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Effect of Extremely Low Frequency of Electromagnetic Fields on Some Toxic Species of Cyan Bacteria

M. A. Fadel[1], *Z. A. Mohamed*[2], *M. A. Abdellateef*[3], *A. A. Hosny*[3*]

[1]Biophysics Department, Faculty of Science, Cairo University, Cairo, Egypt.
[2]Botany & Microbiology Department, Faculty of Science, Sohag University, Egypt.
[3]Physics Department' Faculty of Science, Sohag University, Egypt.

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Abstract: This study investigates the extremely low-frequency electromagnetic fields (0.1-1Hz) on the growth of two species of toxic cyanobacteria with different habits including the colonial *Microcystis aeruginosa* and the filamentous *Anabaena circinals*, commonly found in drinking water sources. The results showed that *Anabaena* cultures exposed to ELF-EMF exhibited a sharp reduction in the cell numbers at frequency of 0.7, compared to control cultures. On the other hand, ELF-EMF had no significant effect on the growth of *M. aeruginosa*. The inhibitory effect of ELF-EMF on the growth of *Anabaena* increased with the increase in exposure time, with complete cell death obtained in 2-hour-exposed cultures. Moreover, ELF-EMF induced morphological changes in *Anabaena* cells and filaments. The study suggests further investigation of the effect of ELF-EMF for inactivation and inhibition of other toxic cyanobacterial species.
Keywords: Electromagnetic fields; Inhibition; Toxic cyan bacteria; Water sources.

1 Introduction

In recent years, greater use of technologies increases the potential exposure to non-ionizing, extremely low frequency (<300 Hz) electromagnetic waves (ELF-EMWs) generated by structures and appliances such as power lines and ordinary devices used inside house and work places [1]. ELF-EMWs have various effects on the biological functions of living organisms represent including induction of DNA damage, cellular changes, and increased risk of cancer [2]. The number of studies of the effect of electromagnetic waves on microorganisms has increased significantly in the last decades. These studies include the effect on the growth of bacteria [3-6] and fungi [7, 8], and a few studies were carried on green algae [9, 10]. It has been reported that electromagnetic fields can negatively or positively affect the cell growth and viability of microorganisms [11]. The negative or positive effect of ELF-EMWs on these organisms depends on the strength and frequency of the electromagnetic field applied, and bacterial strain used [12, 13]. To the best of our knowledge, there is no study concerning the effects of ELF-EMWs on cyanobacteria. Cyanobacteria are prokaryotic, oxygen-evolving photosynthetic microorganisms that can live in a wide range of habitats including terrestrial, marine and freshwater environments [14]. Cyanobacteria become problem when their numbers increase under eutrophication conditions (i.e. high nutrient concentrations) and form Harmful blooms in the aquatic ecosystem [15]. These blooms lead to the formation of hypoxic dead zone in lakes, where fish and other aquatic animals cannot survive [16]. Additionally, cyanobacteria can produce different kinds of toxins (hepatotoxins, neurotoxins, irritants and gastrointestinal toxins) that adversely affect animal and human health [17] and deteriorate the water quality [18].

Therefore, we have attempted in the present study to investigate the possible influence of ELF-EMF on two species of cyanobacteria with different habits including the colonial *Microcystis aeruginosa*, and the filamentous *Anabaena circinalis*.

2 Materials and Methods

2.1 Exposure Facility System

Fig. 1 indicates sketch diagram for function generator of the exposure facility of the cyanobacterial culture, tested samples were exposed to different modulating frequencies of Square Amplitude Modulated Waves (QAMW). The modulating waveform was square. The carrier and modulating carried wave were generated by (BK Precision4085-40MHz Arbitrary function Generator with counter, manufactured in Taiwan). The carrier was 10 MHz wave with amplitude 10 V_{pp} and the modulating depth was ± $2V_{pp}$. Electric field strength at the samples was about 100 V/m. The outer opposite sides of the tube containing the microbial suspension were covered by cupper plate electrodes connected to the output of the

*Corresponding author e-mail: asmaa_hosny@rocketmail.com

generator for irradiation of the sample.

2.2 Organism and Culture Conditions

The cyanobacterial strains used in this study were obtained from microalgal culture collection of Botany & Microbiology Department, Faculty of Science, Sohag University, Egypt. These species were isolated from fish ponds in Sohag region, Egypt, and were identified as *Microscystis aeruginosa* with colony form and *Anabaena circinalis* with filamentous form that reported as microcystin producers [19]. To obtain the growth curve, the cyanobacteria species were grown for 21 days in climatic chamber under controlled light (50 µmol m^{-2} s^{-1}), photoperiod (16:8 hours light-dark), pH 8 and temperature (30 ± 0.5°C) in a BG-11 liquid medium [20], with some modification (3% $NaNO_3$ in case of *Anabaena* culturing). Exponentially growing cyanobacterial cells (14-day old cultures) were used for experiments. These cells were then inoculated into glass test tubes (25 ml volume) containing modified BG-11 medium. To determine resonance frequency of growth inhibition of both two species, three of these tubes with cyanobacterial cells were exposed to QAMW for different AM frequencies in the range (0.1-1)Hz in steps of 0.1Hz for 60 min at electric field intensity of 100 V/m. Control cultures (3 tubes) were kept in the same conditions as the exposed ones outside the instrumentation for generating EMFs (i.e. without field application). After exposure, both treated and control cultures were incubated in climatic chamber under the same conditions described above. The growth of treated and control cells was monitored daily by cell counting Sedgwick-rafter counting with chamber under light microscope according to [21]. Then, additional experiments were conducted by exposing cyanobacterial cells to QAMW at the frequency showing the strongest inhibitory effect (0.7Hz) in the former experiment, with different exposure times (1 , 2 and 4 h). After exposure, the three groups of cultures of different exposure times and not-exposed control culture of *Anabaena SP* were incubated under the same conditions mentioned above.

All measurements were made by taking samples from cultures under aseptic conditions.

2.3 Analytical Determinations

Daily samples of treated and control cultures were collected for analysis of cell number. The cell number as an estimator of cyanobacterial species growth, was counted with a Sedgewick Rafter counter under light microscope according to the method used by [21]. *Microscystis* and *Anabaena* were firstly counted as colony or filament respectively. The cell concentrations (cells mL−1) of *Microscystis* and *Anabaena* were calculated by taking the average from counts of 10 colonies or 10 trichomes (unit mL^{-1}).

2.4 Statistical Analysis

All determinations were made in triplicate and data are expressed as means ± the standard deviation (SD). Statistical tests were carried out using the software SPASS version 16.0. Differences between individual means were determined by one-way ANOVA and Tukey's post hoc multiple range test. Significant differences were established at $P < 0.05$.

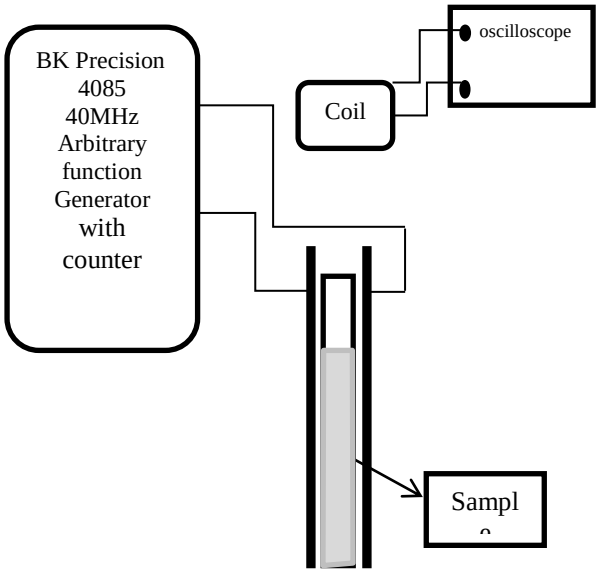

Fig.1 Sketch diagram for the exposure system of cyan bacterium sample.

3 Results

The effect of ELF-QAMW in the range of frequencies (0.1-1.0) Hz on growth (estimated as cell number) of *M. aeruginosa* and *A. circinalis* was studied for exposure period 1hour. As shown in Fig.2, the cell number of *M. aeruginosa* exposed to amplitude modulating frequencies did not differ significantly (P> 0.05) from that of control cultures
along all incubation period (3 days). On the other hands, the cell number of *Anabaena* cultures exposed to electromagnetic fields at 0.7 Hz decreased significantly (P< 0.05) compared with that of control cultures. Meanwhile, the cell count of *Anabaena* cultures exposed to ELF at other frequencies (0.1,0.2,0.3,0.4,0.5,0.6,0.8,0.9,1.0 Hz) did not exhibit any significant variation differ (P>0.05) compared to control cultures all over the incubation period (Figs 3a,b,c).
The results of the additional experiment performed to evaluate the most effective exposure time at 0.7 Hz on the growth of *Anabaena* are presented in Fig. 4a. The results showed a sharp decrease in *Anabaena* cell number with the increase in the exposure time from 1 to 4 h (P< 0.05). The

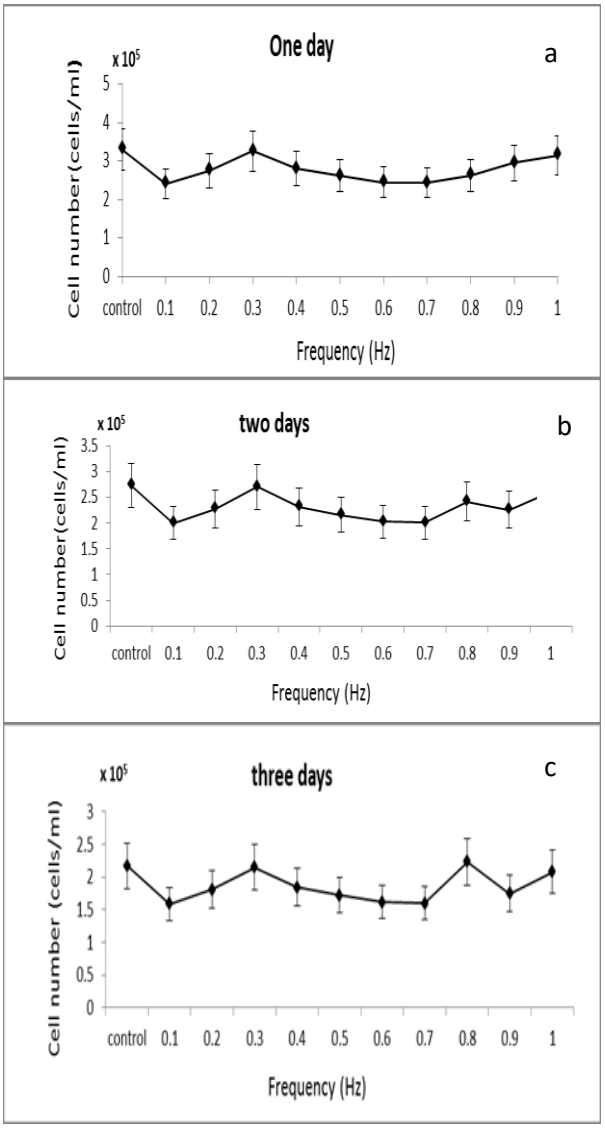

Fig 2. Shows the characteristic curve of cell number for *Microscystis aeruginosa* as a function of frequency at incubation period one day (a),two days (b) and three days (c). Values are the mean of cell number (n=3)±SD.

Complete inhibition of *Anabaena* growth was observed in4-hours exposed cultures after 3 days incubation period. In Addition to growth inhibition, *Anabaena* exhibited morphological changes when exposed to ELF-QAMW at 0.7 Hz for two hours. The cells in control cultures were rounded to barrel-shaped, and the filament is easily recognized by its characteristic coils. Whereas after exposure to electromagnetic field the organism showed cell shrinking, filament fracture and coils dissociation (Fig.5). For *M. aeruginosa*, no morphological changes were observed in treated cells compared to control (Fig.6).

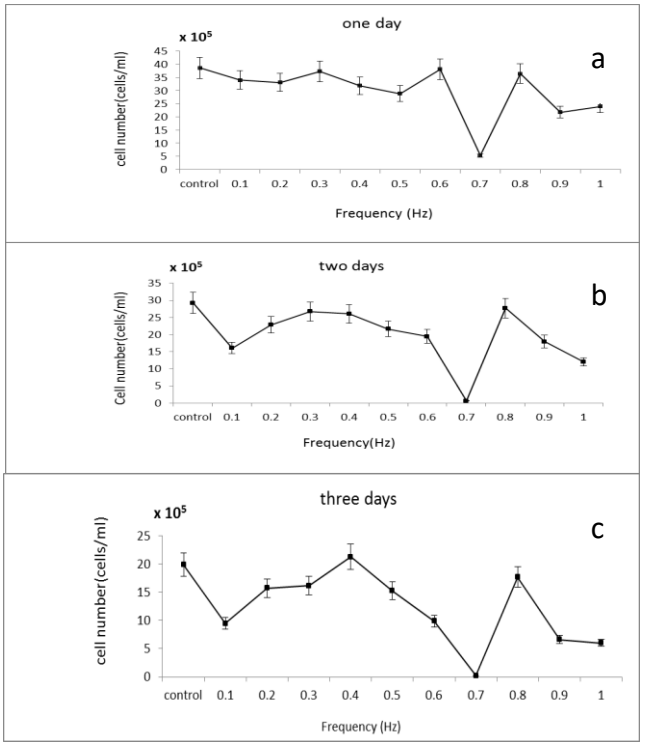

Fig 3. Shows the characteristic curve of cell number for Anabaena *circinalis* as a function of frequency at different incubation periods: one day (a), two days (b) and three days(c) . Values are the mean of cell number (n=3)±SD.

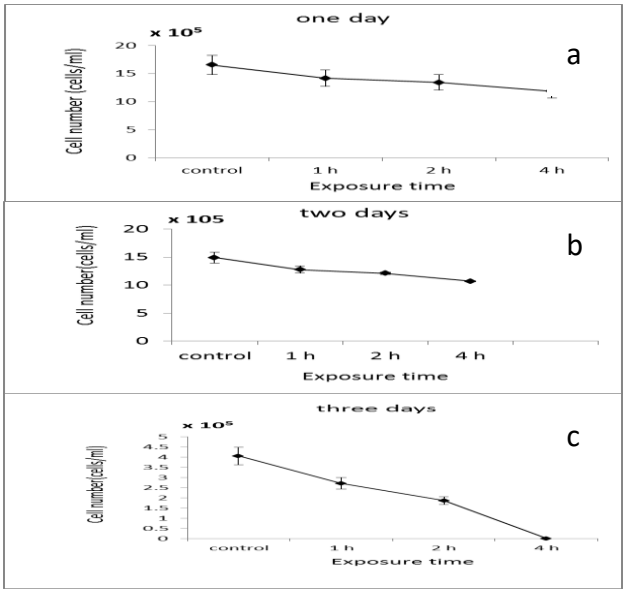

Fig 4. Shows the effect of exposure time to ELFs at 0.7 Hz on cell number of *Anabaena circinalis* at different incubation periods: one day (a), two days(b) and three days (c). Values are the mean of cell number (n=3)±SD.

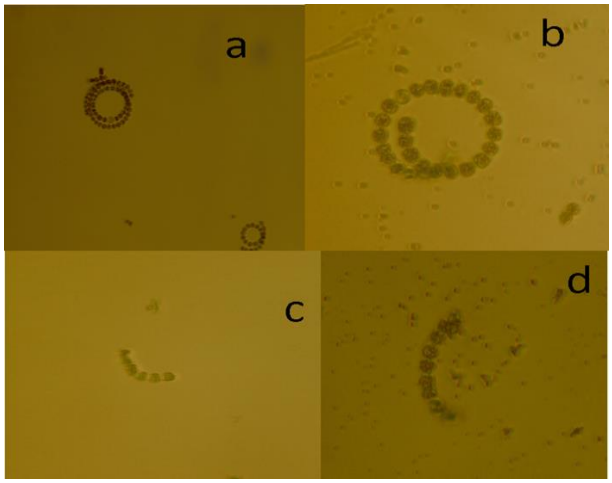

Fig 5. Shows the changes in the morphology *Anabaena circinalis* control (a,b) and exposed to 0.7Hz ELF-EMFs for 2 hour (c,d).

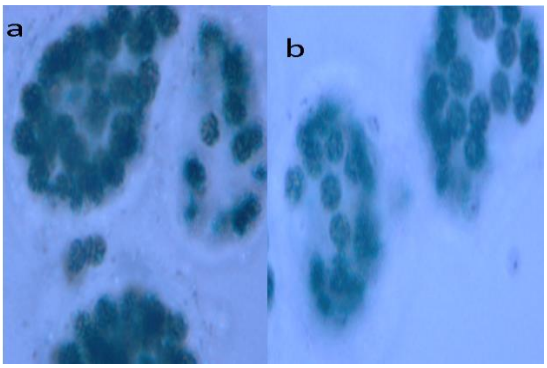

Fig 6. Shows the changes in the morphology *Microscystis aeruginosa* control (a) and exposed to 0.7Hz ELF-EMFs for 1 hour (b).

4 Discussions

In the present study, a new method for controlling the growth of toxic *M. aeruginosa* and *A. circinalis* by using ELF-EMFs has been evaluated. The main objective of this work was to find out the frequency of the ELF-EMFs that resonates with the bioelectric signals generated from microbe during cell division (i.e. cell count) and studying the changes that may occur in its morphology.

In general, Biological systems generate electric currents and signals associated with magnetic signals, resulting from the running physiological mechanisms. In these mechanisms, ionic current loops are involved which are responsible for all bioelectric signals generated. The form, frequency and amplitude of the resulting bio-electric signals are specific and characterize the running physiological processes. Resonance interference between two EM fields can occur when two waves of the same frequency superimpose and the resultant is the algebraic summation of the two waves [5]. Based on this understanding, applied EMFs can interfere with bioelectric signal when they are in resonance and the resultant is the net summation of the two waves which may cause either enhancement or inhibition of the running physiological process. Accordingly, the results of present study revealed no significant electromagnetic effect on the growth of *M. aeruginosa* after exposure to ELF- QAMWs in the frequency range of 0.1 to 1 Hz (Fig. 1). This indicates that the resonance frequency of cellar division of *M. aeruginosa* is not found in this range or there is an adaptive response of the exposed cells to field stress. Conversely, *A. ciricinalis* showed a contradictory response to electromagnetic field, as there is a destructive resonance interference of the applied QAMWs at 0.7Hz (Fig. 2) with the bioelectric signals generated during the microbial cellular division. Such inhibitory effect increased with increasing exposure time and incubation period post exposure (Fig. 3). Our results are in agreement with the results of previous studies reporting the inhibitory effects of ELF-EMFs on growth and viability of heterotrophic bacteria and dependence of this effect on exposure time [3, 5, 11, 12, 22, 23]. The difference in the response to electromagnetic field between *Microcystis* and *Anabaena* may be due to the presence of extracellular polysaccharide sheath surrounding *Microcystis* colonies (but not found in *Anabaena*), which persisted without dissolution against electromagnetic field. This feature could be attributed to scytonemin (yellow-brown hydrophobic pigment) which occurs exclusively in cyanobacterial sheath, and has been reported to absorb UV-A radiation [24] and reduce its entry into the cells [25].In addition to the reduction in the cell number; ELF-EMFs induced morphological changes in *A. circinalis*. Such morphological changes are in agreement with those obtained by [3] for gram negative bacteria induced by ELF-EMFs. The changes in the morphology of ELF-treated-*Anabaena* could be due to the damage and change in permeability of the ionic channels in the membrane induced by ELF-EMFs as suggested by [5,6].

5 Conclusions

The data reported in the current study confirm that ELF-EMFs have the potential to reduce the growth of some toxic cyanobacteria at 0.7 Hz.

However, they did not exhibit any significant effect on other species, indicating the inhibitory effect of ELF-EMFs on toxic cyanobacteria is species-dependent. Therefore, further studies are required to evaluate the influence of different EMF frequencies and intensities values on other toxic cyanobacterial species, particularly those found in drinking water sources. These studies should also be carried out in field to investigate the efficiency of ELF for the inactivation and elimination of such toxic organisms from drinking water during treatment processes.

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