IMPLICATIONS OF VARIOUS INTENSITIES OF RADIO FREQUENCY ELECTROMAGNETIC RADIATION (462 MHZ) IN THE INDUCTION OF OXIDATIVE STRESS DURING THE GERMINATION OF HIPPOPHAE RHAMNOIDES SEEDS

CĂLIN LUCIAN MANIU[1*], EUGEN UNGUREANU[1]

Keywords: electromagnetic field, oxidative stress, reactive oxygen species, superoxide dismutase, catalase, peroxidase.
Abstract. The data accumulated by now shows that the topic of biological effects of electromagnetic radiation is far from being exhausted. Is undoubtedly that a non-ionizing radiation field maintained on a biological entity has some effects on it. To try shaping issues regarding this, this work aims to study the impact of radiation generated by an emission-reception radio station that emits on 462.6875 MHz frequency. For this purpose, were used *Hippophae rhamnoides* L. seeds which germinated in the laboratory, under controlled conditions, concentrically arranged around the radiation source, in which case electromagnetic radiation has a different impact. Seed germination lasted 35 days, while the device has continuously worked, and the seeds were constantly irradiated. It was precisely measured the intensity of the magnetic component of the field in all places where the seeds were placed for germination. It was calculated the percentage of germination and it was determined the enzyme activity involved in eliminating the oxidative stress effects. It was found significant variations of the parameters mentioned above in conjunction with the radiation intensity depending on the distance from the source.

INTRODUCTION

Accelerated and widespread use of different electric and electronic devices increased the exposure to radio and microwave frequency electromagnetic fields (EMFs). These EMFs are classified as non ionizing radiation but they can cause damage depending on the power level, frequency, and the properties of exposed tissue. There is some evidence that microwaves (300 MHz–300 GHz) produces changes in the cell membrane’s permeability and cell growth rate as well as interference with ions and organic molecules, like proteins (Kwee et al., 1998, 2001; de Pomerai et al., 2003; Repacholi, 2001; Pologea-Moraru et al., 2002; Banik et al., 2003). Plants are essential components of a healthy ecosystem and have important role in the living world as main primary producers of food and oxygen; therefore it would be beneficial to investigate their interaction with today’s increased exposure to radio and microwave frequency fields. Additionally, higher plants are useful test organisms for environmental studies because they are eukaryotic multicellular organisms. Many of them are sensitive to different kinds of stresses and are easy to grow in controlled laboratory conditions without too much expense (Wang, 1991). During the years it became more and more interesting to test the effects of EMFs on higher plants (Tkalec et al. 2005, 2007). Considering the increasing interest for the subject, this work focus on the influence of 462.6875 Mhz EMF on the oxidative stress during the *Hippophae rhamnoides* seeds germination. This species was chosen because of the following aspects. Firstly, the period of germination is relatively long, the experiment is held over a period of 35 days, this issue was important because the seeds were irradiated for a long time, unlike other species that germinate very fast (3-5 days). Secondly, sea buckthorn (*Hippophae rhamnoides* L.) is a species which has some interesting biochemical characteristics: vitamins B, C, E, K, carotenoids (the most dominant carotenoid in sea buckthorn, it’s admitted to be associated with reduced risk of breast, stomach, esophageal, and pancreatic cancers), flavonoids (it have been found in controlling arteriosclerosis, reducing cholesterol level, turning hyperthyroidism into euthyroidism and eliminating inflammation), tannins, metallothionein (acts as detoxifying agency for heavy metals and as free radical scavenger for most toxic radical) and 5-hydroxytryptamine (5-HT), a chemical neurotransmitter substances (Lian, 2000; Thomas, 2003).

MATERIALS AND METHODS

To seek evidence of the influence of electromagnetic field (EMF) of radio frequency on oxidative stress, during the germination of seeds, was used a source consisting of two Motorola T5725 emission-reception radio stations that have been programmed to automatically call one another throughout experiment. The communication system frequency is set on channel 6 at 462.6875 MHz with 500mW transmit power. Thus, around the two emission-reception radio stations were delimitated four concentrically levels (different distances from the source), with four groups with five Petri Dishes (A1-A5; B1-B5; C1-C5; D1-D5), in each plate with about 20 seeds. Experiment diagram is depicted in the fig.1 and fig.2.

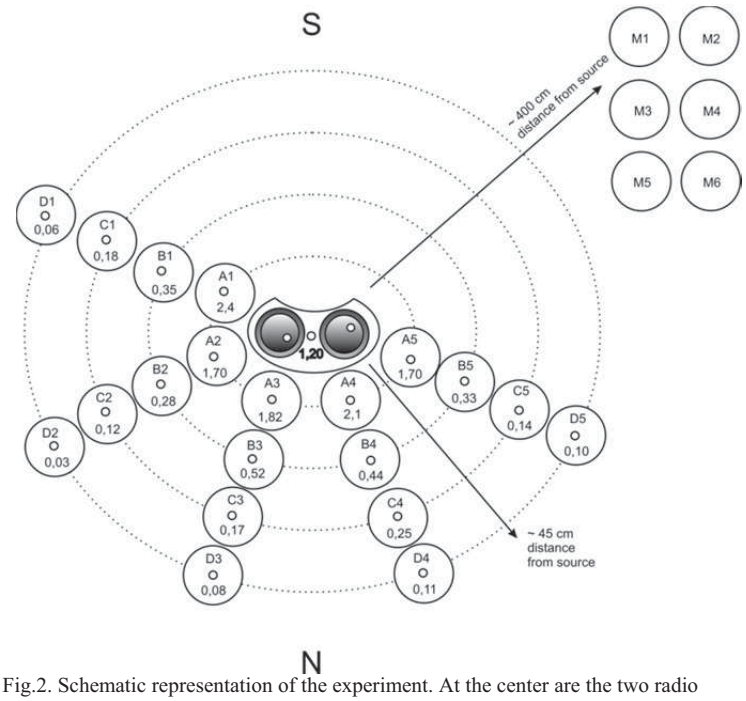

Fig.2. Schematic representation of the experiment. At the center are the two radio stations, around which were arranged the Petri Dishes. In the center of each plate is indicated the magnetic induction in μT.

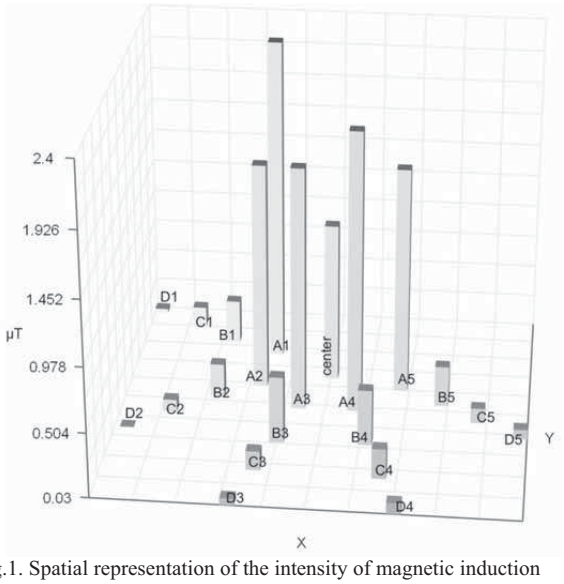

Fig.1. Spatial representation of the intensity of magnetic induction (μT) in relation to the probes arrangement.

The control lots, consisted in six Petri Dishes (M1-M6), were positioned sufficiently far from the EMF source. It was monitored the temperature and the humidity, which were maintained constant in both irradiated and control lots. The magnetic induction (B) of the field was measured with a digital teslameter in the indicated points on the drawing. The values are in μT. After germination period (35 days), the plant material was processed to determine the activity of the superoxide dismutase (SOD), catalase and peroxidase, enzymes involved in the removal of oxidative stress, (Artenie et al., 2008). Also it was determined the total protein synthesis and was calculated the percentage number of the germinated seeds. From each sample was counted the number of germinated seeds and reported to the total number of seeds. Data were represented graphically in the diagrams at the end of the paper, which appear after the statistical processing. On the charts, the vertical error bars shows the 95% (0.05) confidence level for mean. Interval estimates are often desirable because the estimate of the mean varies from sample to sample. The interval estimate gives an indication of how much uncertainty there is in our estimate of the true mean. The narrower the interval, the more precise is our estimate (Kotz et al., 1988-2008).

RESULTS AND DISCUSSION

After investigations, it was obtained a number of results regarding the catalytic activity of SOD, catalase and peroxidase. In mitochondrial electron transport, the participation of flavins, ubisemiquinones, and other electron carriers leads to the formation of superoxide radicals, H_2O_2, and hydroxyl radicals (summarized as ROS, **r**eactive **o**xygen **s**pecies) as by-products. These by-products cause severe cell damage. Since the formation of ROS is especially high, when the components of the respiratory chain are highly reduced, there is a necessity to avoid an over-reduction of the respiratory chain. As regards, the activity of SOD, there was a significant increase in the activity at the A1-A5 and C1-C5 samples, in comparison with the control samples. In case of B1-B5 and D1-D5 samples, it was found no significant differences in relation to the control. It is possible that these variations are due to interference between EMF and the normal light reactions in photosynthesis and electron transport chain, the sequence of reactions is known as generating superoxide ions (O_2^-) (Heldt, 2005; Schulze, Beck, Müler-Hohenstein, 2005). In comparison with the magnetic field induction intensity, it was observed that there is no direct correlation between the induction and enzyme activity. In case of C1-C5 was founded that a small amount of induction caused a significant increase in enzymatic activity comparative to the control as to the A1-A5 lots, undergo on a much higher intensity, being the nearest from the radiation source. The results are presented in the fig 3.

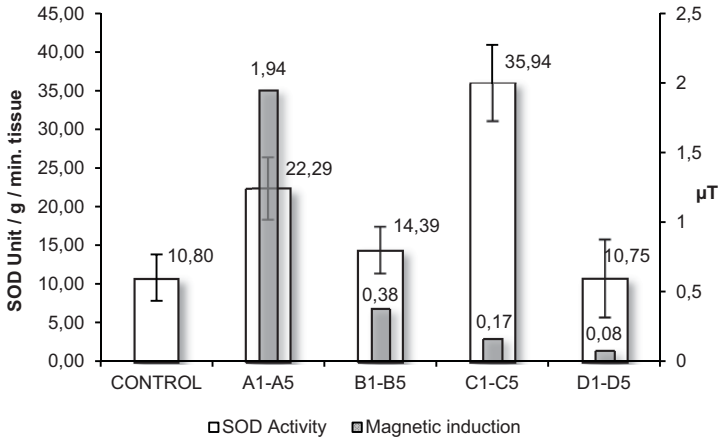

Fig.3. Variations of the SOD activity and magnetic induction.

Catalase is an enzyme present in large quantity in peroxisomes where neutralize H_2O_2 resulting from redox processes. Peroxisomes are a common constituent of eukaryotic cells. In plants there are two important differentiated forms: the leaf peroxisomes, which participate in photorespiration and the glyoxysomes, which are present in seeds containing oils (triacylglycerols) and play a role in the conversion of triacylglycerols to carbohydrates. They contain all the enzymes for fatty acid β-oxidation. (Heldt, 2005). The experiment conducted, shows a significant decrease in the catalase activity in relation to the control (Maniu et al., 2009). The profile of these decrease is similar in both activity expressed in enzyme units at 100g material and for specific activity (enzyme units per 100 mg protein) as is illustrated by the fig. 4 and fig. 5.

Peroxidase, unlike the other two, is an enzyme widespread in all cellular compartments (Bakalovic et al, 2006; Passardi et al., 2007; Koua et al., 2008) where the function is to neutralize hydrogen peroxide using various electron donors (Heldt, 2005). The experiment conducted has shown that activity of this enzyme undergoes major fluctuations, as can be seen in fig. 6 and fig. 7. It might say that this pattern of fluctuation could be due to influences exerted by the different EMF from a cellular compartment to another, which is to have different amounts of enzyme (Ungureanu et al., 2009).

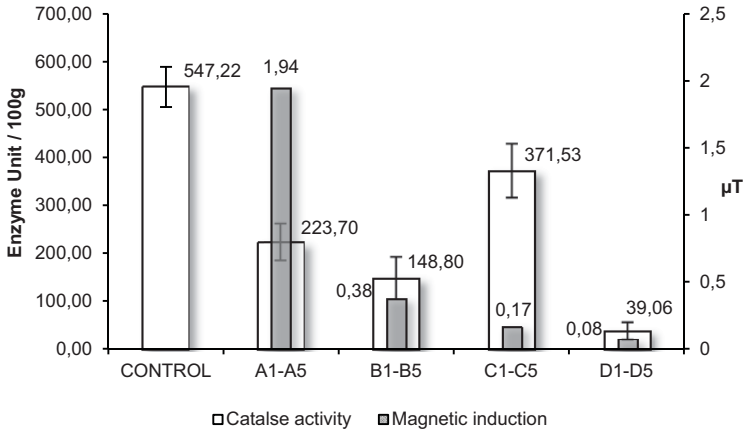

Fig.4. Variation of the catalase activity and magnetic induction.

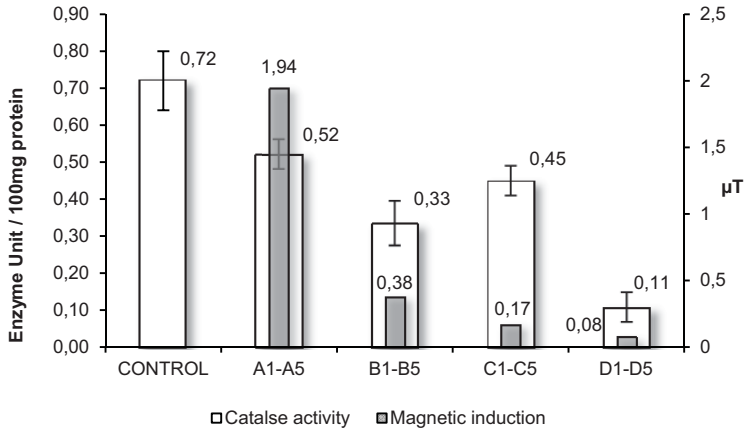

Fig.5. Variation of the catalase specific activity and magnetic induction.

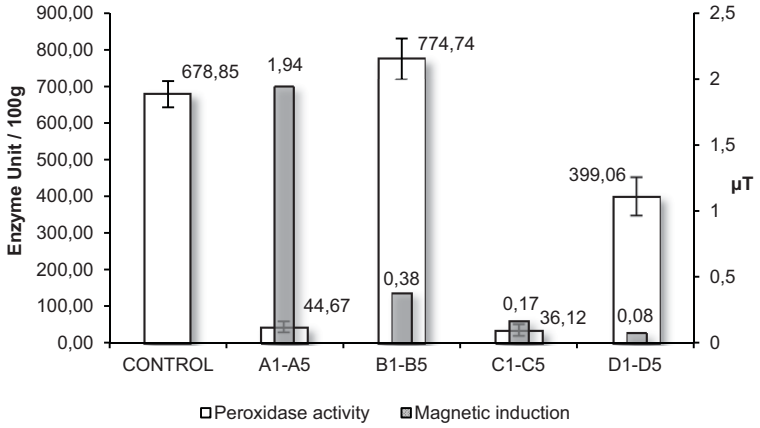

Fig.6. Variation of the peroxidase activity and magnetic induction

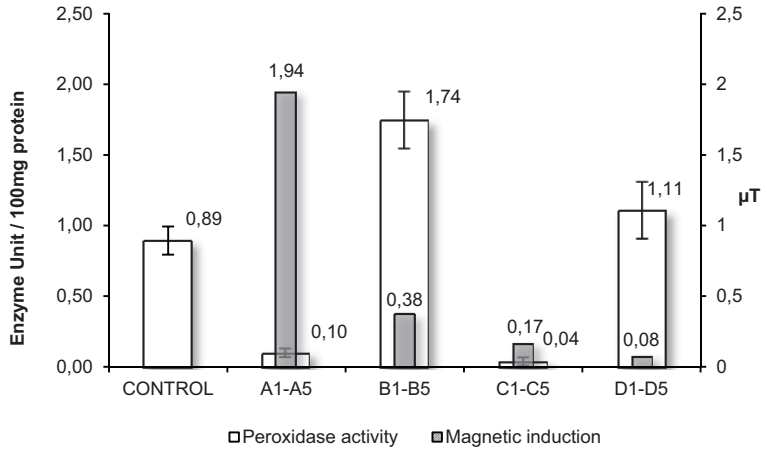

Fig.7. Variation of the peroxidase specific activity and magnetic induction.

The amount of protein highlighted by Bradford method shows a significant variation for A1-A5, B1-B5 and D1-D5 in relation to the control (fig. 8), in such cases were founded decreases. C1-C5 probes, shows an amount of protein approximately equal to control lots. Proteins highlighted in the experiment come both from the reserve proteins in seeds and "de novo" synthesis protein necessary seedlings, especially towards the end of germination.

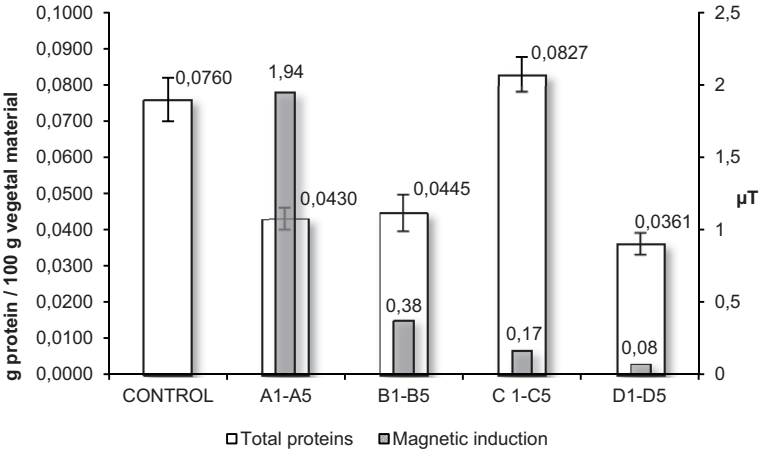

Fig.8. Variation of the total protein quantity and magnetic induction.

The percentage of seeds germinated (fig. 9) during the experiment, indicates that low intensity magnetic induction can have a stimulating effect. This is observed for samples B1-B5 and D1-D5, where the percentage of germination reached very high values, 97% respectively 94%. In the other two cases, compared with the control, there is a negative trend in germination. In case of A1-A5, high intensity magnetic induction does not seem to have affected germination

(percentage difference being only 8% compared to control), where C1-C5 can be considered a decrease by 19%.

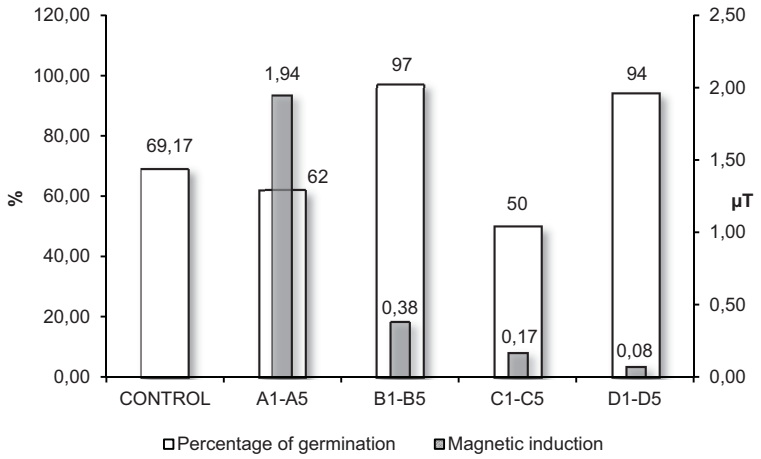

Fig.9. The percentage of seeds germination and the variation of magnetic induction

From germination behavior of toward various intensities of magnetic induction, it may find a correlation between enzyme activities involved in removing the effects of oxidative stress. In cases B1-B5 and D1-D5, a high percentage of germination is correlated with a decreased oxidative stress, due to a weak catalase activity and a normal SOD activity, correlates with a low amount of total protein. Low values identified in the total proteins quantity may be due to a greater consumption of resources during germination. Since peroxidase takes part to other processes, very high activity in cases B1-B5 and D1-D5 in consistency with high rates of germination may be due to the involvement of this enzyme into other metabolic processes closely related to germination and growth (Atak et al., 2007). From this perspective, the low activities recorded in samples A1-A5 and C1-C5 cannot be attributed to the direct effect of EMF. On the other hand, cases A1-A5 and C1-C5, recorded lower rates germination, in line with a higher oxidative stress, indicated by a significant increase in SOD activity. In these cases, catalase activity although lower than the control, is significantly higher than in cases B1-B5 and D1-D5, which shows clearly that it took part in countering the stress in close correlation with SOD.

CONCLUSIONS

The performed experiment, with 462.6875 MHz electromagnetic radiation frequency, obtained from two emission-reception radio stations, has demonstrated that there is no direct correlation between the intensity of induction and the effects caused by the different magnetic induction during the seeds germination. Thus, there are cases where electromagnetic radiation may be used as a stimulating agent since two cases were found with a very high percentage of germination in correlation with a normal SOD activity, low catalase activity and low total protein amount. In these circumstances, it is required more detailed investigations, particularly targeted on that induction values that caused the stimulation of germination.

REFERENCES

Artenie V., Ungureanu E., Negură Anca, Metode de investigare a metabolismului glucidic şi lipidic, Ed. Pim, Iaşi, 2008.
Atak Ç., Çelik Ö., Olgun A., Alikamanoğlu S., Rzakoulieva A., *Effect of magnetic field on peroxidase activities of soybean tissue culture*, Biotechnol. & Biotechnol. Eq. 21/2007/2.
Bakalovic N, Passardi F, Ioannidis V, Cosio C, Penel C, Falquet L, Dunand C., PeroxiBase: a class III plant peroxidase database, Phytochemistry 2006 Mar; 67(6):534-9.
Banik S, Bandyopadhyay S, Ganguly S., Bioeffects of microwave. A brief review. Bioresource Technol, 2003, 87:155–159.
de Pomerai DI, Daniells C, David H, Allan J, Duce I, Mutwakil M, Thomas D, Sewell P, Tattersall J, Jones D, Peter E, Candido M., Microwave radiation can alter protein conformation without bulk heating. FEBS 2003, 543:93–97.
Heldt H.W., Plant Biochemistry 3rd ed., Elsevier Academic Press, 2005.
Kotz, S., Campbell B. Read, N. Balakrishnan, Brani Vidakovic, (Ed. & Co-Ed.), Encyclopedia of Statistical Sciences, vol. 1-9, plus supplements. New York: Wiley-Interscience, 1988-2008.
Koua D, Cerutti L, Falquet L, Sigrist CJ, Theiler G, Hulo N, Dunand C., PeroxiBase: a database with new tools for peroxidase family classification, Nucleic Acids Res. 2008 Oct 23.
Kwee S, Raskmark P, Velizarov S., Changes in cellular proteins due to environmental non-ionizing radiation. I. Heatshock proteins. Electro Magnetobiol 2001, 20:141–152.
KWEE S, RASKMARK P., Changes in cell proliferation due to environmental non-ionizing radiation II. Microwave radiation. Bioelectrochem Bioenerg, 1998, 44:251–255.
Lian Yongshan (Editor-in-chief), Biology and Chemistry of Hippophae. L., Gansu Science & Technology Press, 2000.
Maniu C.L., Ungureanu E., Vântu Smaranda, Creţescu I., Preliminary study concerning the radiofrequency electromagnetic field influence on the catalase activity in the Hippophae rhamnoides seeds germination. Analele Ştiinţifice ale Universităţii „Alexandru Ioan Cuza" Iaşi, (S.N.) Genetică şi Biologie Moleculară, 2009, Tom. X.
Passardi F, Theiler G, Zamocky M, Cosio C, Rouhier N, Teixera F, Margis-Pinheiro M, Ioannidis V, Penel C, Falquet L, Dunand C., 2007, PeroxiBase: The peroxidase database, Phytochemistry 2007 June;68(12):1605-11.
Pologea-Moraru R, Kovacs E, Iliescu KR, Calota V, Sajin G., The effects of low level microwaves on the fluidity of photoreceptor cell membrane. Bioelectrochemistry, 2002, 56:223–225.
Repacholi MH., Health risk from the use of mobile phones. Toxicol Lett, 2001, 120:323–331.
Schulze E.D., Beck E., Müler-Hohenstein K., Plant Ecology, Springer Berlin – Heidelberg, 2005.
Thomas S.C. Li, Thomas H.J. Beveridge, Sea buckthorn (Hippophae rhamnoides L.): production and utilization, NRC Research Press, Ottawa 2003.
Tkalec, M.; Malaric, K. & Pevalek-Kozlina, B., Influence of 400, 900, and 1900MHz Electromagnetic Fields on Lemna minor Growth and Peroxidase Activity Bioelectromagnetics, 2005, 26, 185-193
Tkalec, M.; Malaric, K. & Pevalek-Kozlina, B., Exposure to radiofrequency radiation induces oxidative stress in duckweed Lemna minor L. Science of the Total Environment, 2007, 338, 78-89
Ungureanu E., Maniu C.L., Vântu Smaranda, Creţescu I., Consideration on the peroxidase activity during Hippophae rhamnoides seeds germination exposed to radiofrequency electromagnetic field influence. Analele Ştiinţifice ale Universităţii „Alexandru Ioan Cuza" Iaşi, (S.N.) Genetică şi Biologie Moleculară, 2009, Tom. X.
Wang W., Literature review on duckweed toxicity testing. Environ Res, 1991, 52:7–22.
Xia L, Guo J., Effect of magnetic field on peroxidase activity and isozyme in Leymus chinensis. Ying Yong Sheng Tai Xue Bao (The journal of applied ecology), 2000, 11:699–702.
Xiujuan W, Bochu W, Yi J, Defang L, Chuanren D, Xiaocheng Y, Sakanishi A, Effects of sound stimulation on protective enzyme activities and peroxidase isoenzymes of chrysanthemum. Colloid Surface B 27:59–63, 2003.

1"Alexandru Ioan Cuza" University, Faculty of Biology, Address: 20A Blvd. Carol I; 700505; Iaşi, Romania
* cmaniu@uaic.ro