Reaction of Biofilms (Cyanobacterial, Diatom and Green Algae) from Lake Balaton and River Danube to Herbicide Preparation ROUNDUP and its Components (*Glyphosate, POEA*)

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Abstract
The behavior of biofilms developing during 6 weeks on sheets of glass fixed to AKK-1® type carrier buoy in River Danube (Green Island, Budapest) and in Lake Balaton (Tihany Bay) in spring time was investigated in aquaria exposed to *glyphosate* at concentrations of 100 and 1000 µg/l for 6 and 4 weeks, respectively. The effects of adjuvant *POEA* and formulated herbicide ROUNDUP® at *glyphosate* equivalent concentrations were also tested. Sensitivities of biofilms from River Danube and Lake Balaton were different under laboratory conditions. No adverse effects of *POEA* were determined on the well-estimable biofilm originated from River Danube, while *glyphosate* caused a 30% decrease in 10 weeks. Realignment of biofilms was typical, the initial ratio of diatom (Bacillariophyceae) and cyanobacteria (*Cyanobacteria*) decreased, and these species were replaced by a filamentous green alga (*Chlorophyta*) population.

Introduction
Pesticides applied in chemical plant protection contain various additives, beside their active ingredient(s) [1]. Additives have been considered as inert/inactive components. Authorization requires simplified risk assessment (RA) for additives compared to RA of the active ingredients [2], even though several studies proved the toxicity not only of the leading herbicide active ingredient *glyphosate*, but also of polyethoxylated tallowamines (*POEA*) applied as adjuvants in *glyphosate*-based formulations [3-6]. Biofilms developing on stone surfaces in water media play an important role in the biogeochemical cycles of water ecosystems. A significant part of biofilm communities constitute of photoautotrophic benthic species [7-8], thus, effects of herbicides on these algae deserve special attention.

Experimental
The natural biofilms were grown on glass substrates fixed to AKK-1® type carrier buoy (Cséffán, Darvas and Pasaréti) placed in River Danube (Green Island – 47.481641, 19.057645) and in Lake Balaton (Tihany Bay – 46.914190, 17.892916) between early May and mid-June of 2015 for 6 weeks. The AKK-1® buoy includes four algal deposition units (containing no metal or plastic elements) with 6 glass sheets in each unit. After the 6-week developing period, the glass substrates were placed under laboratory conditions into eight aquaria containing water from the original location of the buoy. Water in the aquaria was slowly stirred (oxygen dissolution), temperature-controlled (22±2°C) and illuminated (L:D =

15:9, daily light program 6-9 hrs 400 lux, 9-18 hrs 2000 lux, 18-21 hrs 400 lux; XiLong White T8®). Five biofilm substrates with blasted and smooth surface sides were placed into each aquarium (the sixth substrate was used for analytical and microscopic evaluations). The water in the aquaria was changed weekly, with water of unchanged quality from the original locations, where the biofilm developed, and with treatment concentrations applied. During the first 6 weeks the aquaria were treated with *glyphosate* at 100 µg/l and with adjuvant *POEA* or with formulation ROUNDUP® (*glyphosate* isopropylamine salt) at *glyphosate*-equivalent concentrations, except for the untreated control. After the 6th week the concentration of the *glyphosate*-equivalent treatments was increased to 1000 µg/l. Although the detected *glyphosate* content in Hungarian surface waters (1 ng/ml) is much below our evaluation level [9], mean *glyphosate* concentration in runoff was ~140 µg/l (max. 180-233 µg/l) in USA [10], moreover, the value can exceed it (~5 mg/l) in some cases [11]. The quantity of the diatom (Bacillariophyceae), cyanobacteria (*Cyanobacteria*) and green algae (*Chlorophyta*) species in the untreated and treated units was determined with a BBE MOLDAENKE BENTOTORCH® algae torch instrument. Beside investigation of biofilm composition, weekly degradation of *glyphosate* was determined daily in water samples of River Danube and Lake Balaton, and in cleaned drinking water (CLEARWATER®). The initial concentration of *glyphosate* was 100 µg/l. *Glyphosate* concentration of water samples were measured by HPLC after derivatization. Grubbs-test (XLSTAT®) was applied to eliminate outliers. Effects of treatments were statistically analyzed by ANOVA and Tukey, Spjotvoll/Stoline *post hoc* tests (STATISTICA®). Effectivity was determined by the Henderson-Tilton formula.

Results & Discussion

No significant differences (ANOVA) were seen between cyanobacterial and diatom biofilms formed either on the sand blasted or on the smooth surface sides of the glass substrates fixed on the AKK-1® buoy in the case of River Danube and Lake Balaton during the 6-week exposition period (data not shown). Results indicated no significant differences prior treatment between the quantity of cyanobacterial and diatom biofilm from River Danube and the cyanobacterial biofilm from Lake Balaton, on either sides of the glass substrate (Figs 1, 3-4). In the case of quantity of diatom biofilm from Lake Balaton, the control unit was significantly lower (Fig 2), than the units treated with *glyphosate* and ROUNDUP®.

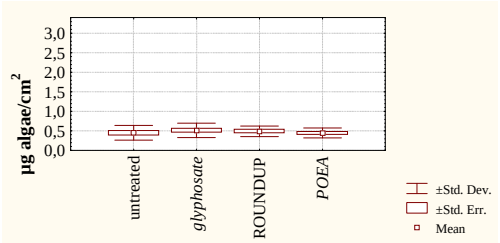

Fig. 1 Cyanobacterial biofilm originated from Lake Balaton, before treatment

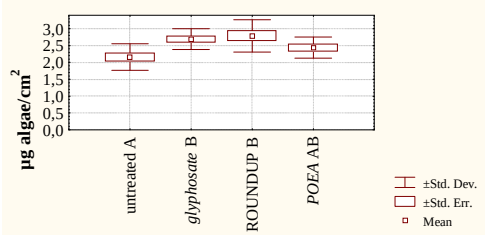

Fig. 2 Diatom biofilm originated from Lake Balaton, before treatment

Different capitals mean significant differences between groups

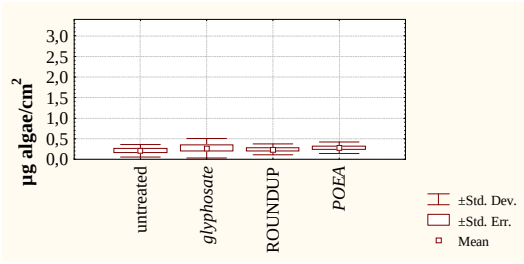

Fig. 3 Cyanobacterial biofilm originated from River Danube, before treatment

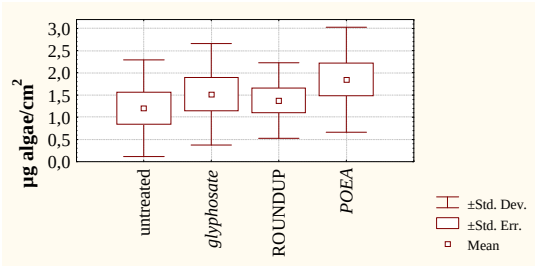

Fig. 4 Diatom biofilm originated from River Danube, before treatment

The untreated control units from River Danube grew from 1.9 to 5.5 µg/cm^2 in 10 weeks (Figs 5-6), while those from Lake Balaton degraded from 2.6 to 1.5 µg/cm^2 (Figs 7-8). The composition of the developing biofilms in the case of River Danube was 12-14% cyanobacterial and 86-88% diatoms, while corresponding values in Lake Balaton were 16-17% cyanobacterial and 83-84% diatoms. *POEA* occurred not to affect the formation of the algal communities. The efficiency of *glyphosate* was 30% at 100 µg/l for cyanobacterial algae species, while 39% for diatoms in River Danube. In contrast to the active ingredient, ROUNDUP® showed a substantially lower efficiency. The value of efficiency did not further increase at the level of 1000 µg/l *glyphosate* (data not shown), presumably due to pre-selection at lower doses. However, during the entire10-week period of the investigation (exposition: 6 weeks at 100 µg/l and 4 weeks at 1000 µg/l), the composition of the algal community was changed to 17% cyanobacterial, 60% diatom and 23% green algae species. Statistical analysis of the apparently more sensitive biofilms from Lake Balaton was feasible only in the first 2 weeks of the experiments. The efficiency of the 100 µg/ml *glyphosate*-equivalent ROUNDUP® treatment was 31% for cyanobacterial algae and 20% for diatoms. From the 4th week filamentous algae species were replacing *glyphosate*-sensitive algal communities (Figs 9-10).

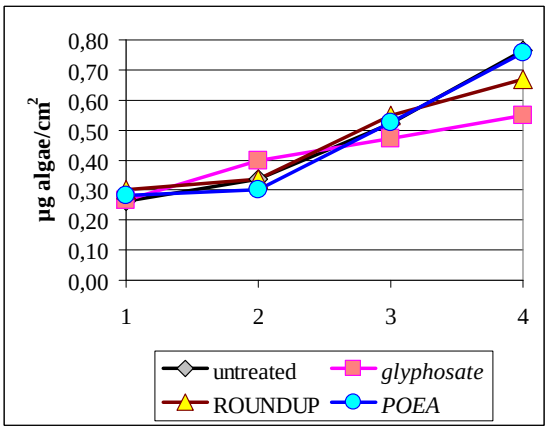

Fig. 5 Cyanobacterial biofilm originated from River Danube after 100 µg/l *glyphosate*-equivalent treatment

Notes: 1 – June 17, 2 – July 1, 3 – July 15, 4 – July 29

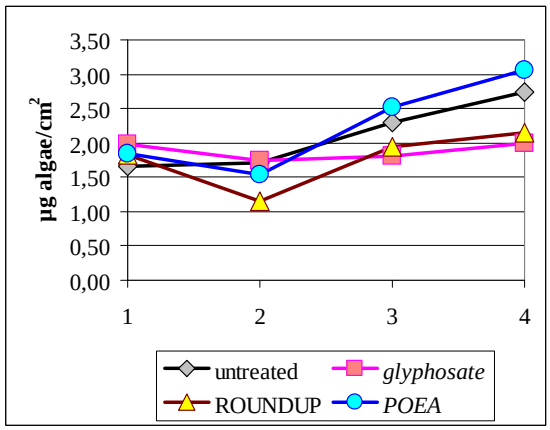

Fig. 6 Diatom biofilm originated from River Danube after 100 µg/l *glyphosate*-equivalent treatment

Notes: 1 – June 17, 2 – July 1, 3 – July 15, 4 – July 29

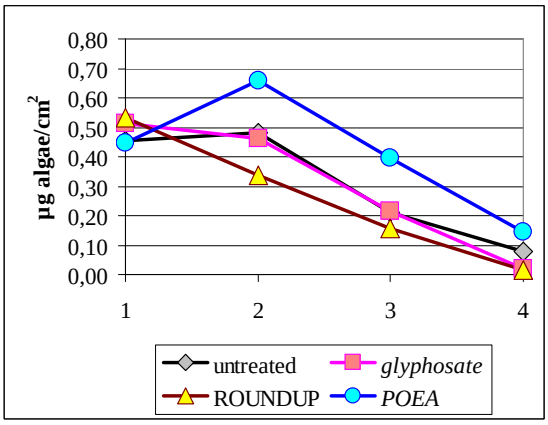

Fig. 7 Cyanobacterial biofilm originated from Lake Balaton after 100 µg/l *glyphosate*-equivalent treatment
Notes: 1 – June 17, 2 – July 1, 3 – July 15, 4 – July 29

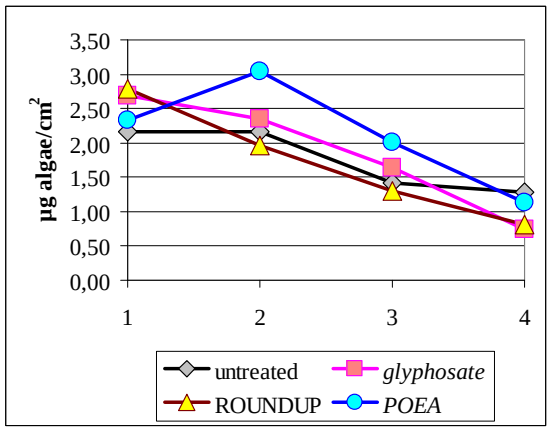

Fig. 8 Diatom biofilm originated from Lake Balaton after 100 µg/ml *glyphosate*-equivalent treatment
Notes: 1 – June 17, 2 – July 1, 3 – July 15, 4 – July 29

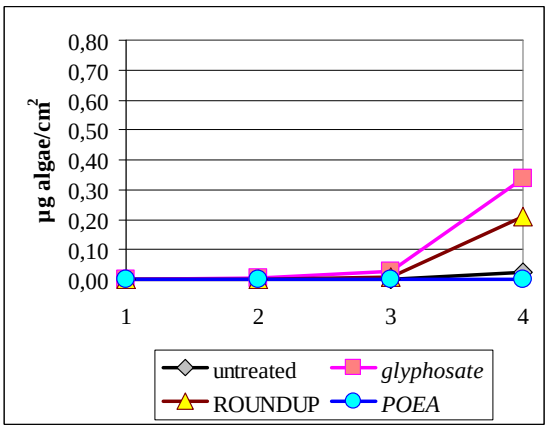

Fig. 9 Green algal biofilm originated from River Danube after 100 µg/l *glyphosate*-equivalent treatment
Notes: 1 – June 17, 2 – July 1, 3 – July 15, 4 – July 29

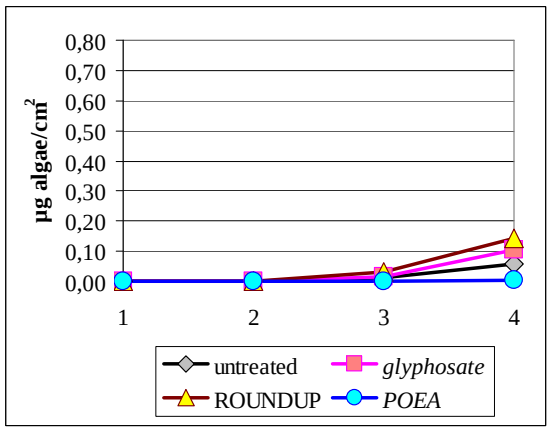

Fig. 10 Green algal biofilm originated from Lake Balaton after 100 µg/ml *glyphosate*-equivalent treatment
Notes: 1 – June 17, 2 – July 1, 3 – July 15, 4 – July 2

Conclusions

In 6 weeks, homogeneous biofilms were formed on the glass substrates fixed to the AKK-1® carrier buoy, and mainly consisted of diatom algae species. Under laboratory conditions (water changed weekly with the same quality as at the original location of the buoy) the biofilms form River Danube adapted well, and the untreated control units continuously thrived. In contrast the biofilm from Lake Balaton (with rich invertebrate fauna including arthropods and worm species) gradually perished within 10 weeks. Identification of the algae species in the various communities with light- and electron microscopy is in progress.

The worldwide detectable water pollution by *glyphosate* can modify the structure of the algal community in biofilms. In biofilms originated from River Danube the filamentous green algae species were integrated into the place of *glyphosate*-sensitive cyanobacterial and diatom algae species. Individual toxicity of surfactant *POEA* was not detectable.

Acknowledgements
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