Extremely low frequency - zero magnetic field and human lymphocytes response

[1]Daniela Ciorba, [2]V. Vasile Morariu, [1] Constantin Cosma, [2]Silvia Neamtu, [3]Eva Fischer-Fodor, [3]Vasile Foris

[1] Environment Science Faculty, Babeş-Bolyai University, Cluj-Napoca, Romania, [2] National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania; [3] Cancer Institute, Prof. Dr. I. Chiricuta, Cluj-Napoca, Romania. Corresponding author: D. Ciorba, ciorbad@yahoo.com

Abstract. The dose-response relationship might be very important for risk modeling and for study of individual sensitivities. In extremely low frequency - zero magnetic fields, (ELF ZMF), this relation is not known and a deterministic or stochastic effect is not established. Inside of ours Helmholtz Coil System, only the natural fluctuation remains active when the static component of geomagnetic field has been compensated. Due to increase areas of relaxed DNA from lymphocyte' nucleus, the increasing of comet tail and score lesions has been observed, which are equivalent with a genotoxic exposure.
Key words: ELF- ZMF, lymphocytes, comet assay, magnetic component.

Introduction. The geomagnetic field (GMF) has been regarded as an environment factor, which accompanied life on Earth, since the beginnings. As we know, many animals are able to orientate with help of the GMF, but it remains unclear which mechanisms may be responsible for reception of these extremely weak magnetic fields (Glaser 1999). Zero magnetic field (ZMF) means the absence of GMF, which can be measured into the space.

The intensity of the main (static) magnetic component of GMF includes a vertical component with a maximum of 60-70 μT at the poles. The horizontal component has its maximum near the equator, with 34-40 μT and at middle latitude, our site, the GMF value on the Earth surface is around 50 μT (http.www.national.atlas.gov).

The natural fluctuations of GMF involve extremely low frequency magnetic field (ELF MF), while the main component of the GMF is static. These fluctuations are known to trigger effects on living systems, despite of their relative low intensity values compared to the main static component of GMF, and are in relation with the natural climate variability and global solar variations (Tahas et al 2012).

A large broad of clinical and epidemiological data proved the effects of geomagnetic perturbations on living systems (Villoresi et al 1998). DNA damages induced by low electromagnetic field were studied earlier (Morariu et al 2000; Ciorba et al 2001).

A reduction of GMF static component up to hundreds times (eg 0.4 -0.5 μT), has happened during reversal of Earth magnetic poles (Raup 1985) and represent a really abnormal environment for life (Nishimura et al 2009).

0.4-0.5 μT magnetic field intensity from anthropogenic sources could be measured sometimes in our environment, both occupational and residential.

Magnetic fields reach as much as approximately 20 μT, close to power lines but, decrease sharply with distance, arriving in many residential places to 0.3-0.4 μT or less. The epidemiological studies on childhood leukemia have focused on average residential ELF MF above 0.3 to 0.4 μT as a risk factor for cancer. Several extensive surveys showed that approximately 0.5-7% of children with leukemia had time-averaged exposures in excess of 0.3 μT and 0.4–3.3% was exposed to in excess of 0.4 μT. Due to increase of leukemia incidence, the ELF MF has been classified as a "possible human carcinogen" (SCENIHR 2015).

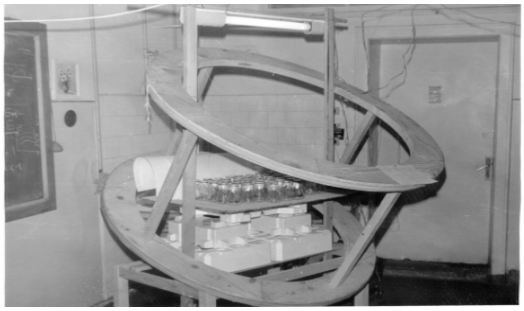
The understanding of cancer promotion in ELF MF, means understanding of involved mechanisms at cellular and molecular levels, when the increasing of DNA damages is associated with increasing of cancer incidence (Gateva et al 2008; Testa et al 2004).

The answer to the question whether the fluctuation's intensity or the absence of the static component of GMF are responsible to induce the effects on proliferation,

differentiation, apoptosis or cells death is still waited. Due to this fact, inside of ours Helmholtz Coil System, we try to observe what happens in absence of static component of GMF, equivalent to an extremely low frequency-zero magnetic fields exposure (ELF-ZMF). DNA damages in human lymphocytes exposed to ELF ZMF have been monitored by comet assay.

Material and Method. The study took place during May-July 2008.

Helmholtz coil system-zero magnetic field exposure, ELF ZMF. Static component of GMF was compensated in a Helmholtz coils system mounted on a wooden frame of 1.2 m in diameter (Picture 1). The resulting magnetic field was a hundred times lower than natural GMF. The sample was exposed in the middle of this system, in a fixed position for a period of three day. The controls were placed in natural magnetic field conditions in the same room at 2 m distance from the Helmholtz coil. The controls were placed in the same room at a 2 m distance from the magnetic field compensating system. All the other experimental conditions as temperature, light and humidity were the same for the sample and control. The trend of natural magnetic fluctuations during the experiment performed in 2008, in National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, was monitored by Ap index of geomagnetic activities which is an average index of the daily geomagnetic activity at certain magnetic observatories around Earth.



Picture 1.

The Ap index data were extracted from the records of the National Geographic Data Center, USA for the periods of concern. According to these data, the samples exposure were done in periods of quiet geomagnetic activity, with low magnetic field variations.

Lymphocytes culture. The human lymphocyte has been exploited by many of the biodosimetry methods including those based on cytogenetic methods. The blood sample was obtained from 4 healthy donors. Lymphocytes were separated using the density gradient protocol, on Histopaque-1077. Briefly, blood is diluted 1:1 with PBS and layered over 600 µl Histopaque and centrifuged at 800 X g for 20 minutes. The 'buffy' coat, an opalescent layer containing mononuclear cells is aspirated into 3-5 mL of PBS and centrifuged at 250 X g for 10 minutes to pellet the lymphocytes and counted over a haemocytometer and viability tested. The pellet is suspended in 1 mL of (RPMI + 15% fetal serum, + 10% glutamine + antibiotics) in test tubes. Aliquots of 2 X 10^6 cells per 100 µL of medium are taken for each dose of the test material. The quantify of inducted effects in lymphocytes cultures after 24 hours, 48 hours, 72 hours of exposure was done through comet assay (CA). The comet assay requires few steps. First, the cells are embedded in agarose on a microscope slide. In the agarose, the cells and nuclear membranes are lysed and the DNA is subjected to alkaline electrophoresis. Individual comet images were captured for digitization with a CCD camera attached to the microscope. Cellular DNA is visualized using a fluorescence microscope after staining cells with an appropriate dye. Etidium Bromide stained nucleoids were examined at 40X objective with a Axioplan, Zeiss epifluorescence microscope, at 460 nm. Visual and computerized image analyses of DNA damage were carried out based on perceived comet tail length migration and relative proportion of DNA in the comet tail, using the next equation:

Scoring for DNA damage - score lesion (SL) = the sum of relative units (UR), means the product between cells number finding in a special stadium and the number of comet class.

SL (UR) =A0 + B1 + C2 + D3 + E4, where A, B, C, D, E, = number of cells in 0, 1, 2, 3, and 4 stadium. A total damage score for each slide was derived by multiplying the number of cells assigned to each grade of damage by the numeric value of the grade and summing over all grades (giving a maximum possible score of 400, corresponding to 100 cells at grade 4).

Tail factor (TF) represents a quantification parameter over 500 of studied cells, meaning the relative percent of DNA in tail. TF(%) = (A F_A + B F_B + C F_C + D F_D + E F_E)/500, where F_A, F_B, F_C, F_D, F_E, is the relative percent of DNA in tail. The working protocol was in accord with ITRC: THE SCGE/ COMET ASSAY PROTOCOL modified after Brie et al (2004).

Results and Discussion. The migration patterns have been graphically expressed in histograms by plotting of comets frequency (*Y* axis) and the cells stadium – according with DNA damaged (*X* axis). After 24 exposure hours the observed effect was increases of DNA damage, corresponding with increased number of cells in stadium 2 (30% from nucleus' DNA damaged) (Figure 1).

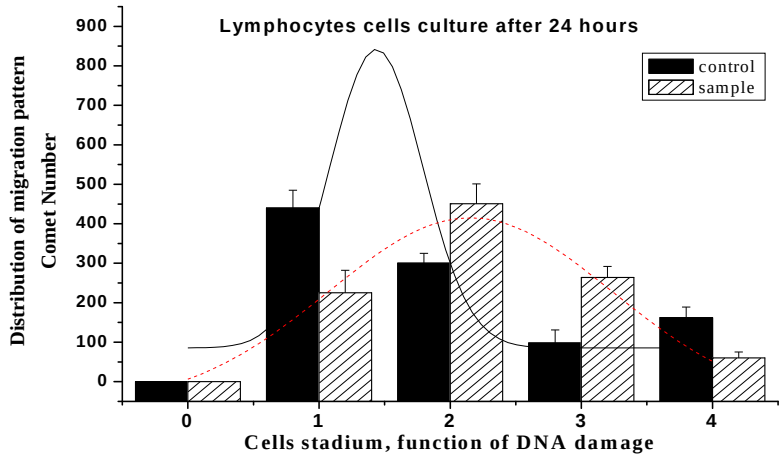


Figure 1. The comet distribution after 24 hours, in ELF ZMF (sample) and geomagnetic field (control), according with stage of lymphocytes damaged.

After 48 exposure hours, the qualitative effect was the increases of sample cells in stadium 2, 3, (30% or 67, 5% of DNA in the tail), while the control stadium was unchanged (Figure 2).

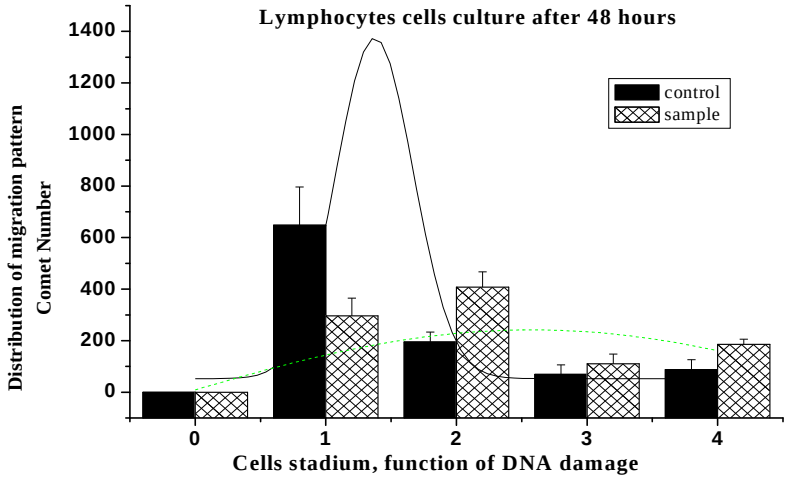


Figure 2. The comet distribution after 48 hours, in ELF ZMF (sample) and geomagnetic field (control), according with stage of lymphocytes damaged.

After 72 exposure hours (Figure 3) the most cells exposed in ELF ZMF were in stadium 3, respectively in stadium 4. That means the increases of damage (67.5% or 97.5% DNA damaged) with moved of DNA in the comet tail.

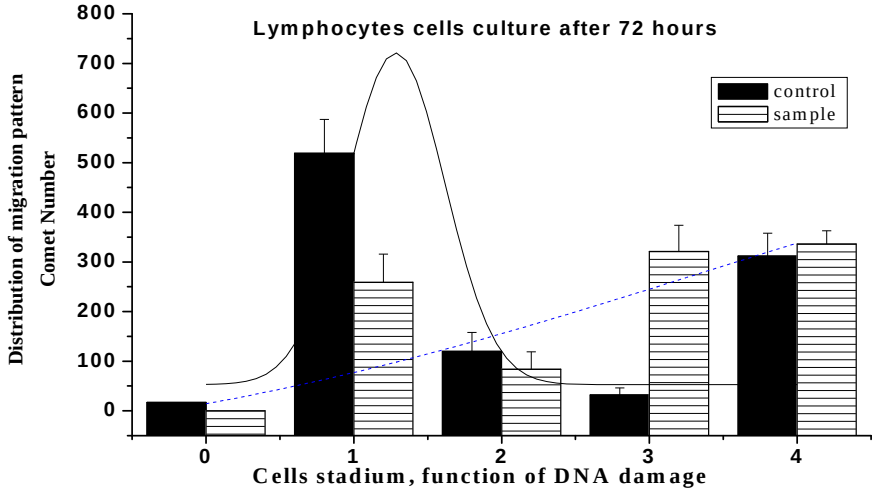


Figure 3. The comet distribution after 72 hours, in ELF ZMF (sample) and geomagnetic field (control), according with stage of lymphocytes damaged.

Quantitative analysis of DNA damage consists in quantifying Score Lesions (SL) and Tail Factor (TF) for control and sample. The results show that both SL and TH were increased for sample exposed in ELF ZMF (Figures 4 and 5).

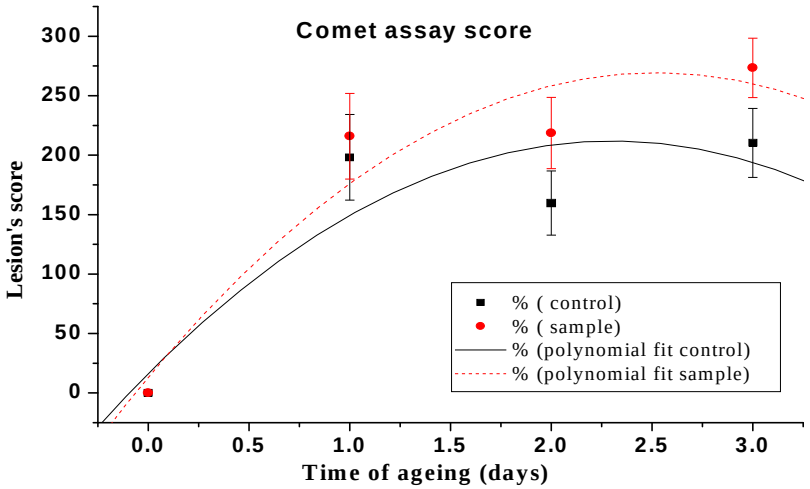


Figure 4. Dose–response relationship of lymphocytes culture exposed in vitro, in ELF ZMF (sample) and geomagnetic field (control), quantified by Lesion's Score, SL (data points are the mean for two independent experiments. *Statistically significant difference from control at p = 0.05).

We observed the increase of DNA damage in human lymphocytes exposed to ELF-ZMF. According with concept of SCGE assay, the undamaged DNA retains a highly organized association with matrix proteins in the nucleus. When damaged are happened, this organization is disrupted. The individual strands of DNA lose their compact structure and relax, expanding out of the cavity into the agarose, during electrophoresis. Undamaged DNA strands are too large and do not leave the cavity, whereas the smaller the fragments, the further they are free to move in a given period of time. Therefore, the amount of DNA that leaves the cavity is a measure of the amount of DNA damage in the cell. A threshold in relation of dose-response could be observed after 48 exposure hours, Figure 4, when the inclination of balance will be for cells in stadium 2 towards stadium 3. By the other way, the score of cells damages (Figure 4) didn't must be complementary

with cell's tail factor (Figure 5). Tail factor means the fragmentation and dispersion of genetic material, according with size of DNA broken from coils and in figure the maximum can't be interpreted like a threshold.

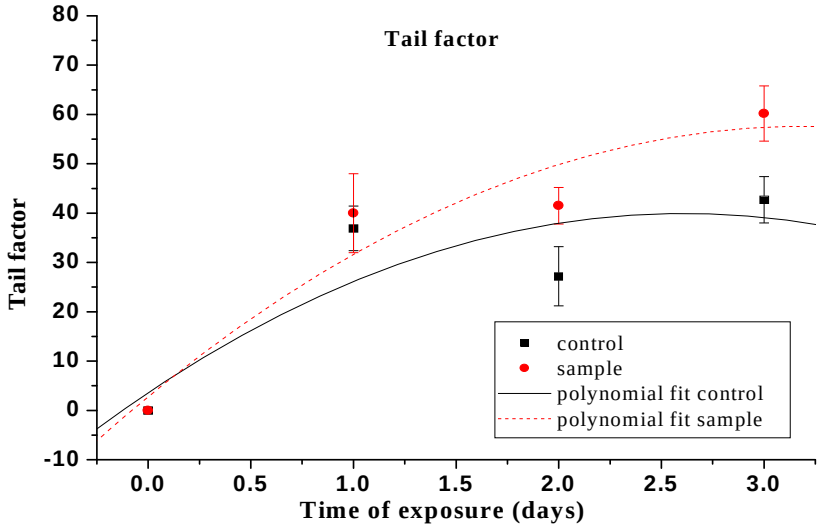


Figure 5. Dose–response relationship of lymphocytes culture exposed in vitro, in ELF ZMF (sample) and geomagnetic field (control), quantified by tail factor (data points are the mean for two independent experiments. *Statistically significant difference from control at p = 0.05).

Extremely low frequency electromagnetic fields resonantly interact with the natural signals, inducing changes in the cellular calcium ion signals, brain waves patterns and reaction times (Cherry 2001). If extreme variations of natural extremely low intensity signals cause human health effects, then it is highly probable that humanly produced extremely low fields that are many orders of magnitude higher than the naturally occurring signals, are also causing significant similar health effects (ICNIRP 2003).

A dose-response relationship due to exposure to non ionizing radiation has not been established, because the genotoxicity of ELF MF is not very much understood, (Ahlbom et al 2000). Although a possible increased risk of childhood leukemia, has been observed at 0.4 μT, the evidence is not strong enough to be considered causal (Ahlbom et al 2001; Salvan et al 2015). Many results of this kind of research indicating some effects on protein expression, on cellular metabolism, on proliferation and differentiation up to process of carcinogenesis, was not enough reproduced and verified (Elwood 2006). Based on the recent studies on magnetic fields and childhood leukemia, Kheifets conclude that magnetic fields are possibly carcinogenic (Keifets et al 2010). The magnetic field can interact directly with the biological system, penetrate the cells, the nucleus and interact with DNA, or other cell constituents (Volpe 2003; Repacholi & Greenebaum 1999; Zhadin 2001). The magnetic intensity of 0.4-0.5 μT and active fluctuations has a particular importance in increasing of DNA damages from cells' nucleus.

According with our results, the assembly of DNA has been changed in absence of static component of GMF. As another, possible explanation, the relaxation of the nucleus areas may be favorable to alkaline treatment during electrophoresis, resulting an increase denaturation and fragmentation of coils. The maxim of relaxation of nucleus area should be touched after 24 exposure hours (Figure 4).

Using the SCGE methods, the detection of cell death by apoptosis is also possible, but is questionable whether apoptotic cells can be distinguished as a special kind of image. Was suggested that the apoptotic cells image have a small head and a long tail (usually with 90% of the DNA in the tail) with the most DNA located in the tail. Anyway, these aspects must be controlled with another method for having a real estimate of cell death process. Decreased of cell's surviving rate in this field was observed beginning with our first studies (Morariu et al 2000; Ciorba et al 2001). The explanation that ELF MF may affect enzymatic processes that involve radical pairs (radical-pair mechanism) must be taken into account.

Conclusions. We used the standard comet assay successfully to generate *in vitro* dose-response curves for human's lymphocytes cells culture under exposure in extremely low frequency zero magnetic field. The absence of static component of geomagnetic field has a great importance in pattern of DNA coils packing. Increasing rate of DNA damage has been observed. This exposure is equivalent with a genotoxic effect, in term of single cells gel electrophoresis. 0.4-0.5 μT magnetic field intensity from our Helmholtz Coil System, a hundred times lower than natural geomagnetic field, may have a special importance through association with the same intensity of anthropic extremely low frequency magnetic field which are correlated with children leukemia incidence. Particularly to our exposure, the threshold appearance is questionable, and should be important in the study of individual sensitivities.

References

Ahlbom A., Day N., Feychting M., Roman E., Skinner J., Dockerty J., Linet M., McBride M., Michaelis J., Olsen J. H., Tynes T., Verkasalo P. K., 2000 A pooled analysis of magnetic fields and childhood leukaemia. British Journal of Cancer 83:692-698.

Ahlbom I. C., Cardis E., Green A., Linet M., Savitz D., Swerdlow A., ICNIRP (International Commission for Non-Ionizing Radiation Protection) Standing Committee on Epidemiology, 2001 Review of the epidemiological literature on EMF and health. Environmental Health Perspectives 109(6):911-933.

Brie I., Perde M., Fischer E., Virag P., Soritau O., Ola R., Postescu I. D., Cernea V., Ghilezan N., 2004 In vitro, evaluation of radiation-induced DNA damage and repair using comet assay. Radiotherapy & Medical Oncology 2:95-104.

Cherry N., 2001 Schumann resonances, a plausible biophysical mechanism for the human health effects of solar/geomagnetic activity. Available at: http://www.salzburg.gv.at/prn/cherry_schumann_ resonances.pdf. Accessed: October, 2016.

Ciorba D., Morariu V. V., 2001 Life in zero magnetic field part IV. Enzymatic activity of aspartat aminotransferaze and alanin aminotransferaze during in vitro ageing of human blood. Electro and Magnetobiology 20(3):313-321.

Elwood J. M., 2006 Childhood leukemia and residential magnetic fields: are pooled analyses more valid than the original studies? Bioelectromagnetics 27:112-118.

Gateva S., Kulekova S., 2008 Chromosome aberrations and apoptosis induced by paraquat corresponding with cell cycle delay in human lymphocytes in vitro. Journal of Environmental Protection and Ecology 9(3):627-633.

Glaser R., 1999 Biophysics. Springer Heidelberg-Berlin, pp. 266-270.

ICNIRP, 2003 Exposure to static and low frequency electromagnetic fields, biological effects and health consequences (0-100 kHz) - review of the scientific evidence and health consequences. Bernhardt J. H., Matthes R., McKinlay A., Vecchia P., Veyret B. (eds), International Commission on Non-Ionizing Radiation Protection, ISBN 3-934994-03-2.

Kheifets L. A., Ahlbom A., Crespi C. M., Draper G., Hagihara J., Lowenthal R. M., Mezei G., Oksuzyan S., Schüz J., Swanson J., Tittarelli A., Vinceti M., Wunsch Filho V., 2010 Pooled analysis of recent studies on magnetic fields and childhood leukaemia. British Journal of Cancer 103:1128-1135.

Morariu V. V., Ciorba D., Neamtu S., 2000 Life in zero magnetic field part II. In vitro human blood aging. Electro and Magnetobiology 19(3):289-302.

Nishimura T., Mohri K., Fukushima M., 2009 The mystery of the dinosaurs: the Earth's electromagnetic field may explain their gigantism and extinction. Available at: www.origin-life.gr.jp/3701/3701007/3701007.pdf. Accessed October, 2016.

Raup D. M., 1985 Magnetic reversal and mass extinctions. Nature 314:341-343.

Repacholi M. H., Greenebaum B., 1999 Interaction of static and extremely low frequency electric and magnetic fields with living systems: health effects and research needs. Bioelectromagnetics 20:133-160.

Salvan A., Ranucci A., Lagorio S., Magnani C., on behalf of the SETIL Research Group, 2015 Childhood leukemia and 50 Hz magnetic fields: findings from the Italian SETIL case-control study. Int J Environ Res Public Health 12:2184-2204.

SCENIHR, 2015 Scientific Committee on Emerging and Newly Identified Health Risks: Potential health effects of exposure to electromagnetic fields (EMF). Available at: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_041.pdf Accessed: October, 2016.

Tahas S. V., Dimen L., Ristoiu D., Cosma C., Muntean L., 2012 Statistical analysis of global solar radiation and temperature trends at Cluj-Napoca, Romania (1921–2009). Journal of Environmental Protection and Ecology 13(4):2404-2415.

Testa A., Cordelli E., Stronati L., Marino C., Lovisolo G. A., Fresegna A. M., Conti D., Villani P., 2004 Evaluation of genotoxic effect of low level 50 Hz magnetic fields on human blood cells using different cytogenetic assays. Bioelectromagnetics 25(8):613-619.

Villoresi G., Ptitsyana N. G., Tyasto M. Y., 1998 Myocardial infarct and geomagnetic disturbances: analyses of data on morbidity and mortality. Biofizika 43:623-631.

Volpe P., 2003 Interactions of zero-frequency and oscillating magnetic fields with biostructures and biosystems. Photochem Photobiol Sci 2:637-648.

Zhadin M. N., 2001 Review of Russian literature on biological action of DC and low-frequency AC magnetic fields. Bioelectromagnetics 22:27-45.

***http://www.nationalatlas.gov/articles/geology/a_geomag.html. Accessed: October, 2016.

Received: 24 September 2016. Accepted: 26 October 2016. Published online: 30 October 2016.
Author:
Daniela Ciorba, Department of Environmental Science, Faculty of Environmental Science and Engineering, Babeş-Bolyai University, Fantanele str., no. 30, 400327 Cluj-Napoca, Romania, e-mail: daniela.ciorba@ubbcluj.ro
Vasile V. Morariu, †, National Institute for Research and Development of Isotopic and Molecular Technologies, Donath str., no. 67-103, 400293 Cluj-Napoca, Romania
Constantin Cosma, †, Department of Environmental Science, Faculty of Environmental Science and Engineering, Babeş-Bolyai University, Fantanele str., no. 30, 400327 Cluj-Napoca, Romania
Silvia Neamtu, National Institute for Research and Development of Isotopic and Molecular Technologies, Donath str., no. 67-103, 400293 Cluj-Napoca, Romania, e-mail: Silvia.Neamtu@itim-cj.ro
Eva Fischer-Fodor, Cancer Institute, Prof. Dr. I. Chiricuta, Republicii str., no. 34-36, 400015 Cluj-Napoca, Romania, e-mail: fischer.eva@iocn.ro.
Vasile Foris, Cancer Institute, Prof. Dr. I. Chiricuta, Republicii str., no. 34-36, 400015 Cluj-Napoca, Romania, e-mail: vasile.foris(at)medunigraz.at

How to cite this article:
Ciorba D., Morariu V. V., Cosma C., Neamtu S., Fischer-Fodor E., Foris V., 2016 Extremely low frequency - zero magnetic field and human lymphocytes response. Ecoterra 13(3):7-13.