Romanian Reports in Physics **69**, 712 (2017)

ULTRAHIGH FREQUENCY-LOW POWER ELECTROMAGNETIC FIELD IMPACT ON PHYSIOLOGICAL PARAMETERS OF TWO TYPES OF CEREALS

MIHAELA RĂCUCIU[1], CORA IFTODE[2], SIMONA MICLĂUŞ[3]

[1]*Lucian Blaga* University of Sibiu, Environmental Sciences and Physics Department, Faculty of Sciences, Applied Ecology Research Center, 5–7 Dr. I. Ratiu Street, 550024, Sibiu, Romania, E-mail: mihaela.racuciu@ulbsibiu.ro
[2] *Politechnica* University, Faculty of Electronics and Telecommunications, 2 Vasile Pârvan Blvd., 300223, Timisoara, Romania, E-mail: cora.iftode@gmail.com
[3] *Nicolae Balcescu* Land Forces Academy, Technical Sciences Department, 3–5 Revoluţiei Street, 550170, Sibiu, Romania, E-mail: simo.miclaus@gmail.com

Received September 2, 2016

Abstract. The aim of this study was to investigate the impact of mobile communication low power radiofrequency radiations on physiological and growth parameters of two types of cereal plantlets developed from exposed seeds (*Zea mays* and *Hordeum vulgare*) in controlled conditions to continuous waves to 850 MHz and 1800 MHz. Exposure of seeds was realized inside a transverse electromagnetic (TEM) cell, for different exposure durations between 0 and 6 hours, and then let to germinate. Computational dosimetry was applied for all experimental cases. Decrease in the photosynthetic pigments levels was found for both frequencies and plant species, respectively. Also, opposite influence was revealed upon level of total nucleic acids depending on plant species and frequency of electromagnetic field.

Key words: athermal, ultrahigh frequency electromagnetic field, assimilatory pigments, seeds.

1. INTRODUCTION

With the development and rapid spreading of wireless communication technology, the upper range of radiofrequency radiation became intensively used. Wireless technologies are increasingly popular due to lower costs for implementation, as compared to other technologies. Most of the mobile communications and wireless data transmission technologies operate in the ultrahigh frequency (UHF) band of electromagnetic spectrum. Thus, the environmental exposure to electromagnetic fields emitted by the afore-mentioned technologies has been gradually intensified in the last decades. A lot of scientific research investigated the ultrahigh frequency electromagnetic fields (UHF-EMF) effects on animals and even on humans, while in just a limited number of published

studies the exposure effects on plants have been addressed. Plants play an important role in different ecosystems being important in food chain; therefore it would be beneficial to study and understand the effects of UHF fields on plants. Many such studies confirm that radiofrequency exposure effects exist and they can cause damages depending on the power density level, exposure duration, frequency, but also on the properties of exposed tissues. UHF field effects on plants have been investigated in some research studies on several species revealing bio-effects such as seed germination inhibition, roots or stem growth inhibition, protein content reduction, variation of photo-assimilatory pigments content, variation of the enzymatic activities, membrane alteration, etc.

Lens culinaris root growth was reduced for seeds exposed to 1800 MHz electromagnetic field [1]. Afzal and Mansoor [2] investigated the effect of 900 MHz cell phone radiation exposure on wheat and mung bean seeds revealing a constant germination rate, growth inhibition, protein content reduction and enzymatic activities increase for both species. Kumar *et al.* showed that total chlorophyll content decreased after 4 h exposure of *Zea mays* seedlings to 1800 MHz and activities of α- and β-amylases significantly increased with prolonging the exposure duration [3]. Some research studies have been carried out on mung bean seeds (*Vigna radiate*) exposed to 900 MHz field emitted by a mobile phone with incident power density of 0.085 W/m^2. They revealed a significant decrease of seed germination capacity and of root growth rate post-exposure [4–5]. An inhibited growth of duckweed after exposure to 900 MHz of 2 hours and to 1900 MHz of 14 hours at power densities less than 1.4 W/m^2 were also reported [6]. In our own previous study we have reported an increase of seeds germination rate and of plantlets growth in case of *Zea mays* seedlings after the seeds exposure to 900 MHz at very low SAR level (0.001 W/kg) [7].

Zea mays and *Hordeum vulgare* are two species coming from the same *Poaceae* family and the *Cyperales* order. They are two of the most used plant species in evaluation of environmental contamination degree [8]. The aim of present study was the assessment of the impact of low power radiofrequency radiation in the frequency band used by mobile communications on physiological and growth parameters of these two types of plantlets developed from pre-exposed seeds.

2. MATERIALS AND METHODS

Due to importance for agriculture and food industry two cereal type seeds (corn – *Zea mays* and barley – *Hordeum vulgare*) were chosen as biological material for electromagnetic exposure to a uniform incident field. Only healthy and non damaged *Zea mays* and *Hordeum vulgare* seeds were provided by an experimental micro population. Biological samples were composed of 30 seeds –

for corn and 60 seeds – for barley. Before use, seeds were superficially disinfected with 0.5 % sodium hypochlorite solution for five minutes and afterwards washed several times with deionized water. UHF exposed seeds and control (not irradiated) samples germination occurred in darkness and peer controlled environmental conditions (temperature: 24 ± 0.5 degC, humidity: 90%) on porous paper support in closed Petri dishes. After germination the seedlings development was accomplished in the same controlled laboratory conditions (22 ± 0.5 degC, 14:10 hours light/dark cycle for illumination and 70% humidity) and each biological sample was daily watered with the same amount of deionized water (10 ml).

Seeds samples were prepared for electromagnetic exposure to continuous waves at two frequencies: f_1 = 850 MHz and f_2 = 1800 MHz. At each four samples were provided from each cereal used for successive exposures in the transverse electromagnetic (TEM) cell, each for a different period of time. The exposure parameters are given in Table 1. The biological sample mass (m) was not identical for the four samples exposed at the same frequency, so that in the table the average mass ± standard deviation over the four samples is indicated. Incident electric field strength (E_{inc}) is specified and it was maintained the same for all samples at the same frequency. The unique variable of the exposure for one grain sample at one frequency was the duration of irradiation.

Table 1

Exposimetric and dosimetric characteristics of the seed samples

Sample type	m [g]	f [MHz]	E_{inc} [V/m]	SAR_{comp} [W/kg]
Zea mays	11.44 ± 0.27	850	20.9	0.006
Zea mays	11.32 ± 0.22	1800	49.3	0.396
Hordeum vulgare	2.85 ± 0.02	850	20.9	0.008
Hordeum vulgare	2.88 ± 0.02	1800	49.3	0.368

Four exposure durations were chosen for each cereal type: 0.5, 1, 2 and 4 hours respectively. Therefore was able to absorb a different energy quantity up to the end of the irradiation process. For the high frequency range, the biological impact on the target is generally quantified by the specific absorption rate (SAR) of energy deposition from the external field [9]:

$$\mathrm{SAR} = \frac{P_a}{m} = \frac{\sigma E_{\mathrm{int}}^2}{\rho} \tag{1}$$

where P_a is the absorbed power in the vegetal sample, m is its mass, σ is the electric conductivity of the seed material (depending on its dielectric loss factor, ε_r'', at the specified frequency) and E_{int} is the root-mean-square of the E-field strength inside

the biological target. E_{int} is not equal to the E_{inc} but is dependent upon the relative dielectric constant of the grain material, ε_r'.

The exposure of the seeds took place in a TEM cell model IFI-CC 104SEXX, in between the floor and the septum of the cell, where E_{inc} is theoretically uniform over the entire surface of the grain sample. Practically the seeds were arranged in a glass Petri dish of 9 cm diameter, so as the entire sample to cover a minimum area inside (tangential seeds positioning), and the location of the dish in the TEM cell was always the same (center, at 4.5 cm above the floor).

The UHF signal was provided by a generator Hameg HM 3184-3 connected to a RF power amplifier Ophir 5150. A bidirectional power sensor model FSH-Z44 from Rhode & Schwarz allowed measurement of the power effectively loaded into the TEM cell (as the difference between forward and reflected power). The sensor was connected to a FSH3 spectrum analyzer Rhode & Schwarz for readings. At the output port of the TEM cell a Keithley RF-3500 power meter with a fixed coaxial attenuator model Mini Circuits BW-N30W20+ (30dB attenuation) was mounted for output power readings. Incident powers were set to: $P_{in1} = 0.46$ W (at f_1) and $P_{in2} = 2.57$ W (at f_2). By the differential power method [9] applied, first – in case when an the empty Petri dish was inserted in the TEM cell and second – with the sample-loaded dish, the total amount of power absorbed in the sample during exposure was finally obtained (P_a).

SAR determination was done by simulation, because this method is more comprehensive and flexible and because it showed to us good agreement with measurements [10]. The simulation of electromagnetic field propagation in the TEM cell was preferred for present dosimetric assessment due to a significant reason – to also visualize the SAR distribution over the sample volume. Direct measurement of local SAR and its distribution over one sample is not possible with the experimental set-up. The method of finite integration technique for solving Maxwell's equations is implemented in CST Microwave Studio [11] which was used as a computational tool. The average SAR value over a sample was also determined and is expressed as SAR_{comp} in Table 1.

The photo-assimilatory pigments (chlorophyll a, chlorophyll b and total carotenoid pigments) and respectively, average nucleic acids (DNA and RNA) levels in the green tissues of all seedlings samples developed from irradiated seeds as well as for the controls, after the 12 days of growth, were assayed by spectrophotometric methods using a JASCO V530 UV-VIS spectrophotometer. The photo-assimilatory pigments extracts in 80% acetone were prepared and, by using the Lichtenthaler and Welburn's calculation formulas [12], their concentrations were obtained. For total nucleic acid level assay preparation, the perchloric acid (6%) extracts were carried out according to the modified Spirin's method [13]. Spectrophotometric measurements were performed at the wavelengths of: 663 nm, 646 nm and 470 nm (for 80% acetone extracts) and, at 270 nm and 290 nm (for 6% perchloric acid extracts) in the case of nucleic acids.

The biological material used in each of the analysis methods conducted in this study, consisted from an amount of green tissue from the total vegetal mass obtained by mixing up of all seedling tissues, grown from each experimental sample. Each mass was quantified with an analytical balance (AS220.R2 – RADWAG, Poland) with 0.1 mg precision. Seedlings individual stem length was measured with 0.1 cm accuracy and the average stem length and the standard errors were calculated for each batch of test seeds. Relative humidity content (moisture) of green tissue for every experimental sample was assayed at 105 degC using an infrared thermobalance (MAC 210 – RADWAG, Poland) with 0.001% accuracy.

The statistical analysis was performed using *SYSTAT v.13* software package. Descriptive statistics parameters were determined for every experimental data set. Using the Student test, the confidence interval was calculated for every batch of plantlets for the confidence levels P = 90%, 95% and 99%. Statistical analysis of the experimental data, resulted from the three repetitions of the biochemical analysis, was accomplished by means of ANOVA test, considering the significance criterion of 0.05 (p value). Regression analyses have been done in ORIGIN 6.0.

3. RESULTS AND DISCUSSIONS

For the dosimetric simulation, the TEM cell model previously characterized by us in [10, 14] was “loaded” by modelled seed samples. Different geometric models were produced for corn and barley, carefully respecting the mean geometric dimensions of one seed in both cases.

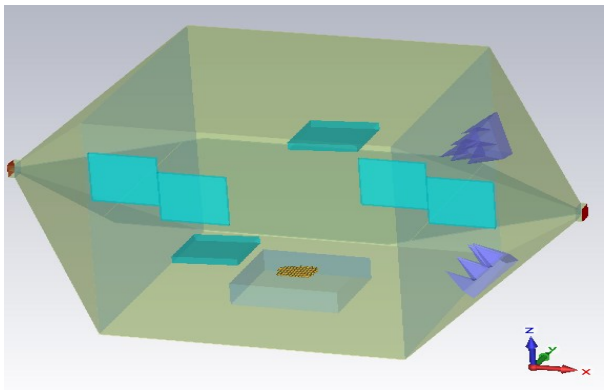

Fig. 1 – Interior of the simulated TEM cell loaded by the sample of 60 barley seeds.

In Fig. 1 the interior of the TEM cell is visible and, near to the floor the seeds were located for all irradiations. On the TEM cell walls one observes the ferrite plates and the absorbent materials, which are exactly simulating the geometry and

material properties of the experimental (real) cell. The geometric, densitometric and dielectric parameters of the seeds were necessary to be set at the computational step. In Table 2 we present the parameters of *Zea Mays* – whose dielectric properties have been extracted from [15] and of *Hordeum vulgare* – whose dielectric properties have been extrapolated from [16].

Table 2

Significant dielectric and physical parameters of the seeds

Sample type	f [MHz]	ε_r'	ε_r"	ρ [kg/m^3]	V_{seed} [cm^3]
Zea mays	850	7.250	1.580	1732.6	0.218
Zea mays	1800	6.600	1.420	1732.6	0.218
Hordeum vulgare	850	2.667	0.136	1268.0	0.077
Hordeum vulgare	1800	2.518	0.140	1268.0	0.077

SAR levels in both corn and barley seeds are two orders of magnitude higher at the highest frequency than at the lowest one. Much more heterogeneous distribution of SAR values over the sample were obtained at the higher frequency, as expected, also. SAR distribution over the modelled seed sample of corn is represented in Fig. 2. The peak values of SAR over corn sample were: $SAR_{max1} = 0.007$ W/kg – at $f_1 = 850$ MHz and $SAR_{max2} = 0.534$ W/kg – at $f_2 = 1800$ MHz. At 1800 MHz a higher non-uniformity of SAR distribution is obvious, than at 850 MHz. SAR variation over the barley sample is observed in Fig. 3. The peak values of SAR over barley sample were: $SAR_{max1} = 0.012$ W/kg – at $f_1 = 850$ MHz and $SAR_{max2} = 0.587$ W/kg – at $f_2 = 1800$ MHz. In this case the SAR non-uniformity is more pregnant also at higher frequency of 1800 MHz.

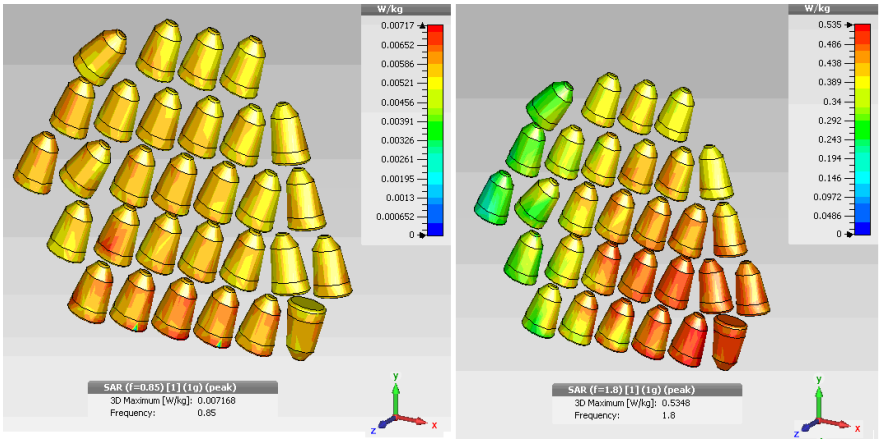

Fig. 2 – SAR distribution in modelled corn (*Zea mays*) seeds,
left – for seeds exposed at 850 MHz and right for seeds exposed at 1.8 GHz.

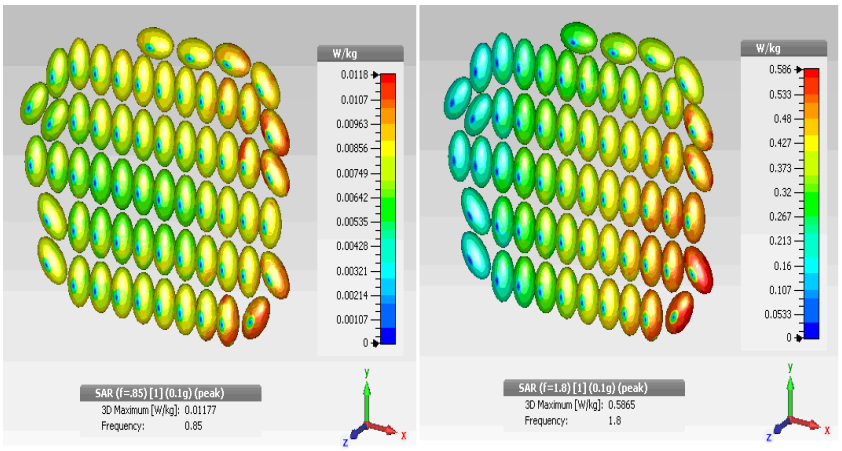

Fig. 3 – SAR distribution in modelled barley (*Hordeum vulgare*) seeds, left – for seeds exposed at 850 MHz and right for seeds exposed at 1.8 GHz.

Concluding the dosimetric assessments, there are two very different situations of energetic rate absorption:

1. At $f_1 = 850$ MHz, an extremely low and practically the same average SAR was produced in both seed types; SAR distribution was not very inhomogeneous throughout the sample.

2. At $f_2 = 1.8$ GHz, a medium level of SAR was produced in the samples, and just slight different for the two plants; in this case however, a significant dispersion of SAR values over the seeds is encountered.

Regarding the biological effects induced by UHF-EMF exposure of seeds, we observed an inhibition of plantlets growth for both plant species at both frequencies.

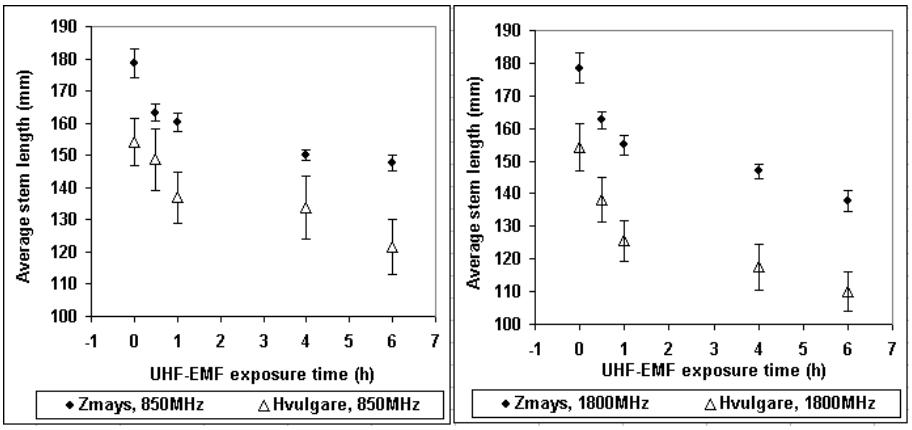

Fig. 4 – The average stem length of 12 days old plantlets in function of high frequency electromagnetic field (UHF-EMF) exposure time.

The average stem lengths of seedlings and afferent standard deviations are represented in Fig. 4 where exposure duration is the experimental variable. All average stem length values are statistically significant ($p < 0.05$) in comparison to control, with one exception in case of *Zea mays* seeds exposed at 850 MHz, 0.5 hours. Regression analysis revealed exponential decay functions over all four experimental groups studied. In Table 3 are given the exponential functions fitting the graphs from Fig. 4. According to these results it seems that similar mechanism of seedlings growth change undergo all experimental cases, resulting in plantlets stem length decreasing at the increase of exposure duration – since the same function type approximates the experimental dependences.

Table 3

Regression functions fitting the dependence between average stem lengths value (L) and UHF-EMF exposure time (t)

Seeds type	*f* [MHz]	Regression function	Determination coefficient R^2, $p < 0.05$
Zea mays	850	$L = 148.78+29.076e^{-t/0.908}$	0.981
Zea mays	1800	$L = 141.815+36.03e^{-t/0.97}$	0.959
Hordeum vulgare	850	$L = 124.72+29.03e^{-t/1.764}$	0.886
Hordeum vulgare	1800	$L = 113.27+41.089e^{-t/0.917}$	0.976

Follow up the green tissue moisture analyses we obtained a slight increase of plantlets tissue humidity percentage with enhanced exposure duration, for all experimental samples (Fig. 5).

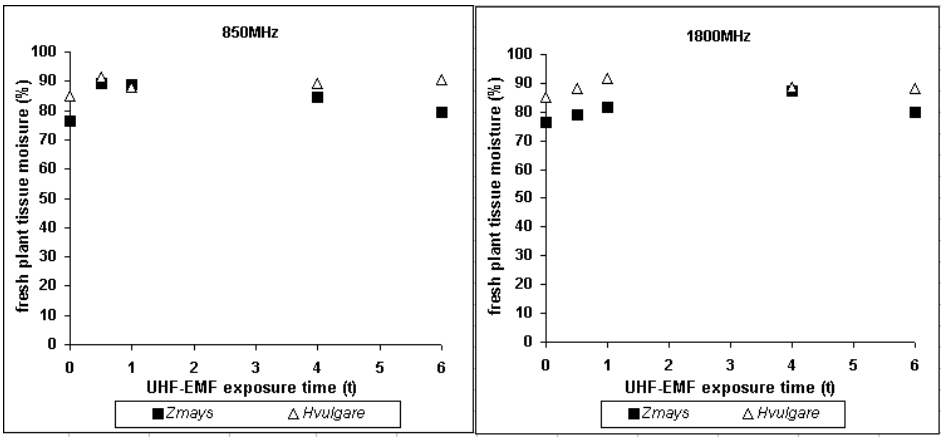

Fig. 5 – The fresh plant tissue moisture (%) of 12 days old plantlets in function of high frequency electromagnetic field (HF-EMF) exposure time.

Chlorophylls content of leaves is an indicator of stress induced by different factors, conducting to important information regarding plant responses to

environmental changes. Lower chlorophylls content are evident in the initial stages of a plant tissue under an environmental stress. For enhanced stress chlorophyll content decreases faster than other pigments, and our results are convergent to others [17]. The chlorophyll a (Chl a) concentrations (Fig. 6) were found decreased for all UHF-EMF exposure times comparatively to the control sample, with one exception for the barley seeds exposed to 1800 MHz EMF for 0.5 hours, in this case concentration of Chl a being larger than of controls (statistically significant in relation to the threshold of 0.05).

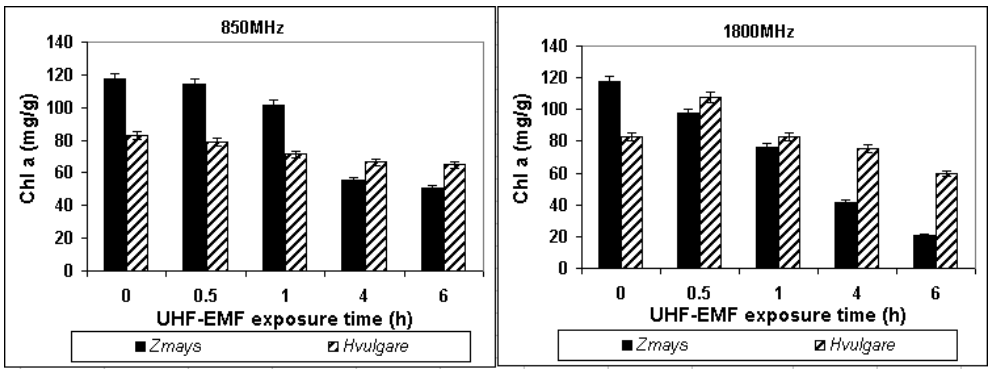

Fig. 6 – The chlorophyll a content (Chl a) of 12 days old plantlets in function of high frequency electromagnetic field (HF-EMF) exposure time.

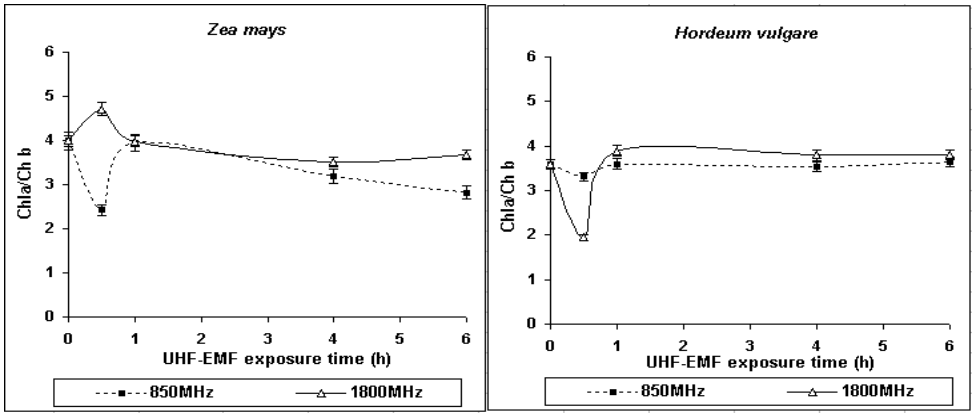

Fig. 7 – The chlorophylls ratio (Chla/Chlb) of 12 days old plantlets dependence on exposure duration of pre-exposed seeds.

The total assimilatory pigments contents had the same variation with increasing of UHF-EMF exposure time as chlorophyll a level. At 1800 MHz, after 6 hours of seeds irradiation the total chlorophylls concentration decreased with 81% for *Zea mays* and with 30% for *Hordeum vulgare* while at 850 MHz after

6 hours of exposure this level decreased with 53% for *Zea mays* and with 22% for *Hordeum vulgare*. Other researchers have been reported a 13% decrease in total chlorophylls level after 4 hours of maize seedlings exposure to 1800 MHz [3].

Also, the chlorophylls ratio (Chl a/Chl b) is considered the best indicator of the photosynthesis process efficiency [18]. Different dependences of chlorophylls ratio were observed for every experimental sample in connection to increasing of UHF-EMF exposure duration (Fig. 7).

It seems that the same environmental constraint is able to have different influences in early ontogenetic stages of development of different plant species, even for species belonging to the same family and order.

The lowest values of chlorophylls ratio were obtained after 0.5 hours of *Zea may* seeds irradiation at 850 MHz (39% decrease) and of *Hordeum vulgare* seeds irradiation at 1800 MHz (45% decrease). On the other hand, the highest amount of chlorophylls ratio was revealed at 1800 MHz after 0.5 hours of *Zea may* seeds irradiation (18% increase).

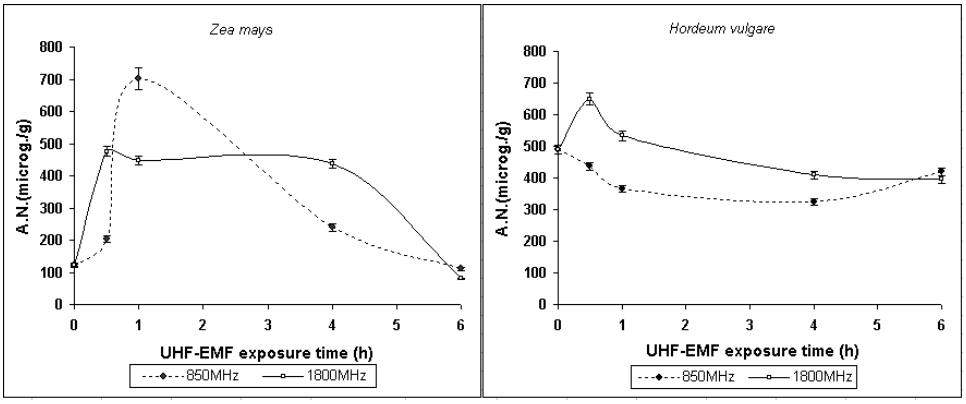

Fig. 8 – The total level of nucleic acids of 12 days old plantlets dependence on exposure duration of pre-exposed seeds.

Analyzing all biological effects highlighted above, one ascertain that at the same frequency and at the same SAR value, the two cereal types behaved differently. Higher SAR level combined with higher frequency applied on the same plant conducted to a more pronounced and a more non-linear biological response for all exposure durations. The most used phenomenological interpretation of UHF biological effects suppose that the photon absorbed from the radiation is leading to complex synergic cellular processes. They could further conduct to molecular damages leading to free radical yielding. Then, free radicals as mediators could interfere with various molecular processes involved in plants growth. Amat *et al*. [19] proposed that light radiation effects on plants appear not only through chromophores, but also through induced alternating electric fields in the medium

which is able to interact with polar structures. On the other hand, non-linearity between electromagnetic exposure and biological effect was reviewed and argued intensively by authors of [20], who also underlined that SAR should not be considered as a proper dosimetric quantity, especially in describing non-thermal or low-thermal effects of EMF such the ones presented here.

4. CONCLUSION

Present experimental study was performed on two different biological species to point out the influence of ultrahigh frequency electromagnetic field on some physiological parameters defining plants growth. The results of the biochemical investigations carried out on *Zea mays* and *Hordeum vulgare* plants developed from pre-exposed seeds to low and very low dosed of radiation, during their early ontogenetic stages, have revealed that growth of different plant species could be influenced in different manner, even for species belonging to the same family and order of taxonomy. Frequencies of 850 MHz and 1800 MHz applied to seeds resulted in inhibitory effects upon plant seedlings growth, the average plants length values being diminished for all exposure durations in exponential decay manner. A slight stimulatory influence of radiation on the moisture content of fresh plant tissue seems to characterize the response of young plantlets from both species. Photo-assimilatory pigments level in both plant species, slightly diminished at both frequencies. Different dependences of chlorophyll pigments ratio evolved for different experimental variables such as plant species, frequency and exposure duration. The photosynthetic processes seem to be influenced by the exposure in the both plant species. The total content of nucleic acids in *Zea mays* plantlets developed from exposed seeds was significantly higher than in control for both frequencies of electromagnetic field. In *Hordeum vulgare* plantlets irradiated at 850 MHz the total nucleic acids level increased while at 1800 MHz the level decreased when compared against the control. Non-thermal or very low thermal effects of ultrahigh frequency electromagnetic field are responsible for the biological influences revealed in the frame of this experimental study. Non-linear responses were emphasized but the triggering mechanism remains unclear. With the very tiny amount of absorbed energy in present cases, which cannot contribute to the biological target temperature increase, we can still assume the hypothesis [20] that the induced forced-oscillation chain will conduct at the end to an exertion of a large resultant force on certain sensors on cell membranes with all biological consequences.

Acknowledgements. This work was funded by *Lucian Blaga* University of Sibiu research grant LBUS-IRG-2015-01/26.

REFERENCES

1. A. Akbal, Y. Kiran, A. Sahin, D. Turgut-Balik, H.H. Balik, Pol. J. Environ. Stud. **21**(*1*), 23–29 (2012).
2. M. Afzal, S. Mansoor, Asian Journal of Agricultural Sciences **4**(*2*), 149–152 (2012).
3. Kumar, H.P. Singh, D.R. Batish, S. Kaur, R.K. Kohli, Protoplasma **2015**, 1–7 (2015).
4. V.P. Sharma, H.P. Singh, R.K. Kohli, D.R. Batish, Sci. Total Environ. **407**, 5543–5547 (2009).
5. V.P. Sharma, H.P. Singh, D.R. Batish, R.K. Kohli, Z. Naturforsch. C **65**, 66–72 (2010).
6. M. Tkalec, K.I. Malaric, B. Pevalek-Kozlina, Bioelectromagnetics **26**, 185–193 (2005).
7. M. Răcuciu, M.S. Miclăuş, Romanian J. Biophys. **17**, 149–156 (2007).
8. W.F. Grant, Mutat. Res. **310**, 175–185 (1994).
9. B.J. Klauenberg, M. Grandolfo, D.N. Erwin (Eds.), *Radiofrequency Radiation Standards: Biological Effects, Dosimetry, Epidemiology and Public Health Policy*, USAF School of Aerospace Medicine, USAFSAM-TR-85-73, Brooks AFB, TX, USA, 1986.
10. S. Miclaus, M. Morega, Rom. J. Phys., **55**(*1–2*), 195–204 (2010).
11. http://www.cst.com/Content/Products/MWS/Overview.aspx
12. H.K. Lichtenthaler, A.R. Wellburn, Biochem. Soc. Trans. **11**, 591–559 (1983).
13. V.A. Struchkov, N.B. Strazhevskaya, R.I. Zhdanov, Bioelectrochem. **58**, 23–30 (2002).
14. S. Miclaus, C. Iftode, M. Colcieru, Proc. ATEE2013, Bucharest, 23–25 May 2013.
15. E. Surducan, C. Neamţu, V. Surducan, J. Phys. Conf. Ser. **182**(*1*), 1–4 (2009).
16. S. O. Nelson, S. Trabelsi, Transactions of the ASABE **54**(*2*), 549–553 (2011).
17. A. Bannari, K.S. Khurshid, K. Staenz, J.W. Schwarz, IEEE Trans. Geosci. Remote Sensing **45**, 3063–3074 (2007).
18. D. Ort, J. Whitmarsh, *Photosynthesis. Encyclopedia of Life Sciences*. Macmillan, London, 2001.
19. A. Amat, J. Rigau, R.W. Waynant, I.K. Ilev, J.J. Anders, J. Photochem. Photobiol. B **82**(*2*), 152–160 (2006).
20. D. J. Panagopoulos, O. Johansson, G.L. Carlo, PLoS ONE **8**(*6*), e62663 (2013).