

# Evaluation of the effects of Extremely Low Frequency (ELF) Pulsed Electromagnetic Fields (PEMF) on survival of the bacterium *Staphylococcus aureus*

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## Abstract

**Background:** This study investigated the effects of extremely low frequency (ELF) pulsed electromagnetic field (PEMF) radiation on the growth of bacterium *Staphylococcus aureus* (ATCC 25923) that plays a versatile role in infecting wounded tissues. The viability of these bacteria (number of live cells as colony-forming units (CFUs)) was measured before and after the ELF PEMF exposures to quantify their survival rate.

**Methods:** *S. aureus* cultures were first cultivated in an agar medium, and then picked and suspended in Columbia broth medium. Optical density reading for the suspended bacteria was measured at 600 nm and adjusted to a specific value of  $0.1 \pm .005A$  prior to experimentation and placement of bacteria into 2.5 mL centrifuge tubes. Sham-exposed tubes filled with bacteria were kept under the same experimental conditions and used as controls. The constructed exposure system, emitting uniform time varying magnetic fields (frequency of 2-500 Hz, and magnetic induction of 0.5-2.5 mT), was employed to irradiate *S. aureus* bacteria for 90 min. To determine the CFU per ml of the exposed bacteria, five serial dilutions were performed. A volume of 100  $\mu$ l from the last tube was suspended onto agar plates by spread plating. After incubation, the colonies formed on the plates were visually counted.

**Results:** All irradiated *S. aureus* bacteria showed decrease in their growth rate compared to control samples. The results demonstrated that ELF PEMF exposures at 150-500 Hz are more effective than exposures at 3-100 Hz in reducing the viability of *S. aureus* in broth. The lowest CFU value was achieved with the exposure at 300 Hz and 1.5 mT. The decrease of at least 20% in CFU value was obtained for frequencies above 200 Hz and all five studied magnetic flux densities (0.5 mT, 1.0 mT, 1.5 mT, 2.0 mT, and 2.5 mT).

**Conclusions:** In summary, the growth rate of the irradiated *S. aureus* bacteria is affected by radiation of particular parameters, thus revealing resonant effects induced by the applied radiation. The decreased CFU values in all irradiated samples compared to control samples (non-exposed) were observed. Findings provide important insight towards selecting the optimal parameters of ELF PEMF for possible treatment of infected tissue and thus, wound healing promotion.

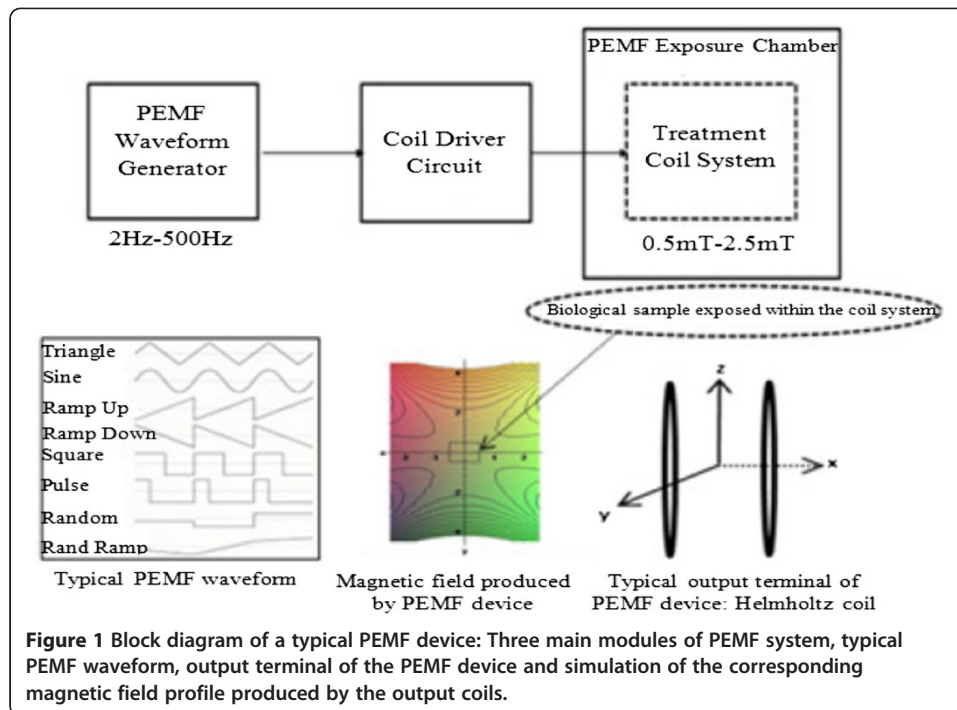
**Keywords:** ELF PEMF system; *Staphylococcus aureus*; Colony-forming units; Wound healing

## Background

There is ongoing interest in applications of pulsed electromagnetic field (PEMF) radiation as an alternative therapy for different medical conditions [1]. Studies have demonstrated that extremely low frequency (ELF) PEMF radiation facilitates the process of wound repair [2,3]. ELF PEMF is a sub-class of electromagnetic field (EMF) that displays frequencies at the lower end of the electromagnetic spectrum [1], from 6 Hz up to 500 Hz. ELF PEMF radiation is non-ionizing radiation that uses electrical energy to direct a series of magnetic pulses through tissue biological media, whereby each magnetic pulse induces a tiny electrical signal that stimulates cellular repair. ELF PEMF radiation produces non-thermal effect on applied biological targets [1]. There are many factors that can affect wound healing and cause improper or impaired tissue repair. One of these factors is wound infection by bacteria. Of particular interest are the infected wounds where bacteria or other microorganisms have colonized that cause either a delay in wound healing or a deterioration of the wound. Types of wounds include: burns, bullet/stab puncture wounds, pet/insect, snake bites, rust nails (tetanus), diabetes foot ulcer, bruising from an assault, amputation due to road side accidents or bombing.

The effects of (ELF EMFs on biological media have been studied by many researchers using a variety of *in vitro* exposure systems [4-12]. The applied radiation produces the energy that is calculated as follows:  $E = h\nu$ , where  $E$  = energy,  $h$  = Planck's constant and equals  $6.626 \times 10^{-34} \text{Js}$  and  $\nu$  = frequency, cycles per second or Hz. For example, radiation at 500Hz (upper limit of the ELF spectrum) would produce an energy equivalent to  $2.068e^{-12} \text{eV}$ . ELF magnetic field therapy is considered as useful and beneficial treatment for different diseases, especially those involving skin and bones [2-4,13]. Almost all diseases are the result of impaired cellular function. A healthy cell operates at a voltage between 70-110 mV in order to produce ATP molecules (Adenosine Triphosphate), which are vital for a healthy body. A damaged cell with voltage range between 40-50 mV loses energy as there is not enough ATP available. Studies have shown that the application of PEMF radiation to damaged skin can accelerate re-establishment of normal potentials, promote cell proliferation, increase the rate of healing; and reduce swelling and bruising [1,3,14]. Almost all of these laboratory-based experiments utilized electric coils to generate electromagnetic radiation to expose biological samples. A classic Helmholtz coil design ascertains that the first and second spatial derivatives of the applied fields are zero at the central point of the applied system [15]. A much larger volume of uniform ELF EM field inside the setup results from the assembly of increased numbers of well-placed coils [16]. Computational modeling (simulation) of the ELF uniform magnetic field exposures, prior to the actual hardware design and development of the exposure system, is extremely important in terms of design flexibility and efficacy.

A PEMF exposure system consists of three modules as can be seen in Figure 1. The first module is the PEMF waveform generator. Typical PEMF wave can be produced via signal generators or microcontrollers. This signal then proceeds to a coil driver circuit (second module of the PEMF system). This circuit typically has a voltage to current amplifier coupled with a current amplifier to aid the production of pulsating current required by a number of current carrying treatment coils systems (output module) to produce PEMF of a defined magnetic flux density at a particular frequency.



The radiation produced by the PEMF is then used to expose biological samples. Typical output module consists of two pairs of Helmholtz coil (Figure 1). The range of frequency and magnetic flux density used in our experiment is also shown in the corresponding module of the PEMF exposure system. The governing equation of the produced PEMF is expressed in terms of Biot-Savart Law (1):

$$B = \frac{2\mu_0 N I R^2}{2(R^2 + x^2)^{3/2}} \quad (1)$$

where,  $B$  = Magnetic flux density (T),  $I$  = coil current (A),  $R$  = coil radius (m),  $x$  = coil distance on axis, to pint, (m),  $N$  = number of wire loops and  $\mu_0$  = permeability constant and equals  $1.257 \times 10^{-6} T \cdot m/A$ .

A good model system for studying effects of electromagnetic fields on micro-organisms is bacterial culture [8,17-20]. It was reported that stimulation or inhibition and proliferation of microbial and bacterial growth were dependent on the field strength of the EM radiation and types of bacteria [21-23]. The quantum nanobiology and biophysical chemistry aid to understand the quantum nature of the interaction between the LF EM radiation and the quantum states of biomolecules responsible for wound healing /repair. Up to date experiments pivot around environmental influences on human health and therefore deal with increasing exposures to man-made ELF generated from appliances at the frequency of 50 Hz [8,18]. Therefore, the focus has predominantly been on the effects of ELF EMF on bacteria adaptability at different time exposures and magnetic flux densities but at the single frequency of 50 Hz. The effects of the entire range of ELF PEMF (2-500 Hz) on bacteria have not been studied as yet. For such experiments, it is important to produce a uniform magnetic field for

irradiating bacterial cultures as this will greatly increase the throughput of replicating results for statistical validation.

In this study, we irradiated the selected bacteria by the constructed ELF PEMFs exposure system. In our previous work we presented the results of software simulation and design of two pairs of air core Helmholtz coils utilized for the development of an ELF PEMF system capable of producing a uniform time varying magnetic field (magnetic flux density,  $B$ , 0.5–2.5 mT over the frequency range,  $f$ , 2-500 Hz) [24]. Our previous study reported the successful application of the device developed for irradiation of Collagenase enzyme which led to significant changes in its kinetics in comparison with the non-exposed enzyme [25,26]. Here, we present the experimental evaluation of the applied ELF PEMF on the survival of the bacterium *Staphylococcus aureus* (ATCC 25923). We selected this gram-positive bacterium because of its prominent role in infecting wound tissues. *S. aureus* is reported to be the almost-universal cause of furuncles, carbuncles, and skin abscesses, and worldwide is the most commonly identified agent responsible for skin and soft tissue infections, which frequently begin as minor boils or abscesses and may progress to severe infections involving muscle or bone and may later spread to the lungs or heart valves [27]. In addition, *S. aureus* is an important pathogen in the dairy farm industry as it causes mastitis in cows [28]. *S. aureus* strains are easy to cultivate since they are facultative microorganisms that can be grown overnight on simple media at 37°C.

## Results and discussion

In this experimental evaluation we examined the effect of ELF PEMF on the viability of *S. aureus*, which is defined in terms of the Colony-Forming Unit (CFU) - a number of live bacterial cells in 1 mL of a sample. Bacterial cultures were irradiated at the selected frequencies in the range of 2 Hz to 500 Hz and five magnetic flux densities (0.5 mT, 1.0 mT, 1.5 mT, 2.0 mT and 2.5 mT). For statistical analysis, each experiment was repeated in triplicate for every unique combination of the selected frequency and magnetic flux density. Due to the absence of *a priori* knowledge of whether the entire range of ELF PEMF would positively or negatively affect the bacterial culture of *S. aureus*, for our null hypothesis, we assumed the mean value of CFU to be equal for both the control and ELF PEMF exposed sample ( $H_0: \mu_1 = \mu_2$ , where mean of the exposed sample,  $\mu_1$  is equal to the mean of the control sample  $\mu_2$ ). Therefore, for statistical credibility and experimental relevance of the conducted study, we tested our hypothesis with the data analysis tool of Microsoft Excel (2010) and used the independent two-sided t- test with sample size ( $n = 3$ ) and degrees of freedom =4. For significance testing, we used alpha ( $\alpha$ ) of 0.05 in the equation,  $100 \cdot (1 - \alpha) \%$ , to determine a 95% confidence interval (data not shown). Each test yielded variance value for both control and exposed data set. Square root of variance resulted in the calculation of standard deviation. Standard deviation values ( $\pm$ ) were then used as upper and lower bound of error bars for plotted data. The results of the t-test showed that the difference between exposed and control samples were always significant ( $P < 0.05$ ).

Experimental data were plotted and presented in Figures 2,3,4,5,6,7,8,9. Figures 2,3,4,5,6 show the significant difference between the CFU values of exposed and control (non-exposed) *S. aureus* cultures. For each measurement, the relative difference in CFU

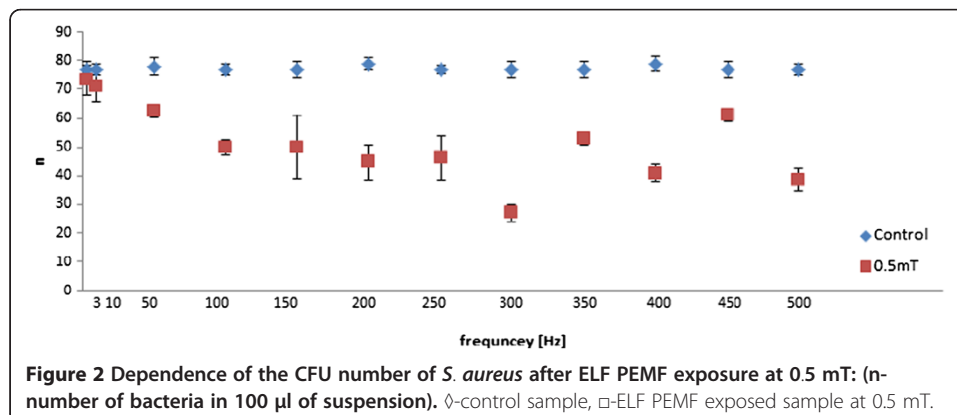
between the exposed and control samples were calculated. These results are presented in Figures 7,8,9. The error bars in the Figures 7,8,9 represent standard deviation at 95% confidence level.

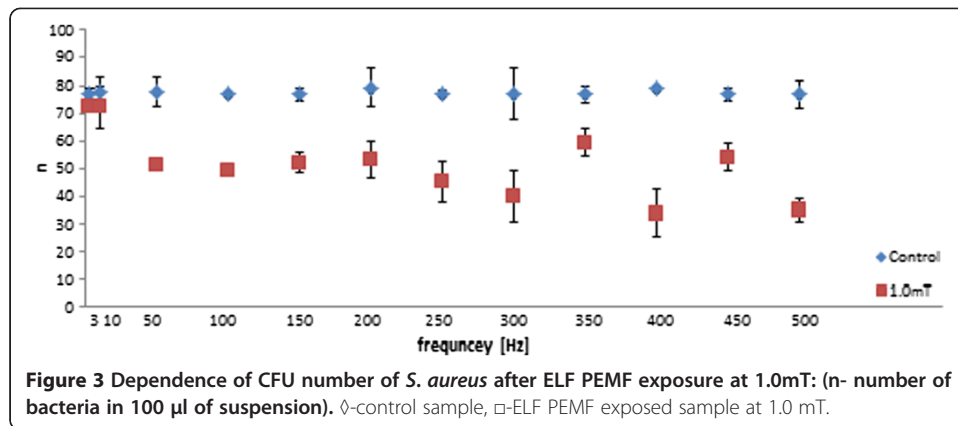
The preliminary results showed that ELF PEMF exposures for less than 1 h produced no effects on bacterial cultures and failed to reduce the number of live cells present. Thus, all further subsequent exposures were set for 90 min. The results revealed an overall decrease of CFU values of bacterial cultures irradiated at frequencies of 100 Hz and above when compared to the control samples. Of note, CFU values for the control samples remained almost constant, around 77, throughout the entire range of different ELF PEMF exposures (Figures 2,3,4,5,6).

The CFU values at the frequencies 3 Hz and 10 Hz and all five studied magnetic flux densities (0.5 mT, 1.0 mT, 1.5 mT, 2.0 mT and 2.5 mT) were consistently higher (minimum decrease) as opposed to the effects observed at the higher frequencies of ELF PEMF. As shown in Figures 2 and 3, CFU values are 73 (cells per mL) and 72 for exposures at 3 Hz and 0.5 mT and 3 Hz and 1.0 mT, respectively. This corresponds to a relative percentage decrease of 4.95% at 0.5 mT and 5.50% at 1.0 mT, respectively (Figure 7). From Figures 2 and 3 we can also observe CFU values of 71 and 72 for exposures at 10 Hz and 0.5 mT, and at 10 Hz and 1.0 mT respectively. This resulted in a relative decrease of 7.52% and 7.25%, respectively (Figure 7). Maximum decreases of CFU values ( $n = 27$  and  $n = 24$ ) were recorded at the frequency 300 Hz and flux densities of 0.5 mT and 1.5 mT (Figures 2 and 4). This corresponds to relative decreases of 64.63% and 68.56%, respectively (Figures 7 and 8).

The CFU values of the exposed and control samples were measured at the selected twelve frequencies and five magnetic flux densities (their different combinations). Higher CFU values indicate less significant effects of ELF PEMF on bacterial cultures and thus yielded a lower relative change. Similarly, lower CFU values indicate the most significant effect of ELF PEMF on bacteria which accounted for a higher value in relative change (%). CFU values, along with corresponding relative change value for studied frequencies and magnetic flux densities are shown in Table 1.

Figures 7,8,9 reveal non-uniform oscillatory patterns for the relative changes (%) of the number of bacteria upon ELF PEMF radiation. Interestingly, similar trends were also observed with our previous findings on the investigation of the effect of ELF PEMF on Collagenase enzyme kinetics [26]. The peak values are not concentrated at a



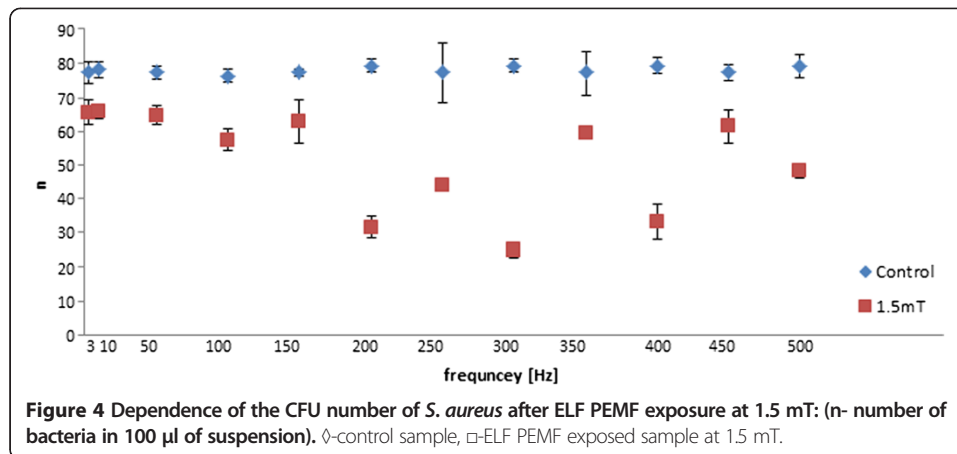


particular frequency but rather spread over a wide range of frequencies. This emphasizes the fact that bacterial cultures are extremely responsive to irradiation with unique combinations of magnetic flux density and frequency. Figure 10 shows the presence of a specific viability pattern “quadrature polynomial” for the ELF PEMF exposures on *S. aureus* cultures at the lower end of the ELF spectrum. The pattern can potentially be used to predict the changes in CFU at the particular frequencies. Concerned patterns occur at the exposures of the following parameters: 0.5 mT for 3-250 Hz, 1.0 mT for 3-200 Hz, and 2.0 mT and 2.5 mT for 3-150 Hz. The coefficients of determination were 0.98, 0.89, 0.91 and 0.98 for 0.5 mT, 1.0 mT, 2.0 mT and 2.5 mT, respectively.

Explanation of the possible ELF PEMF interaction with specific materials, environment, and molecules in broth, media and bacteria in general are presented below:

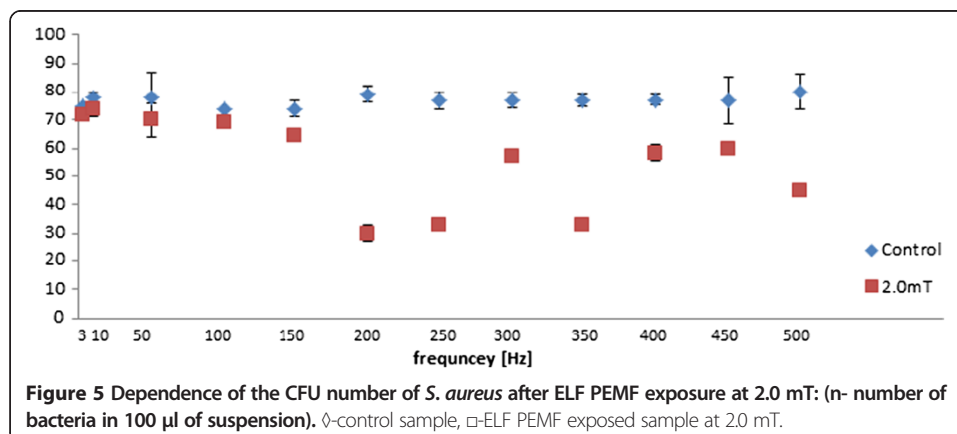
- *ELF PEMF interaction with specific material*: The entire structure of ELF PEMF exposure chamber was constructed using acrylic. Therefore, there is no interaction between the produced ELF PEMF and the concerned material inside the ELF PEMF exposure chamber.
- *ELF PEMF interaction with environment*: Bacteria are known to produce stress protein when exposed to elevated temperature from the environment [29]. This elevated temperature can commonly occur due to resistive heating of the coils used to produce the ELF PEMF radiation. For our experiment, we have controlled the temperature (see Methods) and ascertained that the bacterial cultures were not exposed to heat generated by the current carrying coils.
- *ELF PEMF interaction with molecules in broth and different broth composition*: Columbia broth (ingredients presented in Abstract) is a complex media with undetermined chemical compositions. Due to this undetermined chemical composition, its response to ELF PEMF exposures cannot be established. Of note, only one particular broth composition was used for all conducted experiments.
- *ELF PEMF interaction with media*: Solid media (Columbia agar plates) were never under interaction with the ELF PEMF radiation.

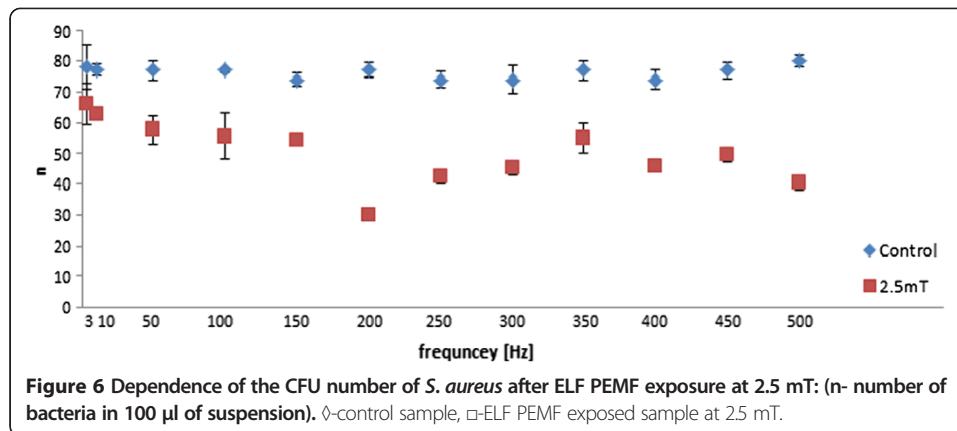
Figure 11 summarizes the results obtained during experimentation. This figure presents the relative changes (%) in CFU values for the selected frequencies. Higher relative change (%) indicates that more bacterial cells have been affected (eliminated)



by ELF PEMF exposures and thus, accounted for a lower CFU value. Minimum relative change (decrease) corresponding to the magnetic flux density range of 0.5-2. 5mT were observed within 3 Hz to 150 Hz and varied from 4.95% to 12.73%. Maximum percentage decrease altered significantly in two phases: (i) a change from 18.27% to 34.61% from 10 Hz-50 Hz and (ii) a change from 35.06% to 62.05% from 150 Hz-200 Hz. The only exception was at 450 Hz, where the maximum decrease of 35.71% was noted. Generally, the most significant changes (increase or decrease) in CFU values on and above 150 Hz are considerably greater as opposed to the changes noticed with the exposures in the lower end of ELF spectrum.

The established method for evaluating the effects of ELF PEMF radiation on the viability of bacterial cultures is the method of CFU- colony forming unit [8], where the decrease in CFU number observed after EMF exposures is correlated with bacterial death (CFU number represents bacteria, which remains alive after all treatments) [8,30,31]. Similar to the above mentioned studies, we have also employed this method to study the viability of bacterial culture of *S. aureus* exposed to ELF PEMF radiation [8]. From the growth curve shown in Figure 12, we can observe that even after exposures bacteria are not in the death phase but rather still growing in their exponential phase. Similar to [32], we found that the the ELF PEMFs effects are not bacteriostatic (blocking their growth during exposures, but rather dynamic, i.e. a number of bacteria still increases upon exposures however, the growth rate of the exposed samples is





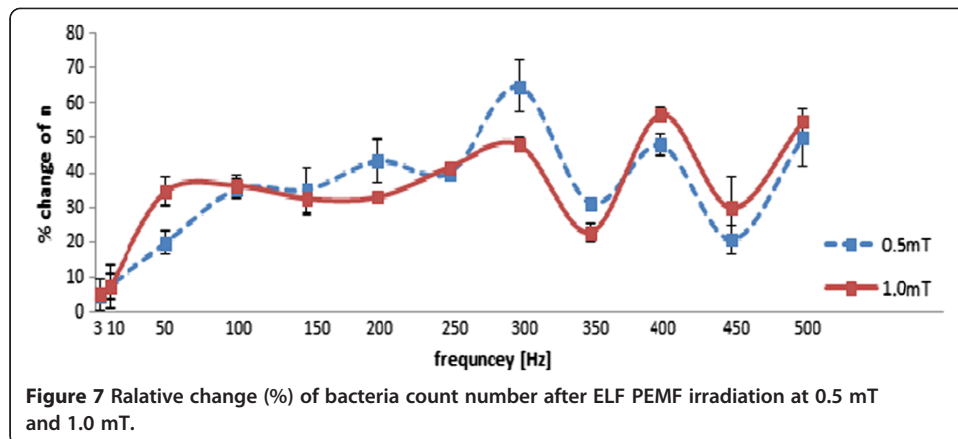
suppressed in comparison to the controls (Figure 12). Therefore, similar [32] we conclude that EMFs can partially eliminate the exposed bacteria.

Similar to other studies [8,31,32] the question of how magnetic field can kill the bacteria has not been solved by our experiments. The mechanism of interaction of ELF PEMFs and biological systems in terms of simple quantitative models is given in [33]. Since an effect can only result from a direct interaction of the EMF with molecular targets, including ions, any adequate description must be of physical chemical nature [34]. Hypothesis of proposed biophysical model theories [33,34] for ELF PEMFs effects are based on the isolated cell culture and are therefore, differ from natural organism [35]. So far, no characteristics physical chemical reaction parameters for bacteria have been established such that they can be compared with those extracted from the molecular physical chemical approach [30].

A decrease in growth rate compared to control samples for all bacterial cultures of *S. aureus* subjected to ELF EMF was reported by [36]. The post exposure effect reported by [36] is comparable to our results. In [36], morphological alteration of *S. aureus* upon exposure to ELF EMF (50 Hz at 0.5 mT) for 120 minutes was presented. An apparent cell wall disruption was not observed but cytoplasmic changes were evident. Studies conducted by [8] have also reported similar morphological changes to *S. aureus* upon exposures to ELF EMF.

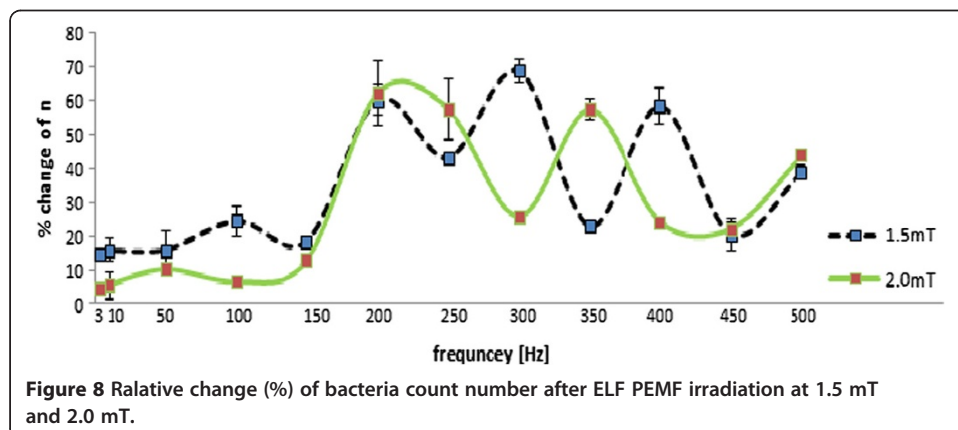
ELF magnetic fields are known to affect biological systems. In many cases, biological effects display "windows" in biologically effective parameters of the magnetic fields: interestingly that weaker magnetic field are more effective [37]. Magnetic fields cause an interference of ion quantum states and change the probability of ion-protein dissociation. This ion-interference mechanism predicts specific magnetic-field frequency and amplitude windows within which the biological effects occur. This type of amplitude phenomenon suggests a nonlinear physical mechanism [37]. Other similar results (absence of correlation between experimental and control samples) suggest that degree of non-thermal effects is almost independent of the absorbed frequency and magnetic flux density. This phenomenon of frequency 'window' of the EMF biological effects was reported for *S. aureus* in [38]. It was suggested that these 'frequency' windows are caused when EMF are creating biological effects through interaction with non-linear and cooperative process within cells [37]. With regards to the results from this study, we observed both frequency and magnetic flux density 'windows,' especially for

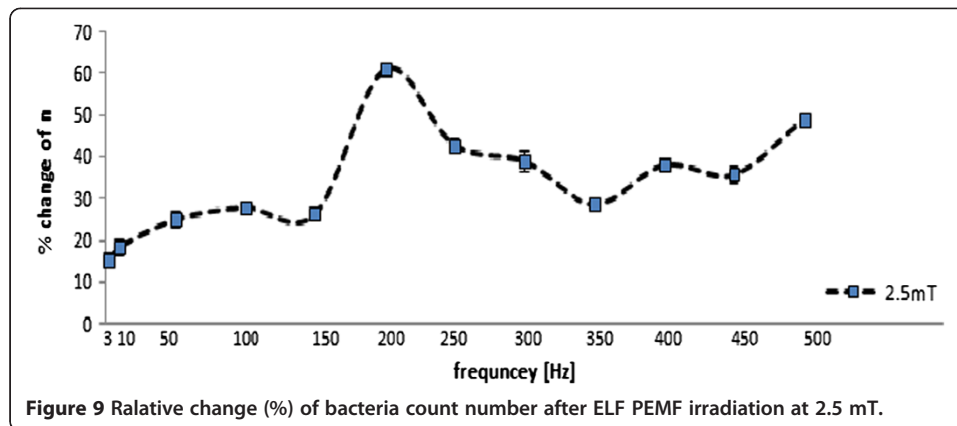




frequency range greater than 250 Hz at 0.5 mT, 200 Hz at 1.0 mT, 150 Hz at 2.0 mT and 2.5 mT. One such example is the effect of ELF PEMF (in terms of CFU number) at 300 Hz for 0.5 mT was 27 (Figure 2) where as for the same frequency at a higher magnetic flux density of 2.5 mT the CFU number was 45 (Figure 6).

The mechanistic aspects of PEMF effects on cells were proposed by different research studies. The interaction of ELF EMF with electrons in DNA of bacterial culture is also suggested by [35]. The biochemical compound in living cells are composed of charges and dipoles that can interact with electric and magnetic field by various mechanisms. For instance, displacement of electrons in DNA could cause local charging that has been shown to lead to disaggregation of biopolymers. Secondly, very weak ELF fields have been shown to affect the rate of electron transfer reaction. [39]. Low EMF energy can move electrons and cause small changes in charge structure [40]. ELF EMF has also shown to interact and accelerate electrons moving within DNA [41]. Applied PEMF can affect the permeability of ion channels in a cell membrane that in turn affects ion transport into a cell and ultimately results in alteration of biological function and/or structure of a cell [21]. Formation of free radicals upon irradiation with a magnetic field is another suggested outcome for EMF interaction with biological samples [21].





## Conclusions

Most of the studies report a decrease in the growth rate of bacteria upon EMF radiation at 50 Hz and 1-10 mT [8,18,22] for 1-24 h. In this study we investigated and evaluated the effects of the entire range of ELF PEMFs (2-500 Hz) at the magnetic flux densities ranging from 0.5 mT to 2.5 mT on the selected bacteria *S. aureus*. In our null hypothesis we assumed that the mean value of CFU is equal for both - the control and exposed samples. We tested this hypothesis using the established method of CFU, which was previously employed by other researchers to evaluate the viability of bacteria upon exposures to ELF EMF [30,34]. The importance of this study is attributed to the emergence of drug resistant bacteria. Clinical studies show beneficial use of PEMF therapy, and thus suggest integration of this form of therapy into the standard of care in inhibiting *Staphylococcus aureus* infections and hence, augmenting antibiotic therapy [42,43]. We investigated the effects of the generated ELF PEMF on the bacterium *S. aureus* in broth cultures and conclude that magnetic flux densities in the range of 0.5-2.5 mT for 2-500 Hz resulted in the decrease of the number of live cells (CFU) in all irradiated samples when compared to the controls. The results are more prominent at the frequencies on or above 100 Hz. The best results were achieved at 300 Hz and 1.5 mT, with the overall decrease in CFU values of at least 20% for frequencies higher than 200 Hz and all five studied magnetic flux densities. We have also shown the specific viability pattern “quadrature polynomial” for *S. aureus* exposed at the following parameters: 3-250 Hz and 0.5 mT, 3-200 Hz and 1.0 mT, 3-150 Hz at 2.0 mT and 2.5 mT. These patterns can potentially be used to predict the changes in CFU for the above mentioned frequency ranges. The presence of non-linear physical responses of bacteria suspended in broth upon exposures to ELF PEMF radiation, and were observed and categorized as the “window” effects. The occurrence of frequency and magnetic flux density “window” was observed at higher frequencies from 250-500 Hz at 0.5 mT, 200-500 Hz at 1.0 mT, 150-500 Hz at 2.0 mT and 2.5 mT, apart from 1.5 mT, where this “window” effect was visible throughout the entire frequency range. Determining the optimal parameters of the applied ELF PEMF for bacteria elimination is important in development of efficient and non-invasive treatment of the infected tissue and thus, wound healing promotion. These ELF PEMF parameters can also be used to explore the possibility of utilizing ELF PEMF as an adjunct treatment on *S. aureus* infected wound treated with antibiotic.

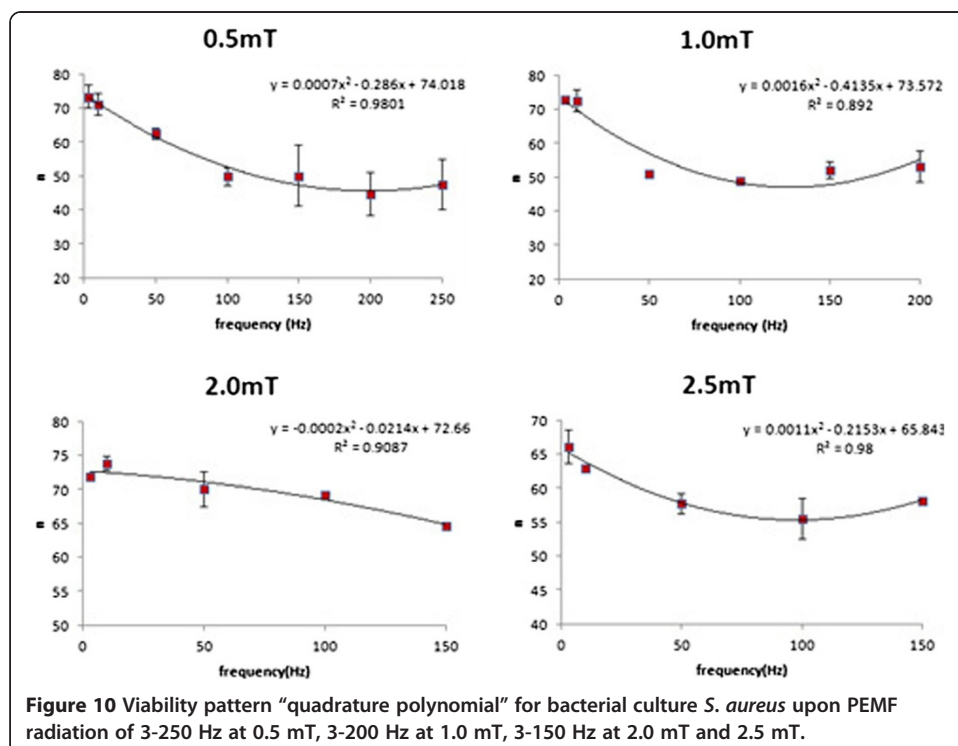
**Table 1 Minimum CFU number and maximum percentage change for ELF PEMF exposure of bacterium *Staphulococcus aureus***

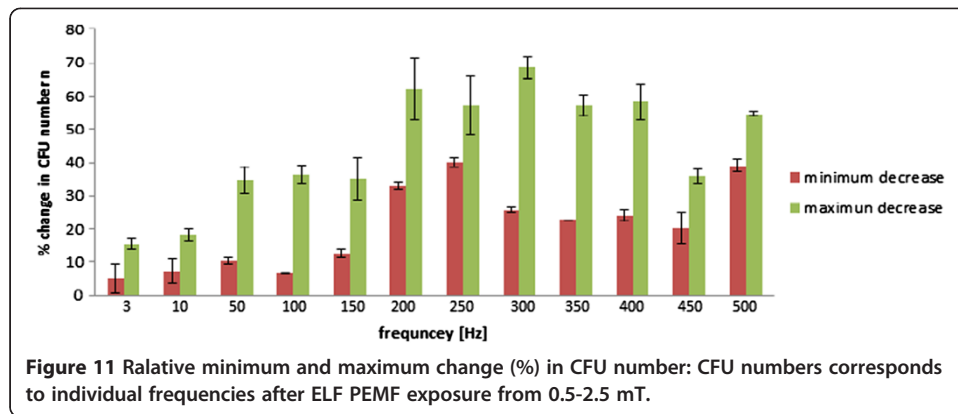
Frequency (Hz)	CFU number, <i>n</i> (minimum)	Percentage change (%) (maximum)	Corresponding value of magnetic flux density (mT)	Corresponding figure numbers
3	66	15.38	2.5	6 & 9
10	63	18.27	2.5	6 & 9
50	51	34.62	1.0	3 & 7
100	49	36.36	1.0	3 & 7
150	50	35.06	0.5	2 & 7
200	30	62.02	2.0	5 & 8
250	33	57.04	2.0	5 & 8
300	25	68.56	1.5	4 & 8
350	33	57.14	2.0	5 & 8
400	33	58.15	1.5	4 & 8
450	49	35.71	2.5	6 & 9
500	35	54.55	1.0	3 & 7

## Methods

### Equipment

The ELF PEMF exposure system (Figure 13) was built and used to generate uniform time varying magnetic fields in the frequency range of 2-500 Hz, with magnetic induction (magnetic flux density) of 0.5-2.5 mT. The developed exposure system was explained in details in [24,25]. EFA-200 EMF Analyzer, fitted with an external B-probe, was used to measure the magnetic flux density produced by two pair of air core Helmholtz coil (Wandel and Golterman). Eppendorf BioPhotometer Spectrophotometer UV/VIS was used to measure the Optical Density (OD) reading of the bacterial culture before ELF PEMF exposures.



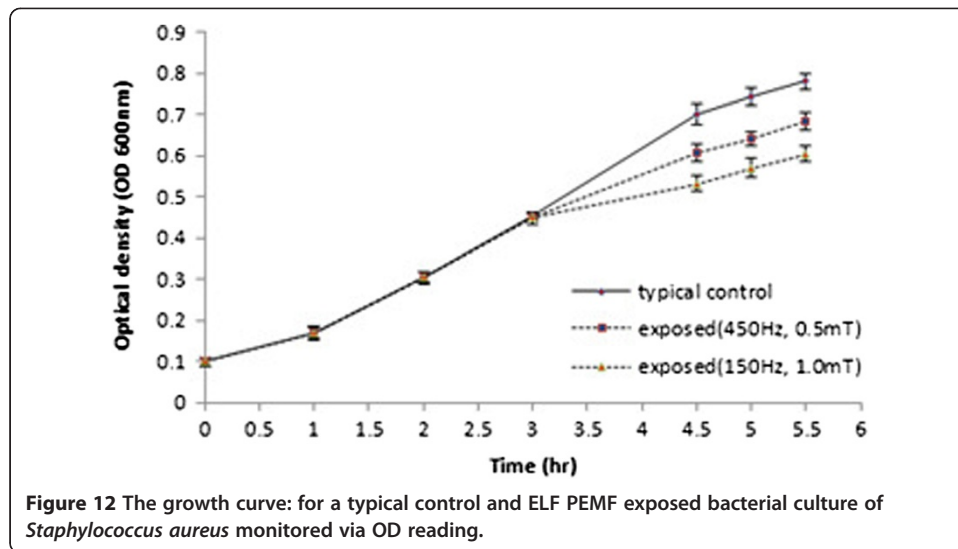


The studied frequencies and magnetic flux densities reported in this study were at all times measured at the center of the ELF PEMF chamber. Therefore, the reported magnetic flux density at a given frequency is exclusively measured for the main field source (air core coil pairs). The versatility of the EMF Analyzer, used here, allowed measuring of the source frequency and its corresponding magnetic flux density (B-field). The analyzer uses an innovative technique of “Shaped Time Domain” (STD) to display frequency of magnetic flux density by directly converting the time-domain limits into frequency domain limits [44].

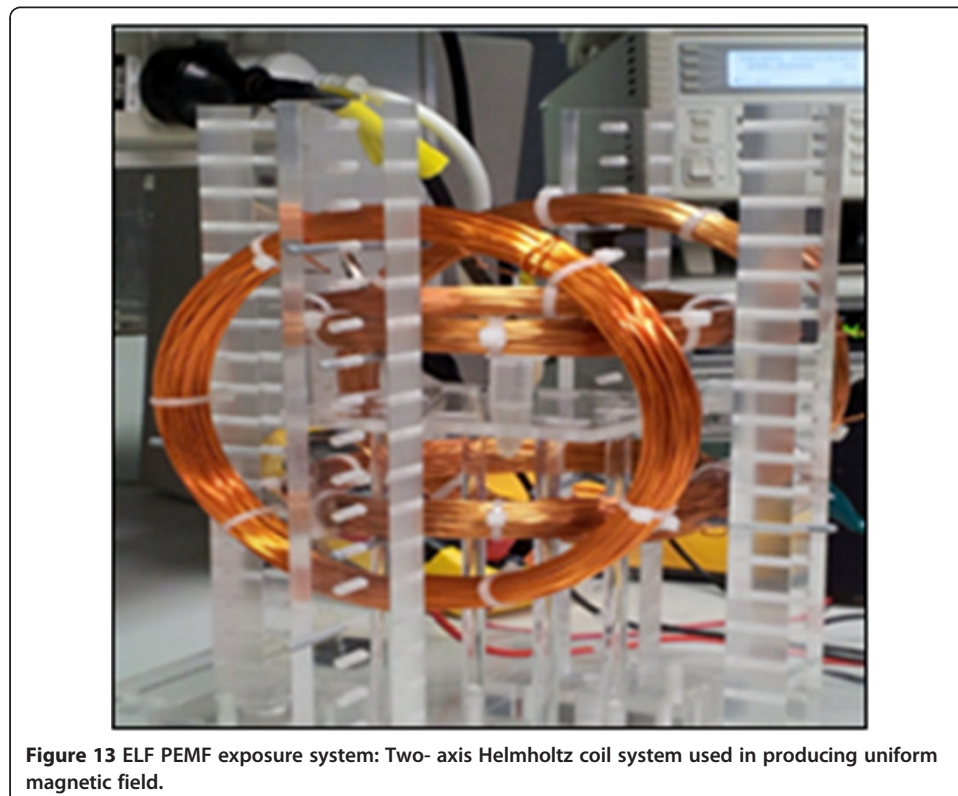
A typical reading measured by the EMF Analyzer for a magnetic flux density of 1.0 mT at 250 Hz during an experimental setup is shown in Figure 14. The magnetic field produced in these experiments is switching with respect to the frequency of the pulsating current that is being fed to the coil pairs from the power supply. Each coil carried a 50% duty cycle square wave. The strength of the magnetic field depends on the magnitude of this pulsating current. For a coil with ferromagnetic core, the inductance will not remain constant but will change with the current getting through the coils and result in distortion of the pulsating current from the power supply via production of higher frequency component. To prevent this, we have used an air core coils. Therefore, the only consideration required to be made, while winding the coil, was for RMS current and not for peak harmonic current (sum of individual harmonic currents), as would have been the case for using a coil with ferromagnetic core. The current waveform from the power supply was at all times observed by an oscilloscope over a 1 $\Omega$  resistor connected in series with the ammeter. The frequency of the current waveform could always be compared to the 50% duty cycle square wave produced by the function generator for a range of 2-500 Hz.

### Temperature control

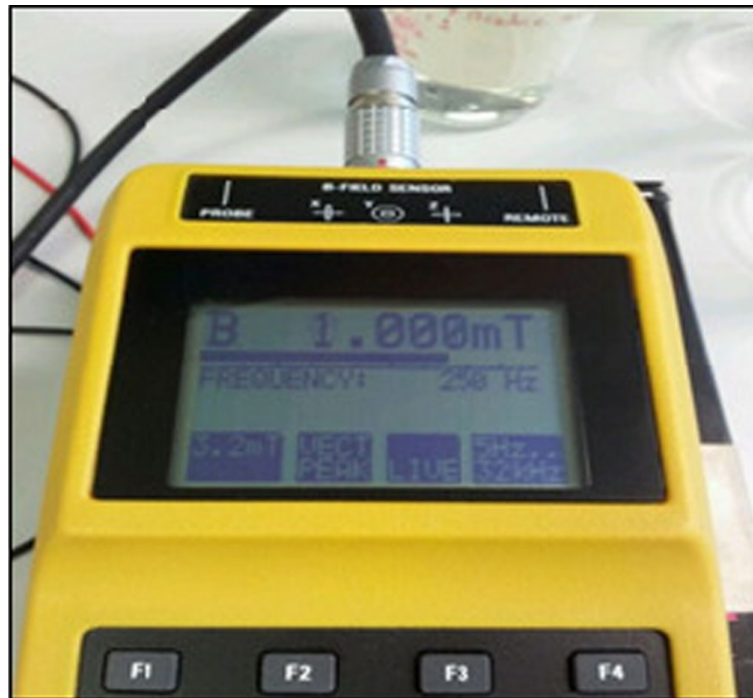
A two-socket digital temperature controller thermostat was connected to a power supply and set at 23°C (lab temperature). A cooling fan was connected to one of the thermostat socket. The temperature sensor of the thermostat was put through one of the inlet of the coil stands, just below the side coil. This sensor picks up the immediate rise in temperature of the surrounding air due to coil heating and matches it with the set temperature of 23°C. A higher temperature reading turns on the airflow of the cooling fan and a lower reading turns it off. Meanwhile, the temperature at the centre



of the exposure chamber (location of exposed samples) was continuously monitored by a digital thermometer. For maximum current supplied to the coils, the maximum temperature increase of 0.2°C above the lab temperature of 23°C was recorded at the centre of the ELF PEMF chamber. It was a good indication that the temperature control setup system efficiently maintains the temperature of exposed samples during the exposures by diffusing any unwanted heat produced via resistive heating of the current carrying coils.



**Figure 13** ELF PEMF exposure system: Two- axis Helmholtz coil system used in producing uniform magnetic field.

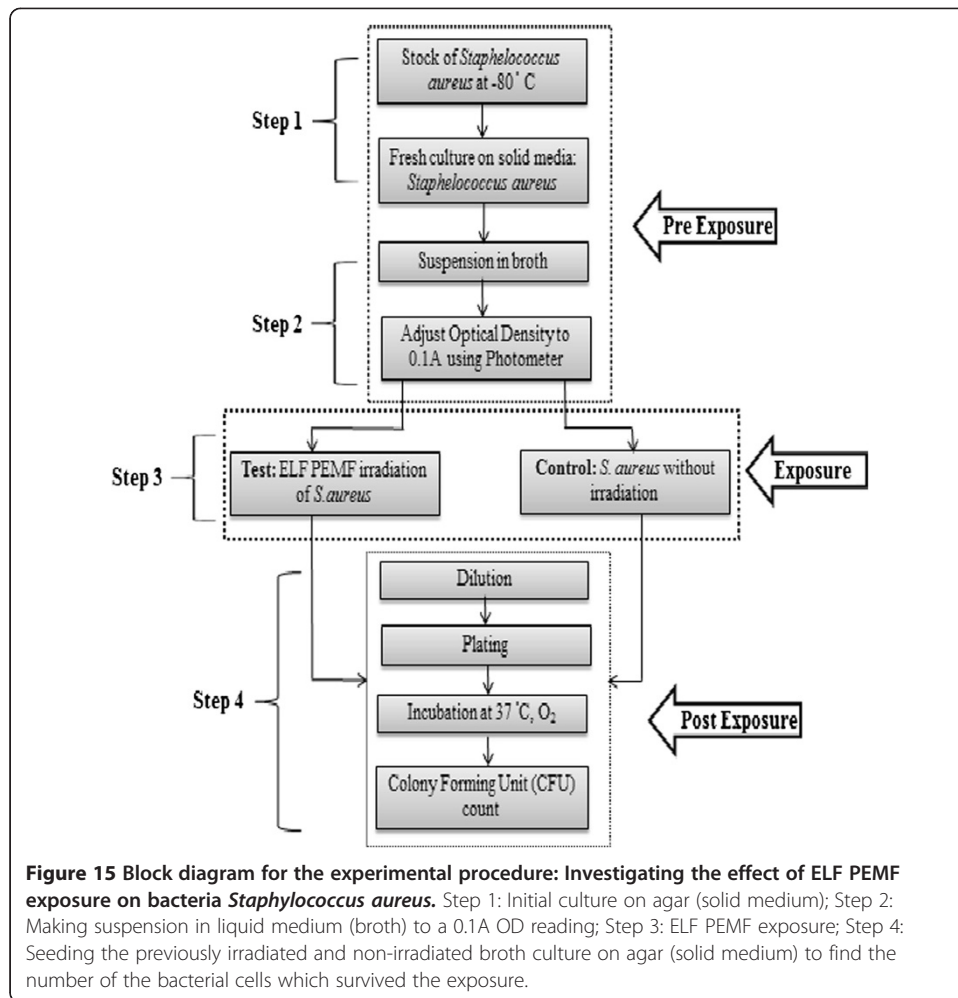


**Figure 14** Measurement of 1 mT at 250 Hz using EFA-200/300 EM Field Analyzer.

### Experimental procedure

In total, 180 experiments were carried out within this study. Figure 15 shows the block diagram of the experimental procedure that was used to expose bacterial culture *S. aureus* to ELF PEMF. The bacterial cultures were first cultivated in an agar medium (Columbia agar) and incubated aerobically for 24 hours at 37°C. Bacterial colonies were then picked and suspended in a broth medium (Columbia broth) to a certain density. Columbia broth composition: 5 g enzymatic digest of casein, 5 g enzymatic digest of animal tissue, 10 g yeast enriched peptone, 3 g enzymatic digest of heart muscle, 5 g sodium chloride, 25 g dextrose, 0.1 g L-cysteine, 0.1 g magnesium sulfate, 0.02 g ferrous sulfate, 0.83 g Tris (hydroxymethyl) aminomethane, 2.86 g Tris (hydroxymethyl) aminomethane-HCL and 0.6 g sodium carbonate.

To investigate the phase of bacterial growth of *Staphylococcus aureus* in suspension, we measured the OD reading for both the controls and exposed samples at 600 nm at every hour within the first 3 h (before ELF PEMF exposure) and then again after the exposures at 4.5 h, 5 h and 5.5 h. The resulting growth curves monitored via OD values for a typical sample and two arbitrarily picked exposed samples at (450 Hz, 0.5 mT) and at (150 Hz, 1.0 mT) are shown in Figure 12. The fact that there is no decrease in the bacterial concentration within the above mentioned time frame implies that bacteria are not in the death phase but rather still growing in their exponential phase. To ensure the controlled initial conditions for all conducted experiments, we always ascertain that bacterial culture is in the exponential phase of growth. Of note, monitoring OD values to study the growth curve of bacteria with and without ELF PEMF exposures was done by [45]. Moreover, [46] also monitored OD values to quantify results from for the effect of 50 Hz magnetic field on bacterial culture *S. aureus*.



Optical density (OD) for the suspended bacterial culture was measured at 600 nm and adjusted to a specific value of  $0.1 \pm .005A$  prior to commencing experimentation. This step was necessary for maintaining and standardization for all conducted experiments. Bacterial cultures were placed in 2.5 mL centrifuge tube and exposed to the ELF PEMF (3 hour since suspension) (Figure 13) of magnetic flux densities,  $B$ , (0.5 mT, 1.0 mT, 1.5 mT, 2.0 mT and 2.5 mT), frequency,  $f$ , range of 2–500 Hz and exposure duration,  $t$ , 90 min.

To determine the CFU value per mL, a serial dilution using the bacterial culture was performed. After five serial dilutions, a volume of 100  $\mu$ l from the last tube was inoculated in the agar plates (dilution factor  $1 \times 10^6$ ) by spread plating. For accuracy of results, it is recommended to make several plates for each dilution. Finally, after incubation, the colonies formed on the plates were visually counted. This is done in order to compare the effectiveness of ELF PEMF exposures on the studied bacterial cultures. Control cultures (non-exposed) for all experiments were kept in the similar conditions as the exposed ones apart from their sole exposition to the magnetic fields. Since the number of colonies growing on the solid agar media represents the number of live bacterial cells, it does not include organisms that may have died during the plating period.

#### Competing interests

The authors declare that there is no competing interests.

#### Authors' contributions

IA conducted the experiments and performed data analysis. TI and EP designed the project and experiments. TI supervised the experiments. The manuscript was compiled mostly by IA with the contribution of the co-authors. EP and TI edited the manuscript. All authors read and approved the final manuscript.

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