 |
| Mercury (mg/kg) | 0.04 | <1 |
| Sum of 16 EPA PAHs (mg/kg) | not detectable | not detectable |

*/a, Total Organic Carbon*
*b, Cation Exchange Capacity/*

**TABLE 3: Elemental composition and pH values of the four additives.**

| | ABC | PBC | Comp | SP |
|---|---|---|---|---|
| pH | 8.14 | 9.35 | 7.41 | 2.77 |
| TC (%) | 11.5 | 81 | | |
| Phosphorus (mg/kg) | 122000 | 1600 | 1800 | 64300 |
| Potassium (mg/kg) | 2640 | 7000 | 12000 | 84700 |
| Calcium (mg/kg) | 279000 | 17000 | 28000 | 122000 |
| Magnesium (mg/kg) | 5650 | 3000 | 7000 | 23300 |
| Copper (mg/kg) | 3 | 28 | 49 | 10 |
| Zinc (mg/kg) | 157 | 176 | 103 | 100 |
| Manganese (mg/kg) | 3 | 1520 | 397 | 530 |
| Strontium (mg/kg) | 126 | 80 | 90 | 390 |
| Cobalt (mg/kg) | <1 | 2 | 3 | <10 |
| Cadmium (mg/kg) | <0.3 | <1 | <1 | <3 |
| Arsenic | <1 | <5 | <5 | <10 |
| Chromium (mg/kg) | 21 | 26 | 27 | 10 |
| Lead (mg/kg) | <1 | 7 | 21 | <10 |
| Mercury (mg/kg) | <0.06 | <1 | <1 | 2.1 |
| Sum of 16 EPA PAHs (mg/kg) | 0.14 | 0.71 | 0.71 | - |

As shown in Table 2, there is a large difference between the soils in terms of their calcium and magnesium contents. Their potassium and phosphorous contents are, however, very similar.

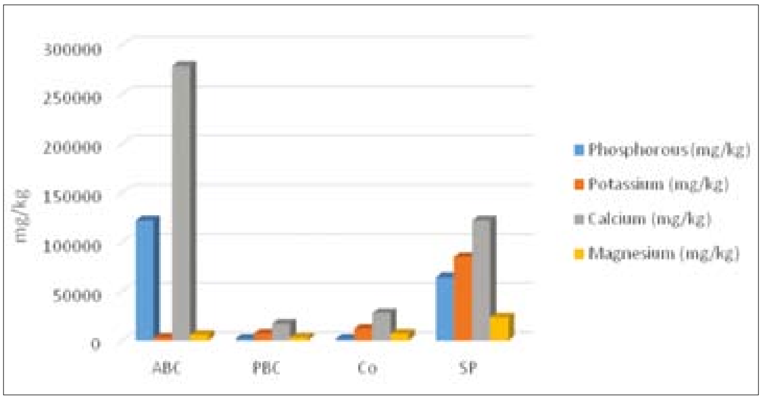


**Figure 1: Primary and secondary nutrient content of the four additives**

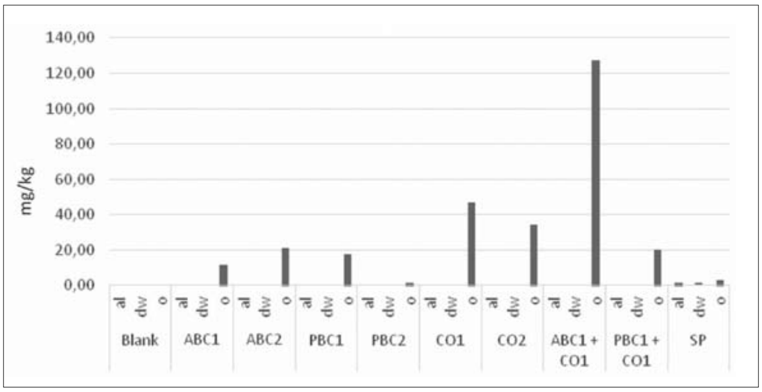


**Figure 2: Phosphorus content of eluates from samples prepared with soil 1.**

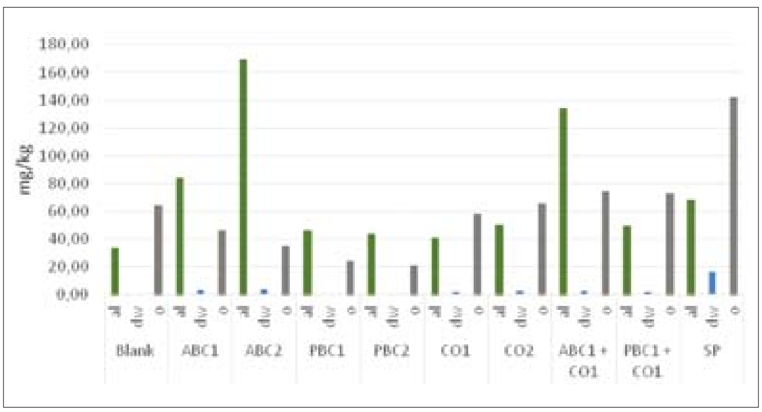


**Figure 3: Phosphorus content of eluates from samples prepared with soil 2.**

In the case of toxic compounds PAHs were not found in the soils. Cadmium and mercury contents were negligible. But there were considerable amounts of them present in the soils.

According to their particle size distributions and the ratio of sand, silt and clay fractions, the two soils were similar from the classification point of view. Soil 1 is a sandy-clay loam type of soil and soil 2 is a sandy loam type of soil.

Chemical information about the additives is summarized in (Table 3). Total carbon (TC) was measured only from the two types of biochars and is given in percent values. As it was expected, total carbon content of PBC is much higher than that of the ABC. The pH value of SP (2.77) is notably lower than the pH of the other amendments. Such acidic conditions may cause changes in soil structure and may pose a risk to groundwaters.

As expected, ABC and SP have the highest nutrient content (Figure 1). Nutrient composition of SP is more diverse than that of ABC, but the latter contains phosphorus and calcium in higher concentrations than SP. It confirms the assumption that ABC can be an appropiate rival to SP, especially if it is applied together with soil improvers such as PBC or compost.

## RESULTS AND DISCUSSION

pH dependency of the leaching of the elements was apparent. In the case of phosphorus, there is a significant difference between the two soils. (Figures 2 and 3).

In both cases, phosphorus was not mobilized by distilled water, except when the additive was SP. The reason for this is that SP is a fast release fertilizer, on the contrary to chars and composts. However, this is a huge advantage of the latter products, because by the application of them, environmental risks, such as eutrophication and contamination of waters can be reduced.

It is interesting that in the case of soil 1 only the alkaline solvent was efficient, while from mixtures made from soil 2 also ammonium lactate could mobilize phosphorus. The explanation for this lies with the pH of the soils: the soil 2 is acidic, while soil 1 is alkaline (Table 2). pH values of the ammonium lactate eluates of samples prepared from soil 2 were about 4.1, while from soil 1 they were about 6. This observation shows that pH~6 is not acidic enough to mobilize acidic phosphorus forms. However, these forms of phosphorus are not able to support plant growth. On alkaline side, pHs of the Olsen solution eluates of samples prepared from soil 2 were about 8.8, while from soil 1 they were about 9.3. In this case, a pH below 9 is more efficient to mobilize phosphorus than one above 9. Phosphorus forms of this pH can be utilized by plants.

Comparing the percentage values of dissolved phosphorus, leaching from soil 2 was obviously more effective (Table 4). The only difference between the two systems is the type of soil, especially their CEC values: their P contents were very similar, but the CEC was significantly higher in soil 1. Therefore, soil 1 has a higher retention capacity. This experiment proves that the expectation that soil type and

**TABLE 4: Absolute and percentage P values in samples of both soils. Percentage values mean how much of the P leached out, compared to the total P content of the sample (soil+additive).**

| | | Dissolved amount of P from soil 1 (mg/kg) | P dissolved/P in soil 1 (%) | Dissolved amount of P from soil 2 (mg/kg) | P dissolved/P in soil 2 (%) |
|---|---|---|---|---|---|
| Blank | Al | <0.1 | <0.1 | 33.4 | 5.8 |
| | Dw | <0.1 | <0.1 | <0.1 | <0.1 |
| | O | <0.1 | <0.1 | 64.2 | 11.2 |
| ABC1 | Al | <0.1 | <0.1 | 84.2 | 14.4 |
| | Dw | <0.1 | <0.1 | 2.8 | 0.5 |
| | O | 11.4 | 1.5 | 46.0 | 7.9 |
| ABC2 | Al | <0.1 | <0.1 | 169.4 | 28.4 |
| | Dw | <0.1 | <0.1 | 3.6 | 0.6 |
| | O | 21.2 | 2.8 | 34.8 | 5.8 |
| PBC1 | Al | <0.1 | <0.1 | 46.6 | 8.1 |
| | Dw | <0.1 | <0.1 | 0.5 | 0.1 |
| | O | 17.4 | 2.3 | 24.4 | 4.2 |
| PBC2 | Al | <0.1 | <0.1 | 43.6 | 7.6 |
| | Dw | <0.1 | <0.1 | 0.7 | 0.1 |
| | O | 1.6 | 0.2 | 20.8 | 3.6 |
| CO1 | Al | <0.1 | <0.1 | 41.2 | 7.1 |
| | Dw | <0.1 | <0.1 | 1..6 | 0.3 |
| | O | 47.0 | 6.3 | 58.4 | 10.1 |
| CO2 | Al | <0.1 | <0.1 | 50.0 | 8.6 |
| | Dw | <0.1 | <0.1 | 2.4 | 0.4 |
| | O | 34.4 | 4.6 | 65.8 | 11.3 |
| ABC1 + CO1 | Al | <0.1 | <0.1 | 133.8 | 22.8 |
| | Dw | <0.1 | <0.1 | 2.4 | 0.4 |
| | O | 127.4 | 16.8 | 74.0 | 12.6 |
| PBC1 + CO1 | Al | <0.1 | <0.1 | 49.4 | 8.5 |
| | Dw | <0.1 | <0.1 | 2.0 | 0.3 |
| | O | 20.4 | 2.7 | 72.8 | 12.6 |
| SP | Al | 1.2 | 0.2 | 68.2 | 11.7 |
| | Dw | 1.6 | 0.2 | 16.6 | 2.9 |
| | O | 3.0 | 0.4 | 142.0 | 24.4 |

soil properties may impact the effectiveness of the additive applied was correct.

In general, the expected tendency among added amendments was observed. On the basis of the amount of phosphorus eluted from samples prepared using soil 2, the biggest amounts leached out from ABC and SP mixtures, then came the combination of ABC and compost, while both PBC and compost gave much lower values than either ABC or SP.

**TABLE 5: Absolute and percentage Ca values in eluates. Percentage values mean how much of the Ca leached out, compared to the total Ca content of the sample (soil+additive).**

| | | Dissolved amount of Ca from soil 1 (mg/kg) | Ca dissolved/Ca in soil 1 % | Dissolved amount of Ca from soil 2 (mg/kg) | Ca dissolved/Ca in soil 2 % |
|---|---|---|---|---|---|
| Blank | al | 14060.0 | 73.6 | 1738.0 | 35.4 |
| | dw | 114.0 | 0.6 | 200.0 | 4.1 |
| | o | <0.1 | <0.1 | 102.0 | 2.1 |
| ABC1 | al | 13860.0 | 72.4 | 1852.0 | 37.6 |
| | dw | 128.2 | 0.7 | 340.0 | 6.9 |
| | o | 31.2 | 0.2 | 92.0 | 1.9 |
| ABC2 | al | 14040.0 | 73.3 | 1958.0 | 39.5 |
| | dw | 122.2 | 0.6 | 272.0 | 5.5 |
| | o | 48.0 | 0.3 | 96.0 | 1.9 |
| PBC1 | al | 13340.0 | 69.8 | 1302.0 | 26.5 |
| | dw | 84.8 | 0.4 | 240.0 | 4.9 |
| | o | 43.6 | 0.2 | 70.0 | 1.4 |
| PBC2 | al | 14060.0 | 73.5 | 1238.0 | 25.1 |
| | dw | 82.0 | 0.4 | 144.0 | 2.9 |
| | o | 48.0 | 0.3 | 98.0 | 2.0 |
| CO1 | al | 13620.0 | 71.1 | 1572.0 | 31.8 |
| | dw | 106.2 | 0.6 | 214.0 | 4.3 |
| | o | 102.0 | 0.5 | 46.0 | 0.9 |
| CO2 | al | 12980.0 | 67.7 | 1986.0 | 39.8 |
| | dw | 100.6 | 0.5 | 298.0 | 6.0 |
| | o | 65.2 | 0.3 | 102.0 | 2.0 |
| ABC1 + CO1 | al | 13000.0 | 67.8 | 1930.0 | 38.8 |
| | dw | 161.2 | 0.8 | 322.0 | 6.5 |
| | o | 62.6 | 0.3 | 98.0 | 2.0 |
| PBC1 + CO1 | al | 14720.0 | 76.8 | 1840.0 | 37.1 |
| | dw | 110.8 | 0.6 | 378.0 | 7.6 |
| | o | 52.0 | 0.3 | 110.0 | 2.2 |
| SP | al | 15320.0 | 80.2 | 1522.0 | 31.0 |
| | dw | 122.2 | 0.6 | 344.0 | 7.0 |
| | o | 60.0 | 0.3 | 76.0 | 1.5 |

The cases of potassium and calcium were similar to that of phosphorus. Elution of potassium from samples of soil 1 was efficient only with AL, but from samples of soil 2 O and DW were also able to leach out potassium (Figures 4 and 5). This is also true in the case of calcium, but in the eluates from samples of soil 1, concentration of Ca was higher by an order of magnitude than in eluates from samples of soil 2 (Figures 6 and 7). The reason is that the original Ca content of soil 1 was fourfold higher than that of soil 2 (Table 2).

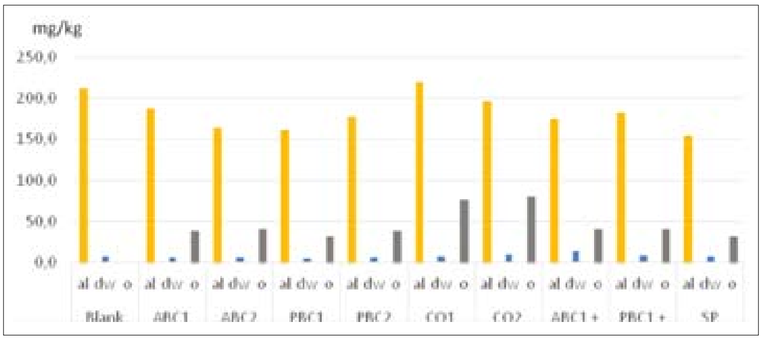


**Figure 4: Potassium content of the eluates from soil 1.**

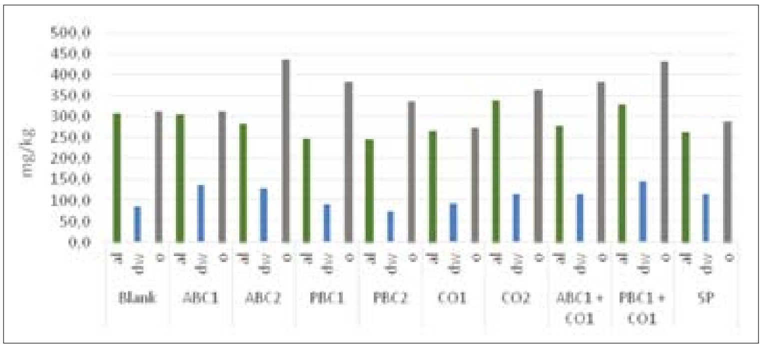


**Figure 5: Potassium content of the eluates from soil 2.**

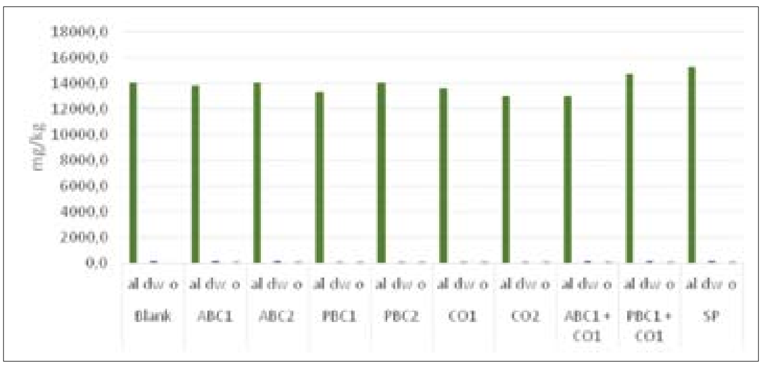


**Figure 6: Calcium content of eluates from soil 1.**

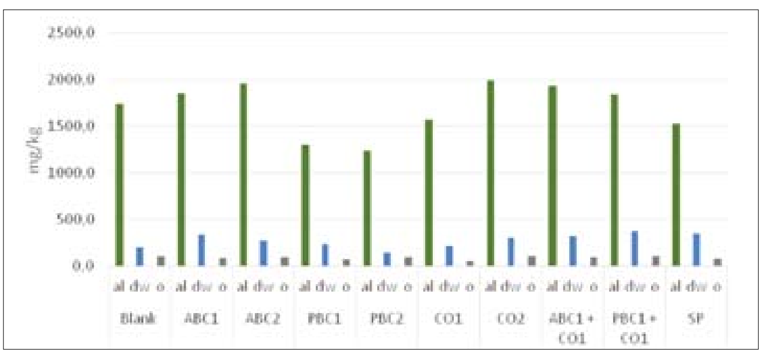


**Figure 7: Calcium content of eluates from soil 2.**

Percentage values of calcium eluteded from both samples prepared using soil 1 and 2 also reflected the much higher Ca content of soil 1. (Table 5).

Except SP samples, in all of other cases, leaching of potassium and calcium from the amended systems was not higher than from the blanks. However, the amended systems contained these elements in higher concentrations than the blank systems because of the added amount contained in them. This means, the additives had an impact on the mobilization, by retaining calcium and potassium.

Copper could mobilize from soil 1 samples at the alkaline pH, and leaching from the amended soils were similar to that from the blank. The only exceptions were the compost mixtures, where leaching of copper was higher than from the other samples. The reason for this lies with the original copper content of the additive. The same trends can be observed in the case of soil 2 samples. There is a difference in the pH values of the soils, and because of this, copper can be mobilized from soil 2 samples at the acidic pH as well, however, the extent of this was lower than at the alkaline pH. The tendency was similar in the case of strontium. Strontium contents of the original soils were approximately the same, but there were difference among the additives (Table 3). Even so, in the case of soil 1, concentrations in the eluates from the blank and the mixtures were very similar. On the contrary, strontium mobilization from soil 2 samples were lower. Mercury was not detected (ND) in any if the eluates, probably because of its low concentration of soils and amendments. Leaching of arsenic, cadmium and lead were negligible, their concentrations in the eluates were very low (Tables 6 and 7).

PAHs were measured only from distilled water eluates because of the pH independency of their leaching. Their concentrations were also extremely low. However, it is shown by actual results, that PBCs pose a higher risk to contamine the enviroment with PAHs (Figure 8), as was suspected on the basis of the additives' PAH contents (Table 3). But it must be noted that - according to our previous studies - this depends on the feedstock. Current PAH values can not be used to determine the dynamics of their mobilization. It would only be possible using additives with higher PAH contents, or by spiking the systems studied with PAHs.

Data presented above prove that the type, pH and CEC of soil to be treated with chars and/or composts has a very significant impact on the results, and the effectiveness of the amendments.

**TABLE 6: Concentrations of Mn, Zn, Cu, Cr, Sr, As, Cd, Pb in the eluates from soil 1 in mg/kg.**

| | | | Mn | Zn | Cu | Cr | Sr | As | Cd | Pb |
|---|---|---|---|---|---|---|---|---|---|---|
| SOIL 1 | Blank | al | 286.0 | 0.2 | ND | 0.1 | 30.4 | ND | ND | ND |
| | | dw | ND | ND | ND | ND | 0.3 | ND | ND | ND |
| | | o | 0.7 | 0.1 | 0.9 | 0.1 | 0.0 | 0.1 | ND | ND |
| | ABC1 | al | 338.0 | 28.6 | ND | 0.1 | 30.8 | ND | ND | ND |
| | | dw | ND | 0.2 | ND | ND | 0.4 | ND | ND | ND |
| | | o | 1.0 | ND | 0.8 | 0.2 | 0.5 | 0.2 | ND | ND |
| | ABC2 | al | 272.0 | 33.0 | ND | 0.1 | 27.8 | 0.0 | ND | ND |
| | | dw | 0.1 | ND | ND | ND | 0.4 | 0.0 | ND | ND |
| | | o | 2.4 | 0.1 | 1.0 | 0.1 | 0.7 | 0.2 | ND | ND |
| | PBC1 | al | 167.4 | 0.1 | ND | 0.1 | 31.2 | ND | ND | ND |
| | | dw | ND | ND | ND | ND | 0.3 | ND | ND | ND |
| | | o | 2.1 | ND | 1.1 | 0.1 | 0.7 | 0.2 | ND | ND |
| | PBC2 | al | 150.8 | 0.1 | ND | 0.1 | 31.6 | ND | ND | ND |
| | | dw | ND | 0.1 | ND | ND | 0.3 | ND | ND | ND |
| | | o | 1.7 | 0.1 | 0.7 | 0.1 | 0.7 | 0.1 | ND | ND |
| | CO1 | al | 254.0 | 0.1 | ND | 0.1 | 32.6 | ND | ND | ND |
| | | dw | ND | ND | ND | ND | 0.4 | ND | ND | ND |
| | | o | 1.6 | 30.8 | 5.0 | 0.4 | 1.5 | 0.3 | ND | ND |
| | CO2 | al | 264.0 | 0.1 | 0.0 | 0.1 | 28.8 | ND | ND | ND |
| | | dw | ND | ND | ND | ND | 0.4 | ND | ND | ND |
| | | o | 1.7 | 1.1 | 3.1 | 0.3 | 0.9 | 0.3 | ND | 0.1 |
| | ABC1 + CO1 | al | 256.0 | 0.1 | ND | ND | 27.8 | ND | ND | ND |
| | | dw | ND | ND | ND | ND | 0.5 | ND | ND | ND |
| | | o | 1.2 | ND | 0.8 | 0.1 | 0.9 | 0.1 | ND | ND |
| | PBC1 + CO1 | al | 250.0 | 0.2 | ND | 0.1 | 34.8 | ND | ND | ND |
| | | dw | ND | ND | ND | ND | 0.4 | ND | ND | ND |
| | | o | 1.4 | 0.1 | 1.1 | 0.1 | 0.7 | 0.2 | ND | ND |
| | SP | al | 242.0 | 0.1 | ND | ND | 36.0 | ND | ND | ND |
| | | dw | ND | ND | ND | ND | 0.4 | ND | ND | ND |
| | | o | 1.2 | ND | 0.9 | 0.1 | 0.7 | 0.2 | ND | ND |

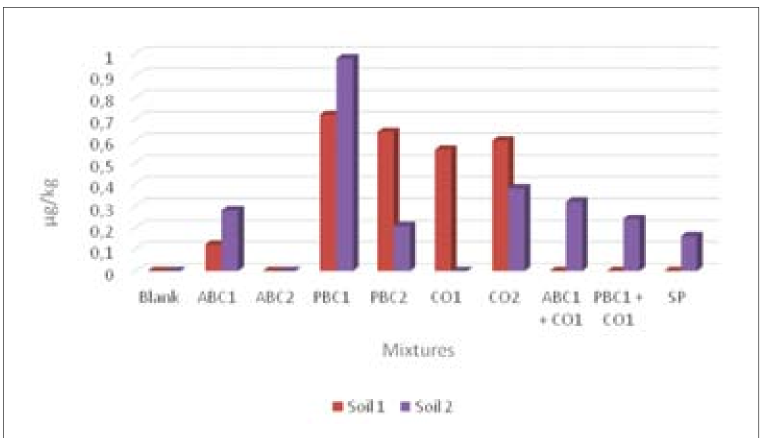


**Figure 8: Concentration of 16 EPA PAHs in distilled water eluates.**

From a practical point of view, it is significant to know how the elements leach out over time. What is the dynamics of leaching in time, does the elution have „chromatographic" properties? To investigate this, we repeated the elution tests with elution volumes of 10 liters instead of 2.5. In this case elution was performed only with distilled water. Because of the large amounts of solvents, the test was carried out only on two mixtures, which were prepared from soil 2: on the blank system for reference and on the lower dosed ABC mixture. At the end of the elution, a total of 9 and 8.5 liters of DW leached out, respectively. To find out whether separation of the components occured this time, eluates were collected in 5 fractions. Because volumes collected during the different stages were not identical, comparison of the absolute values was not trivial. Therefore, for easier understanding, percentage values of the amounts eluted are also shown (Table 8).In this case, we can examine 5 points in time to see the evolution of concentrations. Phosphorus is the most interesting element, because its leaching from

**TABLE 7: Concentrations of Mn, Zn, Cu, Cr, Sr, As, Cd, Pb in the eluates from soil 2 in mg/kg.**

| | | | Mn | Zn | Cu | Cr | Sr | As | Cd | Pb |
|---|---|---|---|---|---|---|---|---|---|---|
| SOIL 2 | Blank | al | 84.4 | 8.5 | 0.2 | 0.2 | 7.5 | ND | ND | 0.1 |
| | | dw | 0.2 | 0.1 | 0.0 | 0.0 | 1.0 | ND | ND | ND |
| | | o | 0.3 | 0.1 | 0.2 | 0.1 | 0.7 | 0.1 | ND | ND |
| | ABC1 | al | 92.8 | 95.8 | 0.2 | 0.2 | 7.1 | 0.1 | ND | 0.1 |
| | | dw | 1.6 | 0.1 | 0.0 | 0.0 | 1.6 | ND | ND | ND |
| | | o | 1.5 | 2.7 | 0.8 | 0.1 | 0.6 | 0.1 | ND | ND |
| | ABC2 | al | 78.0 | 6.8 | 0.1 | 0.2 | 6.7 | 0.1 | ND | ND |
| | | dw | 2.4 | 0.1 | 0.0 | 0.0 | 1.4 | ND | ND | ND |
| | | o | 0.7 | 0.2 | 0.8 | 0.1 | 0.7 | 0.1 | ND | ND |
| | PBC1 | al | 131.0 | 17.7 | 0.4 | 0.3 | 5.6 | ND | ND | 0.1 |
| | | dw | ND | ND | ND | ND | 1.0 | ND | ND | ND |
| | | o | 0.1 | 0.1 | 0.7 | 0.0 | 0.6 | 0.1 | ND | ND |
| | PBC2 | al | 116.8 | 82.6 | 0.3 | 0.3 | 5.2 | ND | ND | 0.1 |
| | | dw | 0.2 | ND | ND | ND | 0.6 | ND | ND | ND |
| | | o | 0.1 | 0.2 | 0.8 | 0.1 | 0.8 | 0.1 | ND | ND |
| | CO1 | al | 120.2 | 54.0 | 0.3 | 0.2 | 6.1 | ND | ND | 0.1 |
| | | dw | 0.8 | 0.1 | 0.0 | 0.0 | 1.1 | ND | ND | ND |
| | | o | 1.0 | 0.2 | 1.1 | 0.1 | 0.3 | 0.1 | ND | ND |
| | CO2 | al | 101.8 | 8.7 | 0.2 | 0.2 | 7.8 | 0.1 | ND | 0.1 |
| | | dw | 0.6 | 0.1 | 0.0 | 0.0 | 1.5 | ND | ND | 0.1 |
| | | o | 2.4 | 0.4 | 0.7 | 0.1 | 0.4 | 0.2 | ND | ND |
| | ABC1 + CO1 | al | 70.4 | 6.7 | 0.1 | 0.2 | 6.6 | 0.1 | ND | 0.1 |
| | | dw | 1.2 | ND | ND | ND | 1.6 | ND | ND | ND |
| | | o | 0.2 | 0.2 | 0.6 | 0.1 | 0.6 | 0.1 | ND | ND |
| | PBC1 + CO1 | al | 94.0 | 8.7 | 0.2 | 0.2 | 7.2 | 0.1 | ND | 0.1 |
| | | dw | 1.2 | ND | ND | ND | 1.7 | ND | ND | ND |
| | | o | 0.2 | 0.2 | 0.6 | 0.1 | 0.9 | 0.1 | ND | ND |
| | SP | al | 74.0 | 72.2 | 0.2 | 0.2 | 6.1 | 0.1 | ND | 0.1 |
| | | dw | 1.2 | ND | ND | ND | 1.5 | ND | ND | ND |
| | | o | 0.7 | 0.3 | 0.7 | 0.1 | 0.5 | 0.1 | ND | ND |

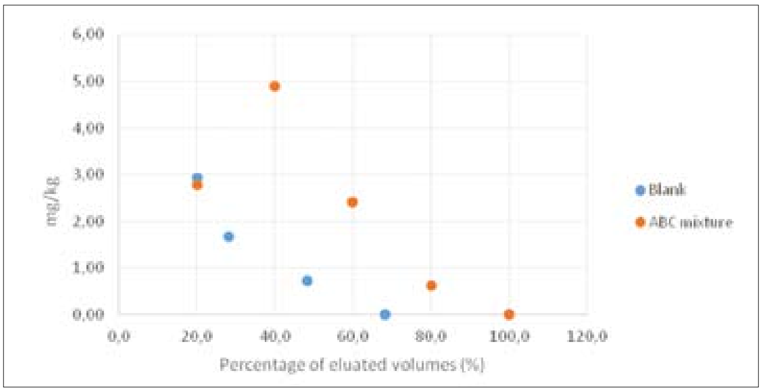


**Figure 9: Dynamics of phosphorus elution in the blank and the amended soil.**

**TABLE 8: Elution volumes in the „chromatographic" test, in absolute as well as percentage values.**

| BLANK - Volumes (l) | % | Soil 2 + ABC - Volumes (l) | % |
|---|---|---|---|
| 1,7 | 20,0 | 1,8 | 20,0 |
| 2,4 | 28,2 | 3,6 | 40,0 |
| 4,1 | 48,2 | 5,4 | 60,0 |
| 5,8 | 68,2 | 7,2 | 80,0 |
| 8,5 | 100,0 | 9 | 100,0 |

amended soil changed in time. The leaching curve has a maximum, it is not a strictly decreasing function (Figure 9). Concentration of phosphorus eluted from the blank system decreased continuously, while mobilization of phosphorus from the amended system was increasing for a certain volume and then it also decreased.

All other elements studied presented strictly decreasing leaching curves (Figures 10, 11, 12). However, in all of the cases (Figures 9, 10, 11, 12), this decrease was steeper in the blank than in the amended soil, showing the retention capability of the additive. Be-

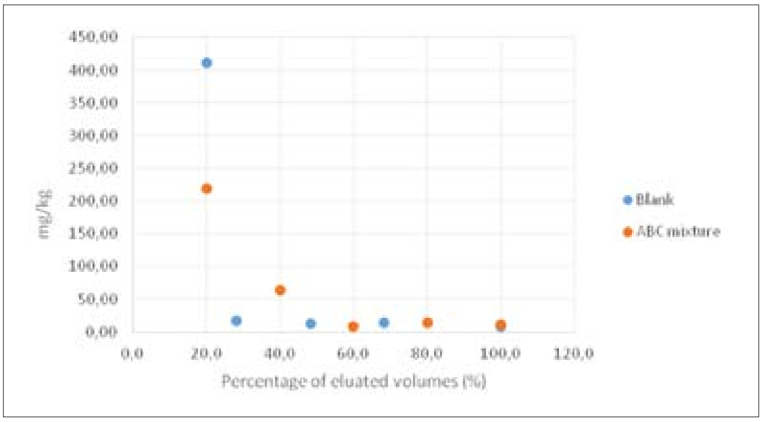


**Figure 10: Dynamics of calcium elution in the blank and the amended soil.**

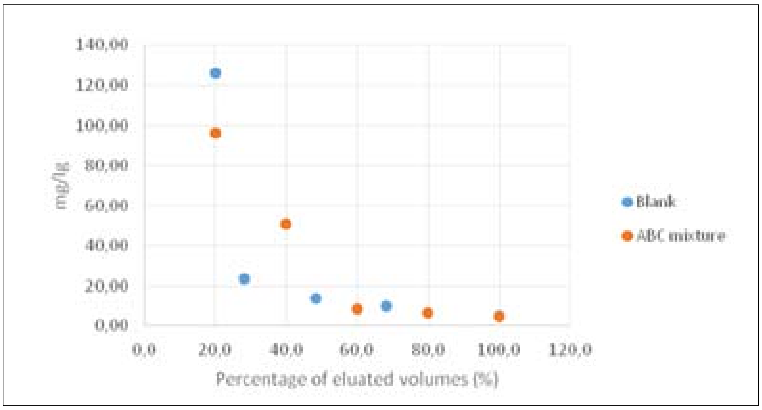


**Figure 11: Dynamics of potassium elution in the blank and the amended soil.**

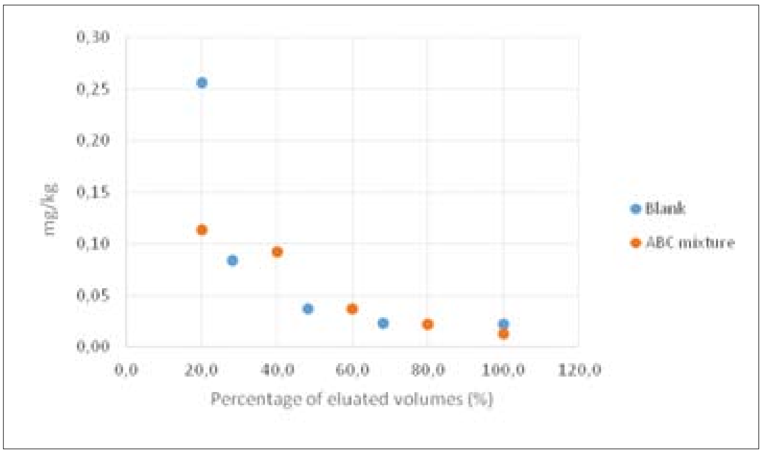


**Figure 12: Dynamics of zinc elution in the blank and the amended soil.**

sides, during the second stage of leaching, the amounts leached from the amended soil were always higher than those leached from the blank. Most likely, this was because addition of the amendment also meant nutrient addition as well.

## CONCLUSIONS

The results presented here and the consequences are promising, as the impact of soil on the effects of additives, and on their nutrient and water retention capabilities were confirmed. However, these experiments should be considered as preliminary experiments, since they raised many further questions. First, it is necessary to spike the studied systems with the target components, especially heavy metals and PAHs, to see clearly the possible dynamics of leaching. Also, using additives with much higher toxic component concentrations can also provide an answer to that question. Another very important issue is the effect of soil type on the amendment. We need to conduct more experiments with further different types of soils and determine how soil properties affect leaching. Then, we will be able to recommend amendment expected to be effective for certain types of soils. In addition, each biochar and compost soil amendment produced from different feedstocks or under different technological conditions should be analyzed thoroughly to prevent possible environmental contamination.

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# PROFESSOR JÓZSEF ANTAL – A PROMINENT SCHOLAR OF CROP SCIENCE

MÁRTON **JOLÁNKAI**

József Antal was born in a nobleman's family on the 18th of March 1919 in Marosbogát, Transylvania. He was educated in a straighforwarding way in the János Bólyai Protestant College at Marosvásárhely. He has begun his agricultural studies at the Agricultural Faculty of the Kolozsvár University in 1937, wherefrom he graduated in 1943. From 1948 to 1952 he was employed by the administration of Csongrád County in Hungary. His was engaged in various fields of education and public administration.

In a long period between 1952 and 1974 he was a researcher of the Agricultural Research Institute of the Southern Great Plain, which was the succeeded by the present Cereal Research Institute in Szeged. In this institute he has experienced all levels of a scientific career; from research assistant to the position of the director of research.

Professor József Antal (Photo: M.Birkás)

In 1974 he was appointed by the Gödöllő University of Agricultural Sciences. As an experienced and renowned scientist he was invited to be the head of the Crop Production Institute of the university. Since that time he labels the scientific and educational activities of Gödöllő. He had been the professor of crop production, head of department and then of institute, and today he is a highly appreciated professor emeritus, - simply a father of all crop science scientists.

A brief summary of his exceptional scientific work. In the beginning he has taken part in the mapping of the highland pastures in the area of the Eastern Carpathian Mountains. Later he was engaged in scientific research related to potato and sunflower production. Most of his active life was dedicated to the research of sandy soil's agronomy; he used to be a forerunner in cultivation of sandy soil areas as well as a successful breeder of bean varieties adapted to such conditions. He had run extensive crop rotation trials as well as green manure field experiments. There were a wide range of field crops he worked with; legumes, fodder crops, medicinal plants and potato secondary cropping. His results in the field of oil seed radish are of international importance. Also he has contributed plant nutrition practices with his research results. In the field of agro-ecology he has established a series of basic principles. He has been a diligent and successful writer all along his life. His main works are the „Növénytermesztés homokon" (Crop production on sandy soils) and the „Növénytermesztők zsebkönyve" (Crop production vademecum) – the „antal" – as it is labelled by generations of agronomists and farmers. The crown of his lifelong work is the recent edition of the national handbook „Növénytermesztés" (Crop production), which serves the education of four universities and six agric colleges as well as thousands of farmers.

In 1956 he got his CSc degree, and in 1974 he was given the DSc degree of the Hungarian Academy of Sciences. He was decorated by so many national and international scientific awards. Some of the most important ones: Westsik Vilmos insignum (1989), Beethoven-Brunswick plaque (1989), Gisevius Prize (1994), Baross László insignum (1994), and last but not least the highest award of crop production of Hungary, the Surányi insignum (2005).

Professor József Antal is 95 years old by now. However he is active, as he has always been during his life. A loving society of colleagues, students, theoretical and practical agriculturists surround him, and he broadcasts miracles of his personality and rudiments of a wisdom he possesses.

# THE ANAC042 TRANSCRITION FACTOR GENE IS RESPONSIVE TO POWDERY MILDEW INFECTION IN ARABIDOPSIS THALIANA

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## ABSTRACT

Powdery mildews (PMs) are obligate plant pathogenic fungi that cause major economic losses worldwide. These pathogens trigger a complex defense reaction in plants, which involves the activation of stress-related proteins, transcription factors, and signaling pathways. The PM/plant interaction has been under intense scientific investigation, but some of the key mechanisms of pathogenesis and plant resistance remains to be answered. Numerous plant genes have been identified to play a role in pathogenesis and defense, including genes encoding NAC transcription factors. Earlier studies of the model plant Arabidopsis thaliana showed that AtNAC transcription factors increased in abundance during fungal infection. A recent study demonstrated that the expression of ANAC042 was induced in response to abiotic stresses, and infection by Alternaria brassicicola and Pseudomonas syringae pv. tomato. This study examined whether ANAC042 also responds to a biotrophic fungus, powdery mildew, and whether the signaling molecule, SA is required for the pathogen-responsiveness of this gene. To study the regulation of ANAC042, we isolated the promoter (pANAC042) of this gene and fused it to the reporter gene encoding the beta-glucuronidase (GUS) enzyme. We transferred this construct into three (wild type, nim1-1/SA signaling deficient, nahG/lack in SA) Arabidopsis thaliana plants. We found that pANAC042 provides constitutive GUS expression at a basal level when the plants are not challenged by a pathogen or are not exposed to abiotic stress. We also determined that GUS expression from the fusion construct was induced significantly above the basal level only at those areas of leaves, where the epidermal cells were directly attacked by PM. The basal as well as the pathogen-triggered expression were similar in the wild-type, nim1-1, and nahG Arabidopsis lines. Based on the results and previous studies, we conclude that the ANAC042 promoter directs transcription of the reporter constitutively at a basal level, responds to PM infection by up-regulation, and that neither SA, nor the SA-mediated signal transduction is required its regulatory function.

**keywords: NAC transcription factor, pathogen, signaling pathway, Arabidopsis**

## INTRODUCTION

Biotrophic fungal pathogens, such as powdery mildews (PMs) cause substantial losses in agriculture. The over 700 known PM fungi are adapted to a broad range of host plants including both monocotyledonous and dicotyledonous species. All PM pathogens are obligate parasites. They establish a feeding structure, named haustorium in the epidermal cells of photosynthetically active tissues of the host, through which they induce exceedingly complex changes in the plant's physiology. The two most important results from the perspective of fungal pathogenesis are manipulation of the plant's metabolism by which the pathogen to meet its nutritional needs, and the avoidance of the plant defense system.

Upon infection by a pathogen, the plant cell sets into motion a defense response initiated by an oxidative burst, followed by the production of phytoalexins (i.e. camalexin) and the expression of pathogenesis-related and stress resistance genes. The defense response is mediated by one or more of the primary signaling molecules, salicylic acid (SA), ethylene (ET) and/or jasmonic acid (JA), depending on the type of pathogen triggering the response. Furthermore, there appears to be a cross-talk among the various signaling systems (Verhage et al. 2010). SA was identified to be the key signal in the plant's interaction with biotrophic pathogen (Glazebrook 2005; McDowell and Dangl 2000), but it was found to promote pathogenesis by the nectrotophic fungus, Botrytis cinerea through the suppression of JA-dependent genes (Leon-Reyes et al. 2010; Rahman et al. 2012). In SA-treated plants, the early SA-regulated genes were involved mostly in cell protection, and signal transduction via protein kinases and transcription factors (Blanco et al. 2005). Numerous signal-dependent PR protein and transcription factor genes have already been identified as integral part of defense, including PR-1 (SA-dependent), ERF1, COI1, EIN2 (ET-, JA-dependent), PDF1.2, RAP2.6, JIN1 (JA-dependent) (Lorenzo et al. 2004; Lorenzo et al. 2003; Uknes et al. 1992).

The NAC (NAM, no apical meristem; ATAF, Arabidopsis transcription activation factor; CUC, cup-shaped cotyledon) transcription factors (TFs) constitute a large plant-specific gene family. The NAM, ATAF, and CUC genes define the conserved NAC domain on the N-terminal region of these proteins. Numerous NAC members have been characterized in various plant species. The NAC family has 117 members in Arabidopsis thaliana, 151 in Oryza sativa, 74 in Vitis vinifera, 167 in banana (Musa acuminata) and approximately 157 in Populus trichocarpa (Cenci et al. 2014; Grant et al. 2010; Hu et al. 2010; Nuruzzaman et al. 2010; Wang et al. 2013). Functional studies in the model plant Arabidopsis thaliana revealed that several NAC transcription factors respond to pathogen infection. The ATAF1 gene was determined to be a SA-, JA-, and ET-responsive gene, and found to negatively regulate resistance against Botrytis cinerea and Pseudomonas syringae (Wang et al. 2009; Wu et al. 2009). ATAF2 also was determined to be a negative regulator acting to further suppress the expression of pathogenesis-related proteins. The TaNAC4 and TaNAC8 genes of wheat responded to Puccinia striiform infection (Xia et al. 2010), while the Brassica napus genes BnNAC5-1, BnNAC5-7, BnNAC5-8, and BnNAC5-11 to Sclerotinia sclerotiorum (Hegedus et al. 2003). The HvNAC6 barley gene was determined to be a positive regulator of defense against powdery mildew, Blumeria graminis f. sp. hordei (Jensen et al. 2007).

The ANAC042 transcription factor was found to be active during abiotic stresses, such as heat and salt stress (Shahnejat-Bushehri et al. 2012; Wu et al. 2012), during senescence (Wu et al. 2012), and in response to total herbicide application (Saga et al. 2012). Moreover, its expression was found to be induced by biotic stresses, such as attack by the necrotrophic fungal pathogen Alternaria brassicola and the biotrophic bacterial pathogen Pseudomonas syringae pv. tomato (Saga et al. 2012). The rice ortholog of this gene responded to drought stress (Nuruzzaman et al. 2012), and the banana ortholog (MaNAC2, MaNAC4) played a role in fruit ripening (Shan et al. 2012). Based on the preliminary studies presented here, we conclude that the transcription of ANAC042 is up-regulated under abiotic and biotic stresses as well. It likely plays multiple roles, but its contribution to the regulation of the defense system remains to be conclusively demonstrated. The goal of this study is to observe how the plant regulates the expression of the ANAC042 gene in response to PM infection.

## MATERIAL AND METHODS

The regulation of ANAC042 gene was studies indirectly by analyzing the activity of its promoter. Based on genome sequence data in The Arabidopsis Information Resource (TAIR) database (www.arabidopsis.org), we designed primers (F-5'TTACAGCGAGGGAGATAATGA3' and R-5'TCGATCTCTTTAGAACACCAATCA3') to amplify a 3814 bp fragment upstream from transcription start site. This promoter fragment was then inserted into the pGWB633 (Nakamura et al. 2010) binary vector using the Gateway® technology (Invitrogen™). The promoter, named pANAC042, was cloned in front of the GUS reporter gene in pGWB633. The T-DNA of this binary vector contains bar gene, the protein product of which provides resistance against the herbicide, and thus serves as selectable marker. In addition to the pANAC042::GUS, a promoterless reporter construct, named pØ::GUS, was also created to serve as negative control. The pANAC042::GUS and pØ::GUS fusion-containing binary plasmids were transferred in Agrobacterium tumefaciens strain pMP90, which provided virulence functions from a resident helper Ti plasmid. The bacteria were used to transform three different Arabidopsis thaliana lines using the floral dip method (Bent 2006; Clough and Bent 1998). The Arabidopsis thaliana lines were: wild type Wassilewskija ecotype (WT), nim1-1 (non-induced immunity mutant, deficient in SA signaling), nahG (transgenic, containing the salicylate hydroxylase gene and lacks SA). The nahG and nim1-1 lines were both derived from Wassilewskija. Following transformation, the seeds of the T0 plants were germinated in soil in Petri dishes and cultivated under cool white light illumination, 16-8 hours light/dark diurnal cycle and at a temperature of 22°C. Positive selection for T1 transformants was performed by spraying 10 mg/l glufosinate-ammonium-containing herbicide (Finale) on two-week old seedlings (Nakamura et al. 2010). The spray was repeated three times. The surviving plants were selected and transplanted to produce T2 seeds. The transgenic lines were brought to T3 generation. T3 plants were tested for the induction of the pANAC042::GUS reporter construct by pathogen attack. This was accomplished by challenging four-week old transgenic plants with Oidium neolycopersici infection (Huibers et al. 2013). The control treatment was performed by mock infection of four-week old transgenic plants. Fourteen days post-inoculation (dpi), when powdery mildew colonies were readily visible, the leaves were collected from both control and pANAC042::GUS transgenic plants. To investigate GUS expression directed by pANAC042 in response to PM, the leaves were subjected to histochemical GUS assay (Jefferson et al. 1987). OLYMPUS Leica Leitz WILD M3Z stereo-microscope was used to observe the stained leaves. The nucleotide sequence of pANAC042 was analyzed using the PLACE database (Higo et al. 1999) to putatively identify regulatory elements of the promoter.

## RESULTS

During preliminary examination of transgenic plants by histochemical GUS assay, we observed that the positive pANAC042::GUS transformants expressed GUS at a basal level in the absence of environmental or biotic stress influence compared to the promoterless control pØ::GUS transformants. (Promoterless transgenic plants had no detectable GUS expression (Figure 1F). This phenomena was consistent in all three Arabidopsis lines. The GUS staining was observed mostly at leaf margins, root tips, lateral shoot buds and vascular tissues (Figure 1A-E).

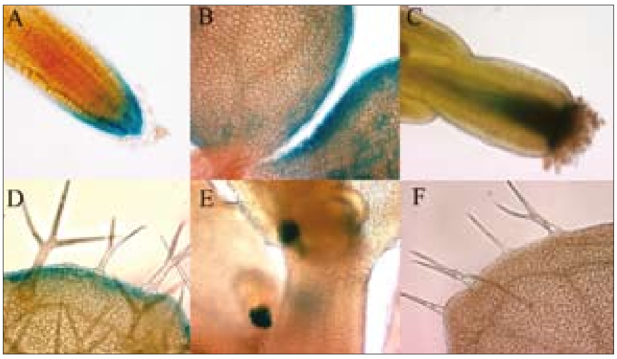


**Figure 1: Microscopic pictures of basal expression of positive transgenic plants. A) Root tip of five-day old seedling; B) Cotyledon of five-day old seedling; C) Septum of silique of five-week old plant; D) Leaf of four-week old plant; E) Lateral buds in flower bolt of four-week old plant; F) pØ::GUS control plant.**

Using GUS staining and microscopy in PM-infected leaves at 14 dpi, we determined that GUS expression significantly increased in those areas of leaf at which the fungus made direct contact with the epidermis (Figure 2). This phenomenon was consistently observed in all three Arabidopsis lines used in this study, and we were unable to detect GUS staining in any of the pØ::GUS control plants

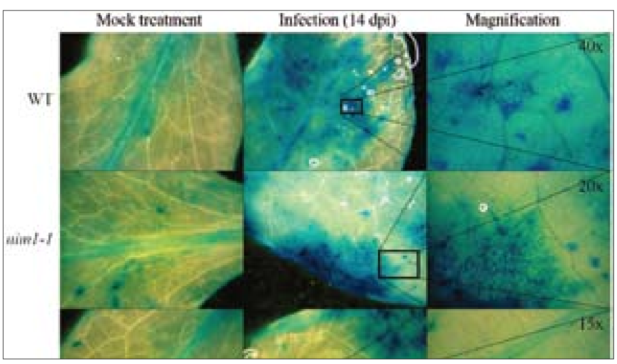


**Figure 2: Microscopic pictures of mock treated and infected plants of all three types of Arabidopsis.**

Scanning the nucleotide sequence of the 3814 bp ANAC042 promoter fragment, we identified putative abiotic-, biotic-stress dependent and hormone-responsive elements. Among the biotic-stress related elements were an ANAERO1 consensus sequence, a CBF-binding site, a GT-1 motif, and several LTRE elements, which are hypothetically involved in regulating gene expression in response to anaerobic conditions, dehydration, salt stress, and cold stress, respectively. We also pinpointed numerous abscisic acid-responsive, and several ethylene-, gibberellic acid-, auxin- and jasmonic acid-dependent consensus elements. The identified biotic-stress sensitive elements were G-box, ELRE, GCC-box, GT-1 motif and numerous W-boxes (Table 1).

## DISCUSSION

The goal of this study was to observe an Arabidopsis NAC transcription factor regulation in response to powdery mildew infection. GUS staining of this NAC promoter-GUS fusion transgenic plants provided indirect evidence that ANAC042 is expressed at a basal level in a variety of tissues. Among these are actively developing tissues that contain actively dividing cells, such as root meristem, flower primordia in developing inflorescence, the septum of siliques, and the edge of expanding young leaves and cotyledons. These expression patterns were observed under normal growth conditions, in the absence of biotic or abiotic stress. In addition to observing wild-type plants, we also studied ANAC042 promoter acivity in nim1-1 mutant and nahG-transgenic plants which are deficient in SA-signaling. ANAC042 basal expression was also present in these SA-deficient mutants, suggesting that this basal expression is independent of SA and SA-mediated signal transduction, and probably mediated by developmental signals. Expression of this NAC gene was identified in the same plant organs in Col-0 wild type Arabidopsis too by Wu (2012), suggesting that this NAC transcription factor is active in actively developing plant tissues. In Oidium neolycopersici-infected leaves at 14 dpi, substantial GUS staining was observed only at the area, where the plant was directly contacted to the fungus. Saga et al. (2012) showed in their study, that ANAC042 transcription factor regulates camalexin biosynthesis and it could also be induced by the general pathogen-associated molecular pattern protein flg22, the necrotrophic fungus Alternaria brassicicola and the biotrophic bacterium, Pseudomonas syringae pv. tomato. PM-triggered GUS expression was detected in all three Arabidopsis lines, proving that the SA-mediated signaling pathway is not the responsible for the pathogen-triggered induction of the ANAC042 gene. This result corroborates Saga's experiment, where the flg22-induced GUS expression in the SA-defective sid2-2 mutant did not differ from the flg22-induced expression in the wild type. Overexpression of ANAC042 gene resulted a reduction of SA and JA signals compared to the control. The anac042 knockout mutant showed a significant increase of ABA compared to the wild type (Wu et al. 2012). In contrast to the ABA-deficient mutants showed substantial increase in camalexin level

**TABLE 1: Putative transcriptional regulatory elements identified in the ANAC042 promoter.**

| Cis-element | Inducer | Cis-element location in the promoter upstream from trancription start site (bp) |
|---|---|---|
| ABRE (ACGTG) | ABA-responsiveness | 789*, 3365, 3757 |
| G-box (CACGTG) | Biotic stress/ABA-resp. | 788***, 3756 |
| DPBF-binding site (ACACNNG) | ABA-responsiveness | 160*, 3756 |
| ELRE (TTGACC) | Elicitor-resp. | 126**, 2951 |
| ERE (AWTTCAAA) | ET-responsiveness | 655*, 2937, 3768 |
| GCC-box (GCCGCC) | Pathogen-resp. | 2320** |
| GT-1 motif (GAAAAA) | Pathogen/salt-resp. | 1463***, 1514***, 1612***, 2055, 2077, 2498, 3227 |
| MYB-binding site (GTTAGTT) | Biotic stress | 3292 |
| T/G-box (AACGTG) | JA-responsiveness | 3364 |
| WB-box (TTTGACY) | Biotic stress | 143**, 1872**, 2014, 2951 |
| W-box (CTGACY) | Elicitor-responsiveness | 1439** |

**Putative reponisble elements: *Abiotic stress-related regulatory element consensus sequence; **Biotic stress-related regulatory element consensus sequence; *** Consensus sequence implicated in both biotic and abiotic stress regulation**

resulting resistance (Kaliff et al. 2007) suggesting that ABA signal could not be indifferent in regulation of ANAC042 gene.

Analyzing the ANAC042 promoter sequence, we identified hormone signal-dependent consensus regulatory elements, such as abscisic acid-, auxin-, giberrellic acid- and ethylene-responsive elements, which are located relatively close to transcriptional start site. The results by Saga et al. (2012) demonstrated that flg22-induced expression was significantly lower in ein2-1 (ethylene-insensitive) mutant compared to wild type and sid2-2 mutant. Taken together results by Saga et al. (2012) and results presented in this communication, we conclude that ethylene may be one of the probable signaling molecules involved in the induction of the ANAC042 gene during fungal infection. The elicitor responsive elements (W-boxes) identified by our in silico promoter scan may also contribute to the regulation of ANAC042 during biotic stress.

## CONCLUSIONS

Our study goal was to observe regulation of ANAC042 gene during biotrophic fungal infection. We determined evidence that ANAC042 can be induced by PM infection, and the increased expression is localized only at the infected areas of leaves. The PM-dependent induction of this gene is independent from SA and SA-mediated signal pathway.

## ACKNOWLEDGEMENTS

The research was supported by the Hungarian Scientific Research Fund (OTKA 77867), the TÁMOP-4.2.2.B-10/1 project („Development of a complex educational assistance system for talented students and prospective researchers at the Szent István University") and the Research Centre of Excellence (17586-4/2013/TUDPOL). We thank Dr. Tsuyoshi Nakagawa (Shimane University) for providing Gateway binary vector pGWB633 which contain the bar gene identified by Meiji Seika, Ltd.

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# BORN TO BE A SCIENTIST

## CONGRATULATIONS NOTE ON THE 70TH BIRTHDAY OF PROFESSOR DÉNES DUDITS

ERVIN **BALÁZS**

Dénes Dudits (Mosonmagyaróvár, 1943), a young enthusiastic graduate student at the Agricultural University, Gödöllő, has started his scientific carrier studying genetics with pea mutants in the Department of Genetics and Plant Breeding, in an era when this discipline has been badly influenced by Lysenko's unscholarly ideas. His professor, Andor Bálint, recommended to professors István Láng and Brunó F. Straub to invite him, the talented young scientist to join the Biological Research Center (BRC) in Szeged. Following the request from his director, professor Lajos Alföldi in the Institute of Genetics, Dénes Dudits established a tissue culture laboratory for the in vitro regeneration of wheat plants from somatic tissues (Dudits et al., 1975, Canadian Journal of Botany 53:957-963). His efforts were strengthened by new technologies after joining the Prairie Regional Laboratory in Saskatoon, Canada, with an UNESCO fellowship. There he learned the most efficient technique of plant protoplast fusion from K. N. Kao, and among the firsts in Europe he introduced this genome engineering technology to his laboratory in the BRC, making it competitive in this research area. During this period he and his team produced asymmetric hybrids between distantly related plant species (Dudits et al., 1987, Proceedings of the National Academy of Sciences USA, 84:8434-8438) and they were able to transfer intact isolated plant chromosomes (Szabados et al., 1981, Planta, 151:141-145).

He invited to his laboratory Dr Csaba Koncz, a young student of professor Pál Venetianer, who already had experience in bacterial genetic engineering. As the start of the recombinant DNA work with plants in Hungary, a male sterile maize mitochondrial plasmid was cloned (Koncz et al., 1981, Molecular General Genetics, 183:449-458). His interests in gene technology lead him to Boston as a Harvard University guest professor for two years. Upon his return to Szeged his research focused on plant genetic engineering that has resulted in the first published transgenic alfalfa in the world (Deák et. al., 1986, Plant Cell Report 5:97-100). Later in collaboration with professor Ervin Balázs his group also produced transgenic potato plants, resistant to potato virus X (Fehér et. al., 1992, Plant Cell Report, 11:48-52). During this very active period his laboratory has developed a highly efficient maize transformation system with Dr Sándor Mórocz from the Cereal Research Institute (CRI), Szeged (Omirulleh et al., 1993, Plant Molecular Biology 21:415-28). The related patent owned by the BRC, CRI and Hoechst AG was used for the breeding of herbicide tolerant maize genotypes presently cultivated as LibertyLink® hybrids. He extended his research portfolio to studies on cell cycle control genes, protein complexes (Dudits et al., 2011, Annals of Botany, 107:1193-202), and the molecular basis of the totipotency of plant cells (Dudits et al., 1991, Journal of Cell Science, 99: 475–484).

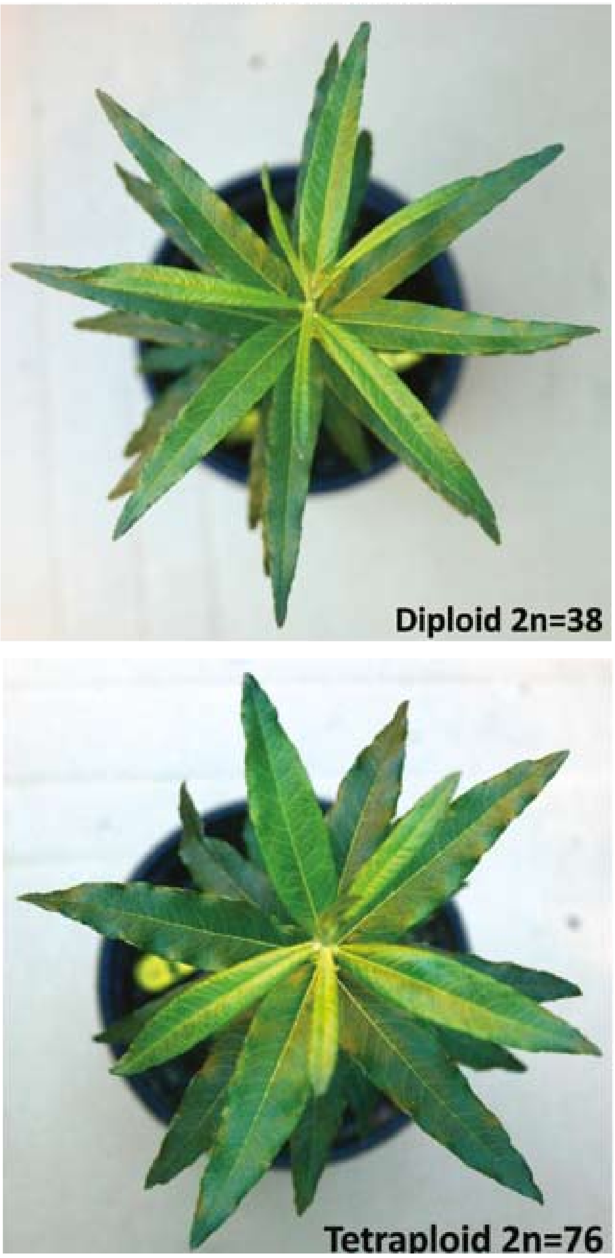
During these years he earned greater international recognition. His total citation stands at 4.386, his Hirsch index is 38. Meanwhile he went through the scientific evaluation steps by obtaining the Candidate then the Doctor of Sciences degrees. He became the correspondent member of the Hungarian Academy of Sciences (1990) and five years later he has received the full membership (1995). He is also the member of the European Molecular Biology Organization (EMBO), the European Plant Science Organization (EPSO) and the European Academy. In the

BRC, in Szeged he served for 10 years as director of the Institute of Plant Biology and for 13 years as general director of the Biological Research Center. He also has been elected for two cycles as vice-president of the Hungarian Academy of Sciences, representing life sciences (2008-2014). He was rewarded with the highest Hungarian State Award for sciences, the Széchenyi Prize (1995).

In the last two decades realizing the importance of modern plant breeding techniques for the practical agriculture he strengthened his research on transformation of higher plants with useful genetic traits such as drought tolerance in wheat (Fehér et al., 2014, Acta Physiologiae Plantarum 36:663–673). He has initiated the construction of a semi-automatic phenotyping platform for the analysis of the role of allelic variants in the drought response of barley genotypes to water limitation (Cseri et al., 2013, Australian Journal of Crop Science, 7:1560-1570). Presently he is coordinating a polyploidization project to produce tetraploid (2n=76 chromosomes) energy willow. These genotypes show larger leaf area and assimilate more $CO_2$ than the diploid control plants.

He also pioneered in introducing plant biotechnology by establishing the Agricultural Biotechnology Committee under the Department of Agriculture of the Hungarian Academy of Sciences, and in 1999 he founded the Zoltán Barabás Biotechnology Association to promote plant biotechnology. He is an outspoken supporter of genetic engineering, opposing all non-scientific arguments on this hot topic in European societies. This led him to publish several articles and book chapters to educate the public on genetics in a historically repeated unscientific era (Genetically Modified Organisms (GMOs): The Hungarian White Book eds: Ervin Balázs, Dénes Dudits, László Sági; www.zoldbiotech.hu). His scientific commitment to improve the competitiveness of the Hungarian agriculture with novel breeding techniques will never be stopped by the ignorance of the decision makers, and hopefully his optimistic view on this topic will soon crown his efforts with the realization of his vision.

**Expression of a detoxification alfalfa gene in GM wheat (Fehér-Juhász et al. 2014 Acta Physiologiae Plantarum 36:663–673)**



**Larger leaves of the tetraploid 2n=76 energy willow (Dudits et al. unpublished)**

# THE AGROECOLOGICAL ASPECTS OF REGION-SPECIFIC MILLET PRODUCTION

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## ABSTRACT

We have started our studies based on the results saying that negative climatic water balance is typical of most of Hungary including Nagykunság, which means that the yearly amount of potential evapotranspiration exceeds the yearly amount of natural precipitation. Climatic water shortage can be around 200-300 mm depending on the year. In light of this, it can be said that the ecological factor that influences and determines the development of agricultural production and by doing so, environmentally friendly plant cultivation the most is water. Beside all of this, soil with a mechanically heavy composition, high clay amount, disadvantageous water and air circulation and often saline that characterises Nagykunság greatly influences the structure of production. This is precisely why those plants have to be promoted that can cope with such peculiar ecological factors which is in fact the essence of region-specific production. Based on this, the subject of our studies is millet, the cultivation of which has a great tradition dating back to the distant past in Nagykunság – not a bit by chance.

A crucial point in our work was to gather data and information, to make examinations related to millet cultivation. The Karcag Research Institute of the University of Debrecen Centre for Agricultural and Applied Economics Sciences provided an excellent background for us where – taking the aspects of region-specific farming into regard – own-bred millet species are cultivated under special agro-ecological circumstances and millet farmers of the region are provided with seeds.

During our research, we mapped and summarised the peculiarities of cultivating millet and we conducted lysimeter measurements to monitor the effect of millet on water circulation of the soil under the ecological circumstances of Nagykunság. Our goal was to fit millet optimally into region-specific production by harmonising local natural factors and the demands, agro-ecological peculiarities of millet.

**keywords: millet, region-specific production, agro-ecological circumstances, weight lysimeter, water balance, evapotranspiration, drought, native species, Nagykunság, traditions, healthy food, second sowing, main plant**

## INTRODUCTION

Region-specific production is the traditional production structure that has been developed adapted to geographical, climate, biological and soil conditions, a certain regional specialisation of agricultural production, farming that suits natural conditions the best, taking the biological demands of plant and animal species into regard to the fullest extent.

The advantage of region-specific farming is that a relative optimum for the place of cultivation can be defined for all plant and animal species where high quality above-average yield can be achieved with lower input levels.

Choosing species selection appropriate to the place of cultivation is a defining issue of region-specific production that is especially significant in accommodating to hectic, extreme weather.

Promoting region-specific production is an important task from the perspective of environment protection, too, because plant species that can adapt to their environment well can be cultivated without chemical fertilizers and synthetic pesticides.

The motivation for the choice of our topic mainly derives from the fact that in the unfavourable areas of the Northern Great Hungarian Plains – such as for example in Karcag – the potential for plant cultivation is limited, production risk is higher than in other regions, therefore the optimal choice of plant species that can be cultivated successfully is outstandingly important. This is precisely why it is useful to go back to old native species, breeds, to cultivate them again, because hardly any other culture can suit the ecological circumstances of each region better than these. One of such defining plants of Nagykunság was millet that had been sunk into oblivion in recent decades, but nowadays more and more attention is paid to it. Starting with this basic idea, we have built our research work on millet.

According to our observation, it is a plant with huge perspectives from many regards. We think that it can be used in region-specific production excellently thanks to the short time it takes to be grown, its advantageous propagation rate and relatively low demands.

We have also paid bigger attention to millet because we regard balanced, healthy diet important, and it can be fitted into it very well, its physiological effects are outstandingly good for the human body thanks to its excellent content values. Besides, it can enrich the diet of those who have more and more widely occurring gluten intolerance due to its lack of gluten.

During our research, we conducted lysimeter measurements to quantify the effect of millet on water circulation in the Karcag Research Institute with the goal to prove that the plant is really ideal for cultivation under local ecological circumstances

## MATERIAL AND METHODS

Nagykunság and Karcag lie in the middle of the mesoregion Great Hungarian Plain by Tisza, more specifically, in the Middle-Tisza agro-ecological district. This district is characterised by frequent droughts in the summer, a patchwork of different soil qualities and disadvantageous agro-ecological potential (Bocz 1996a).

The area of Nagykunság, therefore for example Karcag is one of the places of Hungary with the most dry, warm climate and with the most extreme temperatures.

With an average precipitation around 500 mm per year, Nagykunság can be regarded as one of the most dry lands of the Great Hungarian Plains and also of the whole country. Beside an extraordinarily low amount of yearly precipitation, its yearly distribution is very disadvantageous, and years with extremely high levels of precipitation also occur.

The yearly amount of potential evapotranspiration is higher than 700-800 mm. Most of Hungary and as such, Nagykunság is characterised with a negative climatic water balance, in other words, the yearly amount of potential evapotranspiration exceeds the yearly value of natural precipitation. Climatic water shortage can be around 200-300 mm depending on the year.

Soil with a mechanically heavy composition, high clay amount, disadvantageous water and air circulation that is typical of Nagykunság and the third of which is saline greatly influences the structure of production. This is precisely why those plants have to be promoted that can cope with such peculiar ecological factors which is in fact the essence of region-specific production.

Millet is the collective name of certain species in the Panicum and Pennisetum genera belonging to the family of true grasses (Poaceae) and the subfamily of Panicoideae. The word is often used as a shorter name of the best known millet species, proso/common millet. It was even cultivated 6000 years ago in Mesopotamia. It has made its way to Asia, Africa and Europe in the classical and medieval period, and in the 16th century to America by Spaniards and Portuguese (I1).

80–85 percent of millet produced in the world is consumed by humans. In Hungary, it is only preferred by the followers of reform cuisine and traditionalists, most people cultivate it for bird fodder.

The share of Hungary in millet production is really small but shows a growing tendency. Even the total production of EU member states is below 0.3% and after a significant drop, nowadays it shows some increase (Lazányi 2010).

Millet was the most significant cereal of medieval Hungary but its consumption have been scaled back after the appearance of cereals for making bread (Léder and Monda 1987).

The physiological effects of millet are outstanding for the human body. It is our only basic cereal (meaning it has alkaline effect), therefore it is particularly appropriate for sick people and youth, children, and it also contains important compounds with antioxidant effect. (Léder 2010). Its protein content is not much higher than what corn has, but it also has significant Fe- and Si-, Mn-, Zn-, and Cu- content; these are all vital microelements for the human body. Its fat content is usually 3-6%, and its content of essential fatty acids is exemplified by its especially high linoleic acid content (I2; Klopfenstein and Hoseney 1995). Beside all, millet protein has a gluten content that is under the threshold (Alinorm 2008), therefore millet-based foodstuff can be fitted into the diet of gluten intolerants excellently. The diet of people with liver problems is the most important among medical diets. The high methionine, cystine and cysteine content of millet helps the regeneration of the liver and thereby the healing of the patient (Lazányi and Gocs 1999).

In Hungary, formerly we had cultivated it in bigger areas for porridge. Currently it is significant only in a small area, mostly in succession cropping, replacing destroyed plantations and places with water pressure thanks to its short lifespan (Bocz 1996b).

### Ecological demands

Easily warming, not really wet but rather dry soil is suitable for cultivating millet. Medium hard field or forest soil with good nutrient content is best for it. It can be sown at sands with humus where nutrient supply is good. Watery and cold soil is unsuitable for its cultivation (Nagy 2005). Millet is often cultivated on areas with a thin fertile layer, places with high water pressure and that warm up late or dried-out soils. Many people think that millet has low demands for soil but its ecological demands can only partially be satisfied in such areas. Millet cannot be sown on soils with extremely unfavourable heat, water, air and nutrition circulation (Chrappán 1997).

Láng et al. (1958) said that "millet is not at all drought resistant as it is thought". Millet does not need much water but precipitation, more specifically the distribution of precipitation that falls in its vegetation period has an important role in its yield. It demands half as much water for germination as wheat, rye, barley or maize. Similarly, its transpiration coefficient is also better both until it shoots up and from then on until it ripens. Therefore the water loss of the soil causes lower yield only in the period after it shoots up. From the perspective of water need, the period between the appearance of panicles and the start of ripening is crucially critical (Bocz 1996b).

The heat need of millet is significantly higher than other cereals. It demands a temperature higher than 10°C for germination. The effective heat sum demand of general cultivation is 1400°C, plenty of which is available in Hungary. The critical periods of the heat demand of millet are germination, flowering and ripening. For germination, it needs a daily average temperature of at least 12-14°C, 16-18°C for tillering and 20-22°C in the flowering period. Millet is sensitive for frost, therefore it is not advisable to sow it before May in the Carpathian Basin (CHRAPPÁN, 1997).

## Weight lysimeter measurements of the water circulation specifics of millet

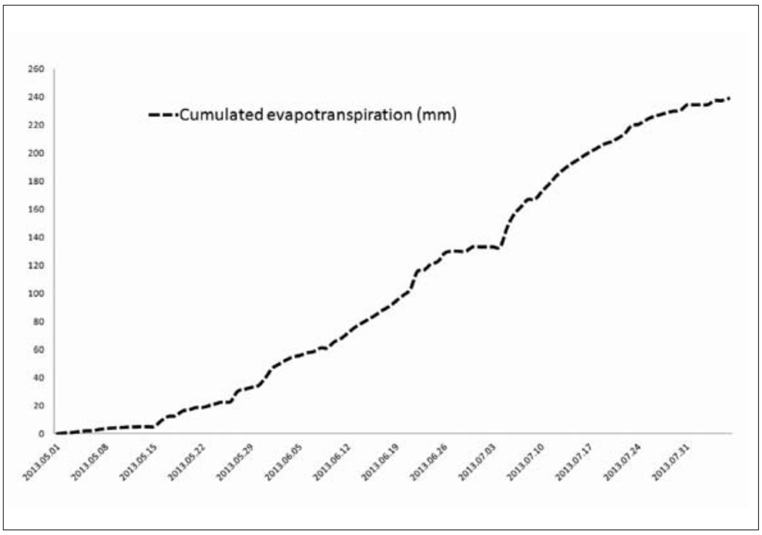
Karcag Research Institute has two own-bred millet strains with which we were able to conduct various experiments, examinations. To create a basis for research in production technology that spares more water, a computer-operated weight lysimeter system was set up in 1992-1993 with six units that is unique in Hungary both regarding its size and measuring sensitivity. With its help, the water consumption and utilisation of plants as well as the water and salt circulation of soils can be measured with high precision.

In 2013, we have set an experiment at three lysimeters of Karcagi Research Institute to monitor the effect of millet on water circulation under the ecological circumstances of Nagykunság (Picture 1). With this, we were able to specify the dynamics of water consumption and its water usage regarding the whole lifespan for the whole year under given circumstances. The seeds of the experimental plant had been sown at the 1st of May and the ripened stock was harvested merely 98 days later, at the 6th of August. During this period, only 153 mm of precipitation fell – most of it in May -, and except that, 10-10-10 millimeters of water was provided for each lysimeter during flowering.

## RESULTS

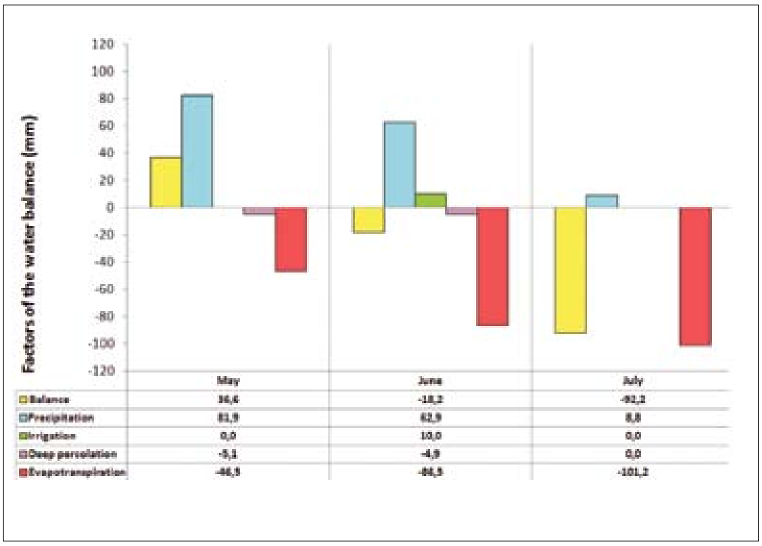
### The cumulative evapotranspiration of millet

In Picture 2, we have graphed the cumulative evapotranspiration results calculated for the whole lifespan based on data from the lysimeter covered with millet. It is apparent that the rate of transpiration is relatively steady for the whole examination period, no real spikes can be observed. based on this, we can draw the conclusion that since the evapotranspiration of the plant is not higher in

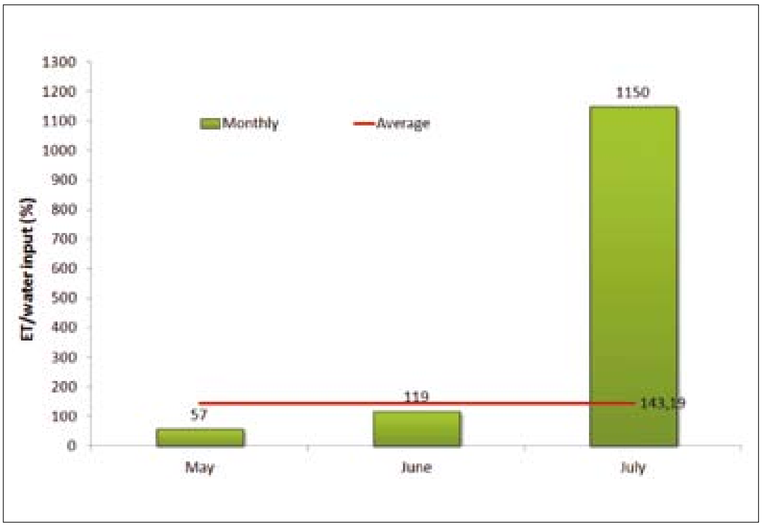



**Picture 2: Values of cumulative evapotranspiration for the whole lifespan of millet (2013. 05. 01. - 08. 06.)**

**Picture 1: Examination of soil circulation with millet by weight lysimeter**




**Picture 3: Monthly water balances of millet between 01.05.2013. - 31.07.**




**Picture 4: Evapotranspiration per water input unit for millet from 01.05.2013. to 31.07. for each month**

warm, dry periods than in milder weather, millet has high tolerance for drought. Therefore its use of water is steady, only a smaller increase can be observed at the time of tillering and between flowering and ripening. The results measured by us are in sync with what has been said by practising millet farmers: millet manages the water content of the soil well, if it gets the water it needs for germination and initial progress, it steadily grows even in dry weather conditions.

### The water balance results of millet

In Picture 3, we have indicated the monthly water balance of three months of the examination period and the values of its contents. On the compound graph, the first period of the parts reflecting each month is the water balance itself, the second two show the inputs, and the last two the depicts the outputs. We have indicated the inflowing water amounts with a positive sign and the outflowing ones with a negative, therefore it is apparent that the inputs increase the balance and the outputs decrease it as well as their amount.

During the three months of the short lifespan of millet, the highest amount of precipitation fell in May. Sprung-up millet that only started to grow used only half of it. The input water amount unused by the plant contributed to the increase of water amount stored by the soil resulting in positive monthly water balance. There was less rain in June than in the previous month but the stock was watered, therefore roughly the same amount of incoming water was available for millet as in May. Despite this, water balance was negative, in other words, the plant used the water stored in the soil beside the total water input to supply the water amount needed for its development. This is due that the phenological phase of millet when it requires the most water is in this period, from flowering to ripening around the end of June – the beginning of July. Therefore the transpiration value for July is almost the same as for June. It is also adds up that drought is typical of this month, the stock was forced to lean on the use of water in the soil completely, therefore the value of the water balance was significantly in negative territory.

## DISCUSSION

### The efficiency of millet water usage

In Picture 4, we have graphed the transpiration for each unit of water input that got in the lysimeters by natural precipitation or watering per month and in total during the three months. This index shows whether the water provision of the plant was enough (in cases where it is 100% or lower) or in other cases how much water it used from the soil. This coefficient practically also shows whether water balance was positive or negative.

Millet reduced the original water supply of soil by 45% of the total amount of water it used for evapotranspiration. This is due to that although in May, weather conditions were considerably advantageous, the plant used only half of the amount of water input; at the end of June – the beginning of July, when it would have needed the most precipitation based on its development condition, millet acquired it from the water supply of the soil because of the great drought. The plant did not suffer from water

shortage in June that was extremely dry; it managed the water supply of the soil well. This also proves the rationality of its cultivation under the examined circumstances.

The drought tolerance of millet is well-exemplified and supported by the crop results under the circumstances with the lysimeter: the 470 g yield from 2 $m^2$ is equivalent to 2.4 t/ha crop result, meaning above-average yield.

## CONCLUSIONS

According to our examinations, we have found out that by cultivating millet as a main crop, it would be possible to utilise many poor-quality areas with dry weather thanks to its relatively low needs and favourable water usage. Although of course higher yields can be attained with it on soils with better quality, but it is unable to compete with other cereals there.

Millet can also be used excellently for second sowing after plants harvested in spring thanks to its short lifespan. It does not exploit the soil, on the opposite, it provides a kind of a cover for the soil. Beside this, in practice it means easily obtained additional income for producers. We highly recommend millet to be cultivated as second sowing for farmers under the agroecological circumstances of Nagykunság.

Since millet can be sown late thanks to its short lifespan, it should be considered for utilising areas suffering from frost or inland inundation.

As a result of examining the water circulation of millet by lysimeter, we have confirmed the extraordinary drought tolerance of millet: Maxi millet bred in Karcag produced 2,4 t/ha yield even in the extremely dry examining period. The increasing occurrence of extreme weather conditions such as droughts confirms the justification of re-including millet in region-specific production.

To sum it all up, we have come to the conclusion that millet can be used ideally as both main and second sowing in the areas of Nagykunság with extremely dry and unfavourable precipitation distribution, on its lower-quality soils thanks to its advantageous water usage, low needs and short lifespan. It also follows that it can be cultivated in other regions with similar agroecological circumstances.

By all means, we regard cultivating and cross-breeding millet in region-specific production to be expedient and perspective since – thanks to its extraordinarily good consumption value – it can not only be key plant of traditional but also modern, functional foodstuff and healthy local commodities.

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# EFFECT OF LONG-TERM SEWAGE SLUDGE COMPOST TREATMENT ON THE Co, Cu, Ni AND Pb CONTENT OF SOIL AND PLANT

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## ABSTRACT

Composted sewage sludge (SSC) can be applied as a nutrient source and organic matter supply to agricultural lands, but the organic and inorganic pollutants in it could cause a danger for soil and plant. The long-term (10 years) regular utilization of SSC was studied in acidic sandy soil at the rate of 0, 9, 17 and 27 t ha$^{-1}$ in every 3$^{rd}$ years. Plant (maize) samples were collected before harvesting while soil was sampled after harvesting. The results show that long-term application of SSC did not cause accumulation of Cobalt (Co), Copper (Cu), Nickel (Ni) and Lead (Pb) in the soil nor in plant, but it increased the pH and humus content of soil, too. These favourable chemical changes increased also the yield of the test plant.

**keywords: composted sewage sludge, long-term experiment, toxic elements**

## INTODUCTION

The amount of municipal sewage sludge generated in Hungary is 120 000-150 000 tonnes yearly. Composting is the most economical way to treat it and compost could be disposed in agricultural lands. SSCs have positive effects on the soil fertility (Jamil et al. 2004; Martinez et al. 2003) but they could contain organic and inorganic contaminants (Gusiatin and Kulikowska 2014; Passuello et al. 2010). If compost has suitable quality according to national or European standards it can be used as a nutrient source or soil improving material.

Toxic elements could cause the large scale of negative effects in the soil-plant system. The distribution, accumulation and leaching of toxic elements depend especially on stabilization process of the sludge and the quality of the organic matter of compost (Richards et al. 1997). There are

some questions arising in the case of toxic elements: are the limits for sewage sludges and SSCs for agricultural use right? Which are the main regulation processes of toxic elements transfer from soil to plants? How can be modified the bioavailability of toxic elements by environmental conditions (climate, soil) and by used agrotechnical methods? Can we give a general threshold for phytotoxic level of different toxic elements considering the combining effects of them? For the answers we need long-term experiments.

The acidic sandy soils in the NE part of Hungary are poorly in organic matter and inorganic colloid content therefore regular addition of these materials to the soil is crucial for the fertility of soil. The SSC as a complex material seems to be an efficient solution for this problem but the safe of its application in the soil-plant-human relation could be decided only in long-term experiments. Our 10 years old experiment is suitable to study the fate of Co, Cu, Ni and Pb in the soil-plant system and to decide whether these elements are accumulate or not in this experimental condition which are similar to a general field application.

## MATERIAL AND METHODS

Municipal wastewater sludge was composted after anaerobically digestion and dewatering. The SSC contain 40% of sewage sludge, 25% of rye straw, 30% of rhyolite and 5% of bentonite and is applied to the 0-30 cm soil layer in every 3$^{rd}$ year at the rate of 0, 9, 18 and 27 t ha$^{-1}$ after harvesting in 2003, 2006, 2009 and 2012. The pH of the compost was 7.2, the Cobalt (Co), Copper (Cu), Nickel

(Ni) and Lead (Pb) contents of it were 2.3, 47.5, 6.6 and 22.5 mg kg$^{-1}$, respectively.

The experimental design is randomized block in five repetitions. Soil is acidic sand (sand: 86.01%, silt: 4.08%, clay: 9.64%) at the University of Debrecen, CAS, Research Institute of Nyíregyháza, in the NE part of Hungary. Plot size is 12 x 18 m. Test plants are maize (Zea mays L.'MV NK 333'), triticale (x Triticosecale Wittmack 'Titán') and green pea (Pisum sativum L. 'Zita'). In this paper we present the toxic elements content of the grain of maize.

Plant samples were collected before harvesting, while soil samples were collected after harvesting. The Co, Cu, Ni and Pb content of soil and plant samples were measured according to the MSZ–08-0012/16-87. Briefly, the samples were digested with cc. $HNO_3$: $H_2O_2$, filtered, and after this procedure the toxic element concentrations were measured by ICP-OES emission spectrometer.

**TABLE 1: Toxic elements content in 0-30 cm soil layer (mean ± S.D.) (n=5)**

| Year | SSC dose | Co | Cu | Ni | Pb |
|---|---|---|---|---|---|
| | | (mg kg$^{-1}$) | | | |
| 2009 | 0 t ha$^{-1}$ | 1.85 ± 0.110 | 5.99 ± 0.665 | 8.51 ± 1.528 | 9.07 ± 1.556 |
| | 9 t ha$^{-1}$ | 2.15 ± 0.317 | 6.03 ± 1.099 | 8.23 ± 1.141 | 11.51 ± 1.775 |
| | 18 t ha$^{-1}$ | 2.07 ± 0.230 | 5.85 ± 0.683 | 8.32 ± 1.680 | 9.56 ± 1.356 |
| | 27 t ha$^{-1}$ | 1.74 ± 0.352 | 5.86 ± 0.824 | 7.70 ± 1.617 | 9.46 ± 2.578 |
| 2010 | 0 t ha$^{-1}$ | 5.02 ± 0.862 | 8.59 ± 0.801 | 10.08 ± 1.729 | 12.11 ± 3.418 |
| | 9 t ha$^{-1}$ | 4.88 ± 1.035 | 8.49 ± 0.396 | 10.04 ± 1.811 | 13.35 ± 5.626 |
| | 18 t ha$^{-1}$ | 4.77 ± 0.916 | 7.80 ± 0.601 | 9.74 ± 1.711 | 10.93 ± 0.550 |
| | 27 t ha$^{-1}$ | 4.66 ± 0.961 | 7.70 ± 0.653 | 9.50 ± 1.622 | 10.69 ±1.168 |
| 2011 | 0 t ha$^{-1}$ | 1.53 ± 0.389 | 4.52 ±0.321 | 6.03 ± 1.230 | 5.26 ± 0.211 |
| | 9 t ha$^{-1}$ | 1.48 ± 0.391 | 5.10 ± 0.555 | 5.98 ± 1.008 | 6.05 ±2.598 |
| | 18 t ha$^{-1}$ | 1.48 ± 0.130 | 4.86 ± 0.816 | 5.96 ± 1.046 | 6.41 ± 1.598 |
| | 27 t ha$^{-1}$ | 1.44 ± 0.392 | 4.63 ± 1.263 | 5.86 ± 1.007 | 4.50 ±1.670 |
| 2012 | 0 t ha$^{-1}$ | 5.63 ± 0.768 | 8.19 ± 0.782 | 9.94 ± 2.478 | 12.10 ±1.536 |
| | 9 t ha$^{-1}$ | 5.58 ± 0.825 | 8.62 ± 0.893 | 9.66 ± 1.937 | 12.48 ± 1.219 |
| | 18 t ha$^{-1}$ | 5.46 ± 1.212 | 8.29 ± 0.932 | 9.48 ± 2.908 | 11.68 ± 0.861 |
| | 27 t ha$^{-1}$ | 5.56 ± 1.003 | 9.88 ± 1.790 | 9.89 ± 1.301 | 12.38 ± 0.942 |
| approved limit (mg kg$^{-1}$) | | 30 | 75 | 40 | 100 |

TABLE 2: Toxic elements content of grain of *Zea mays L.* (mean ± S.D.) (n=5)

| Year | SSC dose | Co | Cu | Ni | Pb |
|---|---|---|---|---|---|
| | | (mg kg$^{-1}$) | | | |
| 2010 | 0 t ha$^{-1}$ | 0.19 ± 0.013 | 3.72 ± 0.557 | 1.74 ± 0.592 | 1.83 ± 0.668 |
| | 9 t ha$^{-1}$ | 0.17 ± 0.030 | 4.17 ± 1.582 | 1.33 ± 0.539 | 1.86 ± 0.698 |
| | 18 t ha$^{-1}$ | 0.21 ± 0.015 | 3.65 ± 0.356 | 1.24 ± 0.115 | 1.77 ± 0.565 |
| | 27 t ha$^{-1}$ | 0.18 ± 0.037 | 3.68 ± 0.454 | 1.76 ± 0.878 | 2.03 ± 0.529 |
| 2011 | 0 t ha$^{-1}$ | Nd | 1.44 ± 0.746 | Nd | Nd |
| | 9 t ha$^{-1}$ | Nd | 1.56 ± 0.700 | Nd | Nd |
| | 18 t ha$^{-1}$ | Nd | 1.39 ± 0.282 | Nd | Nd |
| | 27 t ha$^{-1}$ | Nd | 1.83 ±0.181 | Nd | Nd |
| 2012 | 0 t ha$^{-1}$ | Nd | Nd | Nd | 1.60 ± 1.147 |
| | 9 t ha$^{-1}$ | Nd | Nd | Nd | 0.47 ± 0.590 |
| | 18 t ha$^{-1}$ | Nd | Nd | Nd | 0.62 ± 0.960 |
| | 27 t ha$^{-1}$ | Nd | Nd | Nd | 1.21 ± 1.090 |
| In unpolluted plants (mg kg$^{-1}$) | | <1 | 5-20 | 0.1 - 5 | 0.1 - 10 |

Statistical analysis was done by SPSS 13.0 statistical program. Treatment effect was evaluated for each year separately by one-way ANOVA followed by Tukey's test ($p<0.05$). Pearson correlations were applied to determine the relationships between toxic elements content of soil and plant.

## RESULTS

All of the measured concentrations in the soil samples are rather low, and any significant differences were not found among the treatments (Table 1). In 2010 the measured values are higher than in 2009 because of the SSC treatment in 2009 after harvesting.

There is no significant difference between treatments, according to the Tukey's test ($p<0.05$). Therefore we not signed the "a" index in the table.

The concentrations of Ni decreased from the control to the highest SSC dose while in the case of Co this increase was found in 2010 and 2012. The Cu content increased with SSC doses, except in 2009 and similar increase was found in Pb concentration, except in 2010.

Toxic elements content of maize were not measured in 2009 because the weather conditions caused damage of stands therefore ears have not developed.

Similar to the elements concentrations measured in the soil samples, the test plant samples contain also very low concentrations of the measured elements and any significant treatment effect was not found. Values of the four toxic elements are in the range of plants growing on unpolluted sites (Table 2).

There is no significant difference between treatments, according to the Tukey's test ($p<0.05$). Therefore we not signed the "a"index in the table.

Nd, not detectable, the concentration is under the measuring limit of the instrument (Co<0,050 mg kg$^{-1}$; Cu<0.200 mg kg$^{-1}$; Ni<0.699 mg kg$^{-1}$; Pb <0,180 mg kg$^{-1}$ ).

## DISCUSSION

Agricultural utilization of SSCs has also positive and negative effects. It can improve the soil physical, chemical and biological properties. In our long-term experiment the pH of the control soil has been decreasing (pH KCl: 4.96) continuously, while the pH of the treated soils has been increasing (pH KCl: 5.73-6.53). Similarly, we have found the increase of the organic matter content of treated soils: Hu% is 0.63 and 0.69-0.97 in the control and treated samples, respectively. The forms and other properties (accumulation, leaching, bioavailability) of toxic elements in the soil depend mainly on these factors (Pichtel and Anderson 1997). Generally, increasing pH and organic matter content decrease the bioavailability of toxic elements therefore decrease the danger of food contamination.

The concentrations of all the measured toxic elements are in the range of the unpolluted soils of Hungary in all treatments (Anton et al. 1999). It is known that the Cu content of the Hungarian soils is generally low. The measured values (4.52-9.88 mg $kg^{-1}$) are in the range of unpolluted soils. Total Cu concentration was measured in the experiment therefore the significant increase of soil pH could decrease the bioavailability of this element. According to this statement, the Cu content of maize grain was lower in the treated soils than in control one.

Humus content of soil was positively correlated ($r^2$= 0.778) with Cu content of soil in 2009. We observed similar results between Pb and humus content of soil ($r^2$= 0.606).

Our results show a relatively strong relationship between total Cu concentrations in plant and in soil. But we have not found a plateau effect which is in opposite to the results indicated in the literature (Soon et al. 1980).

All the studied elements were detectable only in 2010 which was a very wet year with double precipitation than in a general year. Wet years could cause greater uptake of toxic elements than dry years (McGrath et al. 2000) which could cause markedly fluctuation in toxic elements uptake from year to year.

The effect of compost on the plant development and on the yield depends on the plant species, too (Latare et al. 2014). In our experiment any toxic effect was not found regarding the maize. Moreover, the increasing yield (not published) indicated the favourable effect of the applied SSC as a nutrient source.

Regarding the possible ecotoxicological effects, different rates (2.7-30 t $ha^{-1}$) of sewage sludges are defined as a safe rate of application (Carbonell et al. 2009; Domene et al. 2008). Our opinion is that the safe rate always depends on the sludge or compost itself, the type of soil amended, the plant species growing on the treated land and the frequency of application.

## CONCLUSIONS

Long-term application of SSC to the acidic sandy soil caused any significant accumulation of Co, Cu, Ni and Pb in the treated soils. The measured low rates of this elements in the upper soil layer (and in the 30-60 cm layer, too) is not enough to state the safe SSC application in long-time period because the sandy soil with fast vertical transport processes needs the study of the deeper soil layers. But the low toxic elements concentrations of test plants in all treatments indicate the low bioavailability of the studied elements. This could support the increasing pH and humus content after regular SSC application.

In the future, study the available forms of different toxic elements in combination with plant uptake bioassays are necessary for determination the safe and economic regular use of the sewage sludge compost.

## ACKNOWLEDGEMENTS

The sustenance of the long-term experiment is co-supported by Nyírségvíz Closed Shareholder Group.

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# THE INVESTIGATION OF RESPIRATION AFTER MECHANICAL BIOLOGICAL TREATMENT OF MUNICIPAL SOLID WASTE

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## ABSTRACT

The mechanical biological treatment (MBT) systems treat and process the mixed municipal solid waste and the residual of other waste treatment processes (e.g.: selective waste collection and sorting). The enrichment and assortment processes are conflated with biological treatments as composting in MBT. The primary aim of these treatments is to reduce the environmental hazards, volume, and mass of wastes, to stabilize the organic residues, and the retrieval of the recyclable waste materials (metals, refuse derived fuel-RDF). One of the largest amount of material originated from the waste treatment is a compost-like material, that may be used to cover the landfill depend on the quality. These types of stabilized biowastes have to meet the legal requirements and have to be stable before utilization.

The aim of our studies was to determine the 4 days respiration index ($AT_4$) of municipal solid waste after MBT with OxiTop Control B6M-2,5 device. Respiration index was determined and followed during the time of stabilization. Preparation of samples and determination of respiration index were made based on different European standards.

**keywords: oxitop, municipal solid waste (MSW), AT4**

## INTODUCTION

Management of municipal solid waste (MSW) has a share in sustainable development. Mechanical-biological treatment (MBT) of waste covers wide range of technologies. MBT plants stabilize and separate waste into less harmful and

more beneficial output streams (refuse derived fuels (RDF)). These systems are the processing or conversion of waste from human settlements with biologically degradable components by a combination of mechanical and physical processes with biological processes. It is a technological alternative to reduce the waste amount on landfill (Soyez 2002, IEA 2007).

One of the largest amount of material originated from the waste treatment is a stable compost-like material, that may be used to cover the landfill depend on the quality. According to Butler et al. 2001 stability can be defined as "The level of activity of the microbial mass". Respiration is directly related to the metabolic activity of a microbial population. Micro-organisms respire at higher rates in the presence of large amounts of bioavailable organic matter while respiration rate is slower if this type of material is scarce. Godley 2003 stated that organic compounds can be classified either as readily degradable, moderately degradable, poorly degradable or recalcitrant depending on how easily they are decomposed. This is a natural aerobic process by which microorganisms transform putrescible organic matter into $CO_2$, $H_2O$ and complex metastable compounds. Respiration activity has become an important parameter for the determination of the stability of MSW. It is also used for the monitoring of the stabilization process and it is considered an important factor for the estimation of the maturity of the material (Raquel et al. 2006).

EU policy prior to emplacement on landfill, the waste will have to be treated and meet the applicable quality norms, such as degree of stability Directives do not provide the methods for assessing the waste stability. Some Member Countries have their own regulations addressing this matter. Works are in progress on unifying the methodology and establishing the limit values for the bioactivity of waste landfilled in the European Union. There are various tests available to determine the bioactivity of waste. Directive on "Biological treatment of waste" sets forth the requirement to determine the respiration activity ($AT_4$) of waste after mechanical and biological treatment, or the dynamic respiration index (DRI). Germany and Austria have introduced the requirement to assess two parameters of biological stability of landfilled waste ($AT_4$ and $GB_{21}$) (Bozym 2012).

## MATERIAL AND METHODS

The mechanically-biologically pretreated waste samples originated from Zöld Híd Régió Environmental and Waste Management Ltd. The facility is located in Hungary, periphery of Kerepes. In the Waste Management Centre the collected mixed municipal solid waste were shredded in the mechanical pretreatment, followed the process the shredded waste separate with a screen to the fractions under 70 mm. A significant part of these materials (particle size below 70 mm) are biodegradable organic waste and it has to get biological stabilization process. Stabilization was going on in special concrete silos. The aeration passed through the aeration channels which built up to the platform (Chart 1.). Aeration system is controlled by feedback control engineering unit which is depended on the temperature of wastes. Temperature was measured locally with mobile probe. Treated waste covered by during treatment a special semipermeable membrane cover. The material was turned twice during the 10 weeks of stabilization, after 5 and 8 weeks. According to the standards (MSZ EN 14899:2006 and MSZE 21420-17:2004) samples were collected weekly.

The biological activities of waste samples were investigated from different stages of biological stabilization process. Activity of samples were tested by OxiTop Control measuring system and determined the respiration activity value $AT_4$ (Chart 2.). A part of the system is a controller which allows collecting recorded data by sensors and transmit these to a computer.

Analysed samples are under aerobic conditions in the reaction vessel. Biologically degradation of samples depended on the stability (respiration activity) of collected waste samples. During the measurement the quantity of oxygen is reducing or running out in the vessel. The evolved carbon-dioxide ($CO_2$) absorbed on the surface of 2M NaOH in this way the pressure will be lower in the vessel. The sensor measures the changing of pressure in the vessel and register it. Pressure reduction is proportional with the activity of sample namely the oxygen consumption. $AT_4$ value assessment method comprises the measurement of $O_2$ consumption during the decomposition of the organic fraction of waste. The parameter can be assessed already

**Chart 1: Heap and concrete silo with the aeration system (Photo: Csaba Sebestyén)**

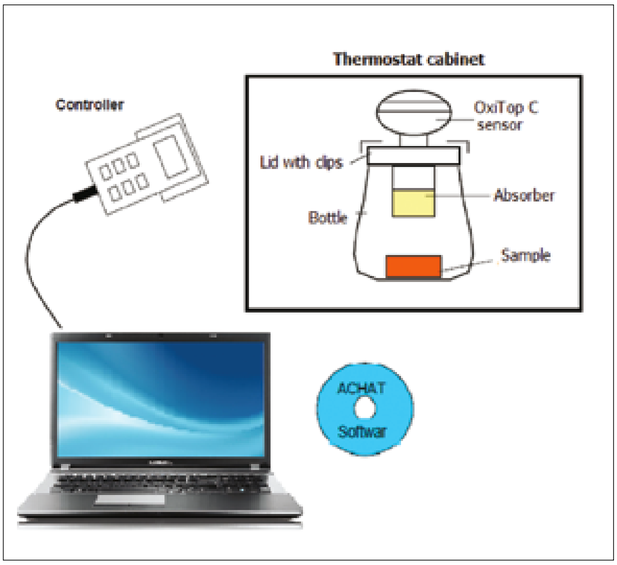


Chart 2: OxiTop Control system (WTW application manual $AT_4$)

after 4 days. Values are given in the dry matter content of samples (mg $O_2$ $g^{-1}$ DM).

In case of investigation of samples sometimes become a period of incubation so called Lag-phase (Chart 3), when the $CO_2$ level is increasing very slowly. Oxygen consumption is increased exponential after the Lag-phase. The $AT_4$ value has to be determine from the end of the Lag-phase.

The impurities (glass, stone, metal, plastic etc.) are removed from the waste samples before preparation (Chart 4). The amounts of these materials are taken account of calculations. Samples are cut until the granulation is under 10 mm (Chart 5) (Verordnung über Deponien und Langzeitlager (Deponieverordnung - DepV) vom 27. April 2009).

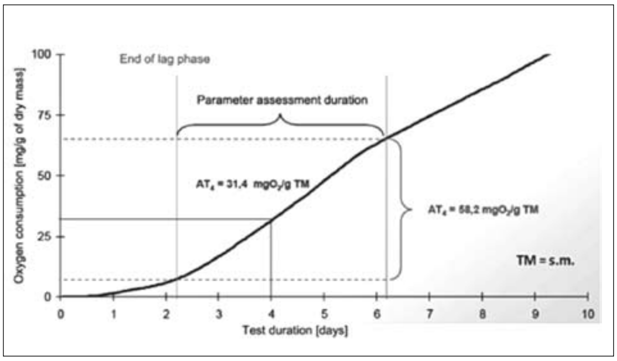


Chart 3: Example diagram of oxygen consumption over time, with marked lag phase and determination of the $AT_4$ parameter; T.M. (total dry mass) = s.m. (dry mass) (Binner 2011)

Chart 4: Original sample from heap and inorganic part of sample (Photo: Miklós Gulyás)

Chart 5: Prepared, homogenized part of sample (Photo: Miklós Gulyás)

The prepared samples are watered. The following applicable standard provided a wetting method: Verordnung über Deponien und Langzeitlager (Deponieverordnung - DepV) vom 27. April 2009). The moisture content of samples were typically between 45-60%. In the course of investigation the moisture content was determined according to standard MSZE 21420-18:2005 and the pH values were determined by MSZE 21420-21:2005. Determination and evaluation of result was carried out according to the method.

## RESULTS

Recorded data could be read by controller from sensors and the assessment of dataset was used MS Excel. In order to prevent any oxygen limiting measuring conditions from occurring, transmit the measurement data of the sensors to the controller at regular intervals. If the oxygen consumption is higher (run out from range) than the selected volume the measuring vessel should be opened namely aeration the system.

During the measuring period (10 days) vessels had to be open typically 2-3 times (Chart 6). If the material will be

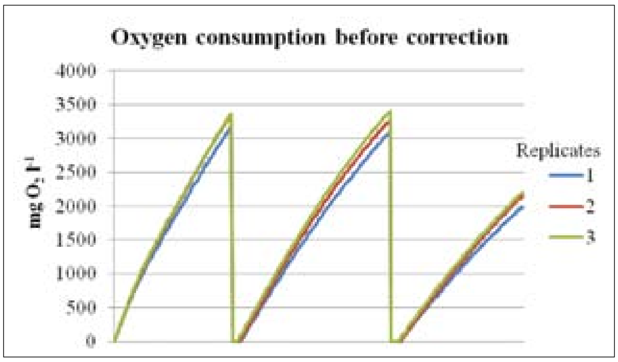


Chart 6: Recorded oxygen consumption of samples by sensors

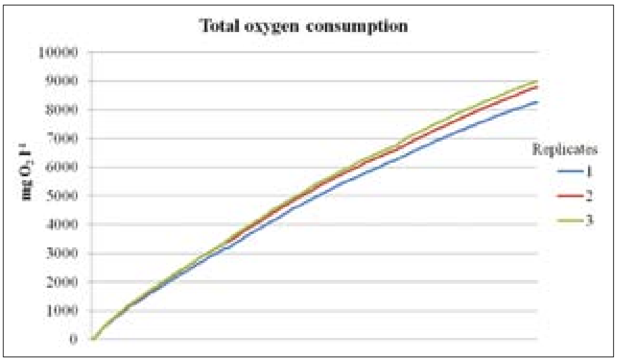


Chart 7: Total amount of consumed oxygen

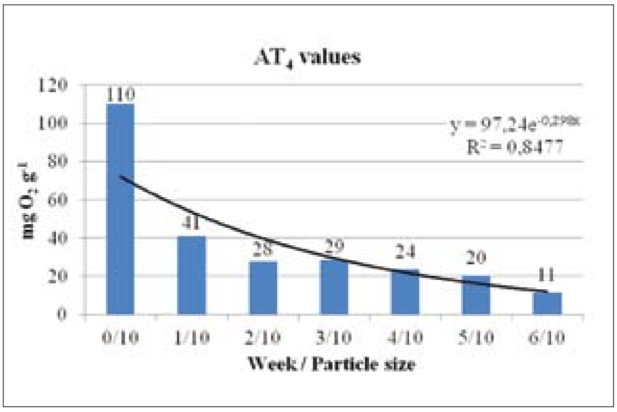


Chart 8: $AT_4$ values after correction between 0-6 weeks

more and more stable the period between two ventilations will be longer. After the measurements the zero values were cut out. As Chart 7 shows a linear growth function that we got after the correction.

When corrections (bulk density, dry matter, impurities) were carried out we got the following results. $AT_4$ values exponential decreased between the 0-6 weeks. Oxygen consumption correlates well with elapsed weeks.

## CONCLUSIONS

There is no standard methodology in EU to determine the oxygen consumption of waste materials and regulation of landfilling the treated materials. Member countries have their own regulations and landfilling directives and these don't correspond with each other. The quality and homogeneity of treated collected waste samples can influence highly the oxygen consumption. Final conclusions can be draw after knowledge of the entire dataset.

## ACKNOWLEDGEMENTS

This research was supported by the by the Hungarian Government and the European Union, with the co-financing of the European Social Fund, within the framework of the KTIA_AIK_12-1-2013-0015 project. Special thanks to the many people who have made this experiment possible.

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# EFFECT OF CULTIVATION AND FERTILIZATION ON THE YEARLY BIOMASS PRODUCTION AND BRIX CONTENT OF SWEET SORGHUM

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## ABSTRACT

Today humanity gets the vast majority of its energy consumption, nearly 75% by burning oil, coal and natural gas. These sources are of course finite, therefore research in renewable energy sources that can satisfy the energy needs of humanity on the long term is more and more urging. Hungary is poor in fossil fuels but half of its territory is under agricultural cultivation and its agroecologic peculiarities are favourable to biomass production. Therefore in the future, energy produced as biomass can be the main perspective in Hungary. This is why Hungarian research and higher education institutes pay more and more attention to an energy crop that shows the way for producing bioethanol and gas, namely sweet sorghum (Sorghum bicolor L. Moench).

**keywords: biomass production, energy crop, sweet sorghum, bioethanol, biogas**

## INTORDUCTION

Sorghums are native to the steppes and savannahs of Africa. Their excellent drought tolerance and adaptability is due to it being endemic to the tropical zone. The drought tolerance of sorghum is thanks to its big root system, its many secondary roots, the structure of its plant stem and leaves, the latter of

which reduce transpiration significantly. The water-absorbing activity of roots is twice of maize while the surface of its leaves is substantially less. The drought tolerance of forage sorghum and its excellent value as green fodder would justify it (Berényi & Szabó 2001) to be cultivated instead of forage maize on our sand and other soils that are prone to drought. Sorghums are undemanding of soil, they usually can be successfully cultivated on soils with low productivity. Successful cultivation is possible on looser soils with more than 1% humus, on moderately salty, lower-productivity pasture, eroded hilly and all other soils where sorghum exceeds the yield of both forage and grain maize. Sorghum is undemanding of precrop, it can follow precrop harvested either in the summer or autumn. Sorghum requires the most careful preparation of the soil. Basic cultivation for the autumn (with ploughing, disk harrow or cultivator) has to be done properly and on time. Today – beside keeping water – increasing the water receiving ability of the soil is a more and more important goal.

Sweet sorghum has a wide range of energetic use, almost all of the plant can be used to produce energy. Beside energy production, sorghum has an important role for human consumption and fodder. Gosse (1996) mentions three options for using sorghum: producing electricity and heat energy by direct burning or indirectly in biogas plants. The third option is its use in first- and second-generation bioethanol plants. Thanks to its high fibre content, sorghum is good for direct burning, too. In burning experiments, with 10.5 percent water content, it has 16,37 MJ/kg energy for one kilogram dry matter which is the same as the heating value of brown coal used in Hungary. The stem of the plant has high sugar content that can easily be extracted by pressure and be used to produce bioethanol (Gnansounou 2005, Tarpley & Vietor 2007, Yu et al. 2010). The stem has two parts, the external supporting tissue that has high cellulose and very low fermentable sugar content (0.51%). The internal sponge-like pith contains 14% dry fibre and 86% juice with 15-17% fermentable sugar. The amount of sugar content in the stem depends greatly on the effect of the year as well as the chosen breed. According to the recommendation of Antonopoulou et al. (2008), sweet sorghum can play an important part in hydrogen production because 10,4 l $H_2$ have been extracted from 1 kg of sweet sorghum juice.

## MATERIALS AND METHODS

### The set-up of the experiment

The experimental area is in the geographic subregion of Gödöllő Hills that is a transition between Cserhát and Duna-Tisza sand ridge. Its height is 247 m above sea level. The yearly duration of sunlight amounts to 1950 hours, yearly mean average temperature of many years is 9.7 °C. The average amount of precipitation of many years is 564 mm, 313 mm of which is during the production period. The predominant direction of wind is NW. The soil of the experimental area is mainly rust-brown forest soil formed on sand (Luvic Calcic Phaeozem). Physically the soil is sandy adobe that is sensitive to compaction. The upper, 20 cm-high layer of the soil contains 53% sand, 26% adobe and 20% clay fraction. The upper soil (0-35 cm) has 26% clay and good water permeability while this ability of the lower soil is weak. Due to degradation processes, a variation with medium thick fertile layer and low humus content had been formed. The area is threatened by erosion, it has increased sensitivity to compaction. In 2009, yearly average mean temperature was 11.5 °C. 265.4 mm precipitation fell in the vegetation that stayed below the average of many years. In 2010, yearly average mean temperature was 10.2 °C. 646.6 mm precipitation fell in the vegetation that that was more than the average of many years. In 2011, yearly average mean temperature was 11.1 °C. Only 172.0 mm precipitation fell, resulting in extremely drought weather. The technology-developing experiment was arranged in split-plot design with three replications in the combination of four different cultivation methods (ploughing, cultivator, disk harrow, direct seeding) and seven different ways of providing nutrients. Nutrient treatments: $N_0K_0$ (control), $N_1K_1$ (50kg/ha N, 40kg $K_2O$), $N_1K_0$ (50kg/ha N), $N_2K_2$ (100kg/ha N, 80kg $K_2O$), $N_2K_0$ (100kg/ha N), $N_0K_1$ (40kg/ha $K_2O$) and $N_0K_2$ (80kg/ha $K_2O$). Cultivation methods: ploughing (22-25cm), cultivator (15-25cm), disk harrow (16-20cm) and direct seeding.

During the experiment, we have examined Sucrosorgo 506 silo sorghum hybrid that has a long vegetation period. We used digital refractometer to define dry matter (Brix). Refractometers are used to measure the dry matter content of transparent liquids (juices , solutions). The sugar content can be measured by refractometer indirectly (Figure 1., 2.).

The biomass of the stem of sweet sorghum was measured as well as its dry matter content. To determine the biomass yield of sweet sorghum, harvesting was done in a 10-meter-long reference area from each parcel. After that, the samples were weighted on a Kern De type industrial scale with a scale measurement of 100 grams. We used IBM SPSS 12 programme for the statistical analysis of the values.

Figure 1

Figure 2

## RESULTS

Figure 3,. 4., 5. shows the value pairs of biomass and Brix in the years of the examination. The effects of yearly weather can be well observed on the graphs. In 2009, the biomass of sweet sorghum was 27.8 t/ha for the average of the treatments. The dry matter content had reached 16.2 % by the time of harvesting. We had different results in 2010 where the measured biomass was 46.6 t/ha for the average of the treatments, and Brix content was 7.3 %. The rainy year of 2010 constitutes a separate dataset where low Brix content was coupled with high biomass. In the extremely rainy year, even summer months were colder than the average, therefore the heat sum of the vegetation period also remained lower which was disadvantageous for the integration of Brix (dry mass) in the stem of the sorghum. In 2011, biomass measured at the time of harvesting was

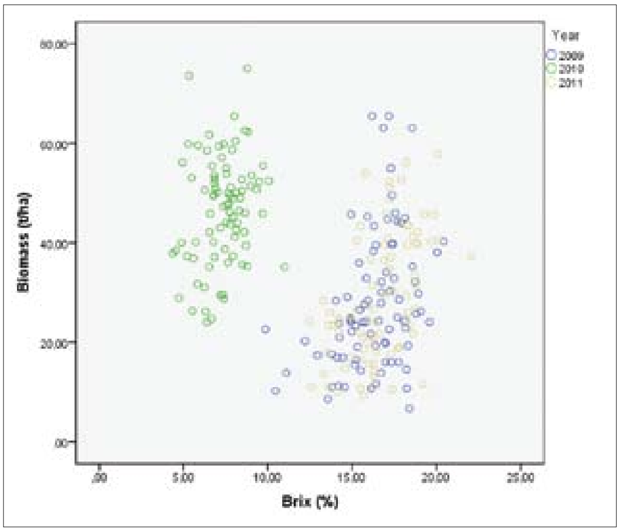


**Figure 3: Correlation examination between biomass and Brix content (effect of yearly weather, Gödöllő 2009-2011)**

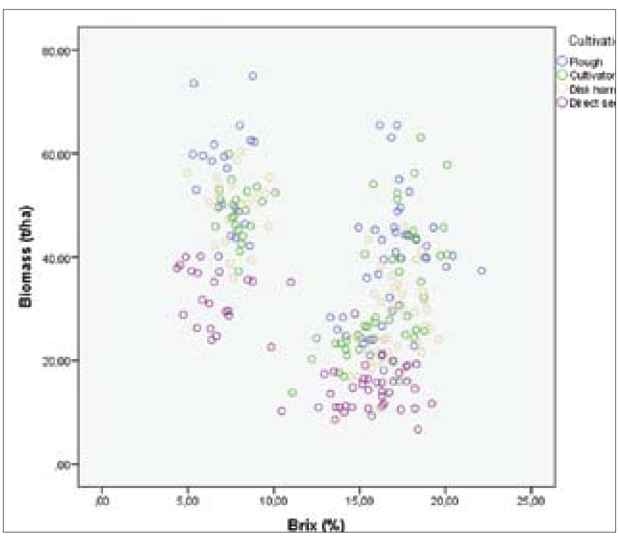


**Figure 4: Correlation examination between biomass and Brix content (cultivation effect, Gödöllő 2009-2011)**

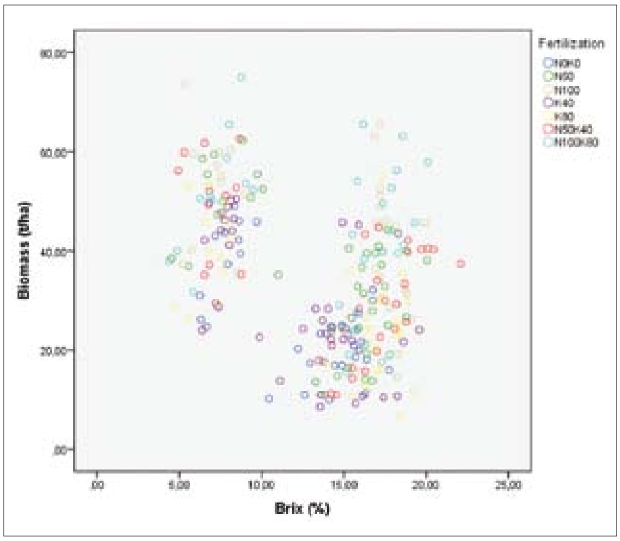


**Figure 5: Correlation examination between biomass and Brix content (fertilization effect, Gödöllő 2009-2011)**

28,8 t/ha and the Brix content was 16,6 %. The years of 2009 and 2011 show many similarities. Both years were dry with high heat sums during vegetation and because of that, biomass had become less but Brix content had increased. The differences between the years can be statistically verified. All in all, it could be stated that the effect of yearly weather significantly influences the quality and quantity parameters of sweet sorghum.

## RESULTS AND DISCUSSION

According to Hungarian and international literature, it can be stated that sweet sorghum can be a good raw material for bioethanol and biogas plants. Sorghum provides an alternative for areas where producing maize is unprofitable, but there is further research needed in harvest, storage, production technology and biological bases to fully make use of the potential opportunities that lie in the plant. Agrotechnical processes have to adapt to the needs of the plant and the soil in the case of sorghum, too. The ecological circumstances of the experimental area were specifically disadvantageous and were further exacerbated by the whimsical weather. The years of 2009 and 2011 were extremely dry while 2010 was the opposite of these. Despite this, according to our examinations sweet sorghum was able to produce the biomass described in international and Hungarian experiments in the disadvantageous place of cultivation in Gödöllő Hills even during years with drought. According to the literature, the applied agricultural technique can significantly influence the physical and biological processes of the soil. In our experiment, rotational and non-rotation techniques – although with a different effect – all proved to be fruitful. The doses of artificial fertilizer influenced positively the yield of the biomass of sweet sorghum. Nitrogen agent had significantly helped the increase of biomass and content parameters in the

first three years of the examination. As a result of artificial fertilizers with nitrogen, the biomass per hectare increased by 25-30% in an average of soil cultivation treatments. Nitrogen agent also helped the absorption of potassium. The biomass yield and Brix content of treatments that received artificial fertilizer with only potassium did not change in a statistically provable way. The cultivation of sugar sorghum for energetic use can be viable in disadvantageous areas in practice, therefore the abovementioned results can show the direction for more favourable years, too. The results of plant cultivation research can be extended to other weather conditions only with restrictions even if the conditions of the place of production are the same, but because of the more and more probable occurrence of dry years, our results for 2009 and 2011 can be the starting point to expand the cultivation of sweet sorghum for energetic use.


## ACKNOWLEDGEMENTS

The study has been conducted as a result of the call for applications KTIA_AIK_12-1-2012-0012 named Basic and industrial studies related to plant and animal-sourced food raw materials in conform with food safety conditions, supported by the National Innovation Office.


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