

Effect of Extremely Low Frequency Magnetic Field on the Growth Rate of Bacteria

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ABSTRACT

Developments in technology has resulted an increase in the use of electrical appliances. Electrical appliances use an alternating current with a frequency of 50 Hz. This alternating current generates an electromagnetic field which is also varies with time. This type of electromagnetic field is classified as a low frequency magnetic field that fills our living environment.

In this thesis, the effect of this low frequency magnetic field is investigated on the biological systems. *Escherichia coli* (prokaryote) (ATCC 25922) and *Drosophila melanogaster* (eukaryote) are used as two different biological systems. *Escherichia coli* is exposed to low frequency magnetic field for three different magnetic field intensities. It is shown that the low frequency magnetic field decreases the growth rate of the bacteria. *Drosophila melanogaster* is exposed to low frequency magnetic field and shown that the low frequency magnetic field affects the number of pupa generation negatively.

Keywords: Extremely low frequency magnetic field, *Escherichia coli* (E.coli ATCC25922), *drosophila melanogaster* (fruit fly).

ÖZ

Teknoloji alanında yaşanan gelişmeler, elektrikle çalışan aletlerin kullanımında artışa neden olmuştur. Elektrikle çalışan aletler, 50 Hz frekansına sahip alternatif akımla çalışmaktadır. Bu alternatif akım, zamanla değişen elektromanyetik alanlar üretmektedir. Bu tip elektromanyetik alanlar düşük frekanslı elektromanyetik alan olarak sınıflandırılmakta, ve yaşadığımız çevreyi sarmalamaktadır.

Bu tezde, düşük frekanslı manyetik alanların biyolojik sistemler üzerindeki etkileri incelenmiştir. Biyolojik sistem olarak prokaryot hücre yapısına sahip koli basili (*Escherichia coli*, ATCC 25922) ile ökoryot hücre yapısına sahip meşe sineği (*Drosophila melanogaster*) kullanılmıştır. Koli basili, düşük frekanslı üç farklı manyetik alan şiddetine maruz bırakılmıştır. Düşük frekanslı manyetik alanların koli basili bakterisinin büyüme hızına olan olumsuz etkisi gösterilmiştir. Meyve sineği de düşük frekanslı manyetik alana maruz bırakılmış ve yavrulama sayısına olan olumsuz etkisi gösterilmiştir.

Anahtar Kelimeler: Düşük frekanslı manyetik alanların, *Escherichia coli*(*E.coli* ATCC 25922), *Drosophila melanogaster* (meyve sineği).

DEDICATION

**To my dear mom, dad, sisters and brothers who always supported
me.**

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Chapter 1

INTRODUCTION

Developments in technology have resulted in an increase in the use of electricity and electrical appliances in our daily life. Today, we are living in such an environment that, the extremely low frequency magnetic fields (< 300 Hz) emanating from the electrical appliances became part of our living environment. This situation is questioned by researchers, whether exposure to low frequency magnetic field is harmful for our health or not.

The primary research in this field was belongs to Wertheimer and Leeper [1]. In their work, they concentrated on the epidemiology and the increased cancer hazard and the rate of leukemia for children and people living or working near power-lines and normal instruments used inside the houses.

In another study, the connection between cancer and children residing near Swedish high-voltage power lines were investigated [2]. A similar study [3] was also devoted to cancer and electrical workers in New Zealand. All these studies are motivated due to the fact that, long period of exposure to low frequency magnetic fields can cause cancer because it is believed that magnetic field can cause a fatal harm to DNA structure. However, all these studies did not give a concrete proof to this assumption and the results are considered to be controversial [2, 3].

In order to explore this problem in more detail, the researchers started to investigate the effect of low frequency magnetic fields on microorganisms, normally on bacteria.

In the last two decades, considerable amount of work was devoted for the investigation of the effects of low frequency magnetic fields on the prokaryotic and eukaryotic microorganisms has been increase.

Strasak et al. applied 5–21 mT magnetic fields to *Escherichia coli* (E.coli) for 0–24 hours and studied a possible decrease in growth rate [4]. Similarly, Fojt et al. discovered at 50 Hz, 10 mT magnetic fields exposed for a period of 30 minute, decreases colony forming units of *Escherichia coli*, *Leclercia adecaboxylata* and *Staphylococcus arueus* [5]. Also El-Sayed et al. was confirmed a decrease in growth rate of *Escherichia coli* focused to 50 Hz, 2 mT magnetic field for 0–6 hours [6].

Garip et al. explored the effect of extremely low frequency (<300 Hz) electromagnetic fields on the growth rate of three Gram- positive and three Gram- negative of bacteria *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* subjected to 50 Hz – 0.5 mT magnetic field for 6 hours. The outcomes showed a decrease in the growth rate of exposed samples with compared to control and determined morphological changes for both bacteria [7].

In this thesis, we are aiming to investigate the effect of low frequency electromagnetic fields on living organisms. In doing this we have used two types of structurally different organisms, prokaryote bacteria *Escherichia coli* and eukaryotic organism, *Drosophila melanogaster*. The reason for choosing *Escherichia coli* is that,

it is easy to find and this strain is not pathogenic, exposure of bacteria was investigated by low frequency 50 Hz for different magnetic fields with intensities ranging from 1 mT, 5 mT, 10 mT. The growth in diameter of the colony is measured every two hour for a period of 12 hours.

We also used *Drosophila melanogaster* (fruit fly). This fly is small 2mm long 1mg in weight, and has a short life cycle. Over 100 flies were collected in field and placed in a glass vial with standard medium food after 24 hours at $22 \pm 3^\circ\text{C}$ room temperature were laid eggs and remained 10 ± 1 days to be adults. After that, we separate 20 virgin females and 20 males. Flies exposed to magnetic fields intensity range for 10 mT, flies exposed for a period time 4 hours.

Emel and Hacer were investigated the effects of microwave frequency electromagnetic fields (EMFs) on the fecundity of *Drosophila melanogaster*. The Oregon strain females of *Drosophila melanogaster* were exposed to 10 GHz electromagnetic field continuously (3, 4 and 5) hours and discontinuously 3 hours exposure + 30 minutes interval + 3 hours exposure. The fecundity of females was determined. It was found a statistically significant decrease in mean fecundity as compared to the control [8].

In our experiments, the growth rates of *E.coli* and reproductive capacity of *Drosophila* were determined under the condition of the low frequency 50 Hz magnetic fields application.

Chapter 2

ELECTROMAGNETIC FIELDS

2.1 Extremely Low Frequency Electromagnetic Fields (ELF-EMF)

In general, the static charged particles create electric fields; on the other hand moving charged particles at constant speed (current) generate magnetic fields. The electromagnetic fields form as a result of the combination of electric and magnetic fields. The electromagnetic wave is characterized as a low frequency electromagnetic wave, if its frequency is less than 300 Hz (< 300 Hz). In this thesis our main concern is to investigate the effect of these waves on living organisms.

Living organisms, human are exposed to electric and magnetic fields from many sources including high or low voltage transmission lines, electric equipments inside buildings and electric appliances.

People are daily exposed to electromagnetic fields. Electromagnetic fields have a very wide range of spectrum. Extremely low frequency has frequencies up to 300 Hz. and have very long wavelengths (6000 Km at 50 Hz and 5000 km at 60 Hz). ELF-EMFs, such as 50 Hz or 60 Hz electric and magnetic fields are created from power transmission lines and the appliances which are used in our living environment.

2.2 Sources of Extremely Low Frequency Electromagnetic Fields

2.2.1 Power Lines

Power stations create large amounts of electric energy. Electric energy is transferred via high voltage transmission lines. The electric and magnetic fields intensity is correlated to distances from transmission lines, the currents and the voltage carried by the lines. The distance is inversely proportional to electric and magnetic fields. The electric and magnetic field under the over head transmission lines may be as high as 12 kV/m and 30 μT respectively [9, 10]. Workers who work in the surrounding area of transmission and distribution lines or people who live around transmission and distribution lines are exposed to larger electric and magnetic fields.

2.2.2 Generating Stations and Substations

Substations are one of the low frequency electromagnetic field sources. Electric and magnetic fields within the generating stations and substations may be as high as 25 kV/m and 2 mT. Similarly, electric and magnetic fields around the generating stations and substations may be as high as 16 kV/m and 270 μT respectively [9, 10].

2.2.3 Home Electrical System and Appliances

The electric and magnetic fields intensity depends on the distance from home appliances, the number and types of electrical devices, the construction and the position of household electrical wiring system.

The electric field produced by home wiring depends on how it is established. Wiring installed in metal trucking or conduit produces very small external fields, and the fields produced by wiring installed within walls are attenuated by an amount depending on the building materials. Generally electric and magnetic field surrounding electrical devices are up to 500 V/m and 150 μT respectively [9, 10].

2.2.3.1 Computers

All parts of computers emit EMF. Most of the computers radiate $0.7 \mu T$ magnetic fields at 0.30 m from the display. The Swedish protection standards determine the protection magnetic fields values for all parts of computer manufacturers. The maximum value is selected as $0.25 \mu T$ and greater values may reason to the leukemia cancer [10, 11].

2.2.3.2 Electric Blankets

Electric blankets radiate $10 \mu T$ magnetic fields at 0.01 m [11]. Its means that if you do not switch off electric blanket in bed it penetrate into the body. It may cause miscarriages and childhood leukemia [12].

2.2.3.3 Electric Clocks

Electric clocks emitted magnetic field up to $0.5 \mu T$ to $1 \mu T$ at 91 cm away. The amount of electromagnetic field emitted by electric clocks are equal to the amount of power lines . If it is continuously, it may cause brain tumors same as miscarriages leukemia and cancer. All clocks and other electrical devices (such as telephones and answering devices) should be placed at least 182cm from your bed [12].

2.2.3.4 Fluorescent Lights

Fluorescents lights produce electromagnetic field much more than bright bulbs. According to studies of photo biologist John Ott, fluorescent lights decrease concentration performance and change behavior of human (1929). A typical fluorescent lamp of an office ceiling has reading of 16 to $20 \mu T$ at 2.5 cm away. If it is continuous, it may cause leukemia cancer [12].

2.2.3.5 Microwave Ovens

Microwave ovens generate radiation. Water molecules absorb radiations and water molecules vibrate. This vibration produces heat that means increases heat of the food. The Russian protection limit is 10 mW/cm^2 . Russian studies have shown that normal microwave cooking may convert food protein molecules into carcinogenic substances. If it is used continuously, it may cause cancer [12].

2.2.3.6 Hair Dryers and Electric Razors

Hair dryers and electric razors radiate EMFs up to $0.01 \mu T$ to $0.15 \mu T$ at 30 cm away. Hair dryer must not be used on children because it may affect the development of brain and nervous system [10].

Table 2.1. Typical field strengths from household appliances compared to International Commission on Non-Ionizing Radiation Protection (ICNIRP) suggested limits [10.13]

Electrical appliance	Magnetic field strength in μT at 0.3 m.	ICNIRP suggested exposure limit in μT .
Electric oven	1 to 50	100
Microwave oven	4 to 8	100
Vacuum cleaner	2 to 20	100
Electric shaver	0.08 to 9	100
Clothes Washer	0.08 to 0.3	100
Clothes Dryer	0.01 to 0.15	100
Fluorescent Fixtures	0.2 to 3.02	100
Hair Dryer	0.01 to 0.15	100
Electric iron	0.12 to 0.31	100
Toasters	0.06 to 0.7	100
Coffee Makers	0.9 to 1.2	100
Blenders	0.52 to 1.7	100
Television	0.04 to 2	100
Electric Range	0.4 to 4	100

2.3 Electric and Magnetic Fields in Biological Systems

In general, electromagnetic fields consist of electric and magnetic fields together. However, if one is interested at distances less than one wavelength from a source, the fields can be considered separately without a significant loss of generality. In our case, since the frequency is 50 Hz, the equivalent wavelength is 6000km and, hence electric and magnetic fields can be divided because we are interested in a distance of 1 – 100 m. Here, we wish to review the theoretical calculation presented in the master thesis [14], to show how the time varying magnetic fields affects the biological systems.

The body is conductor with a mean resistivity $\rho \approx 1 \Omega m$ [15]. If the body is exposed to an external static electric field E_{ext} , it will be oriented in such the positive and negative charges of our away that the positive charges travels in the same direction of E_{ext} , the negative charges will travel in the opposite directions. This movement will generate another electric field inside the body which is in the opposite direction of E_{ext} .

Finally, when electrostatic equilibrium is reached this secondary field will cancel the external field E_{ext} inside the body. This implies that static external field E_{ext} is zero inside the body.

However, if the external field E_{ext} is time-dependent and oscillates with angular frequency ω , the sign of the surface charge will always change, and in turn it will generate a small oscillating current as the charge moves from one side to another inside the body. This current can be measured as the result of a small internal field

that is proportional to the magnitude and frequency of the external field. In order to find the induced internal electric field inside the body, we assume a spherical body in an external oscillating electric field, which varies as $E_0 \cos \omega t$.

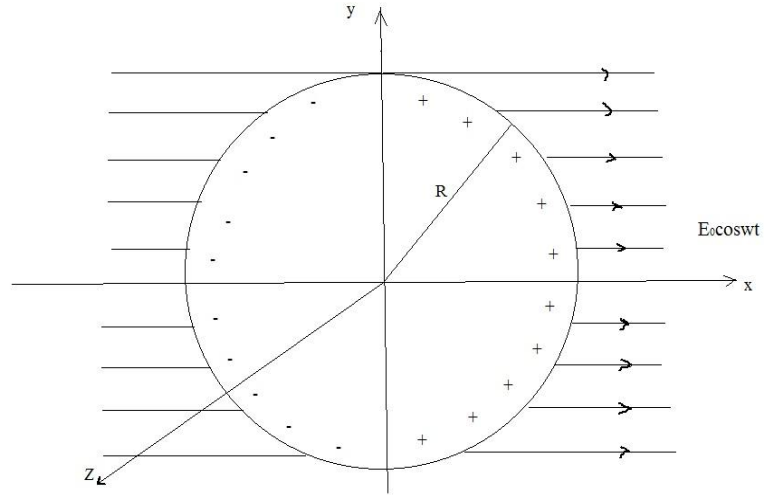


Figure 2.1. Spherical body in an external oscillating electric field which varies as $E_0 \cos \omega t$

Since the external field is oscillating, the sign of the induced charge on the sphere will always change. It was stated that human body is a conductor with conductivity $\sigma = \frac{1}{\rho} \approx 1(\Omega m)^{-1}$. In order to make calculations more precise to solve the resulting mathematical expressions, we assume a spherical body and hence, we employ spherical symmetry [16]. The internal electric field E_{in} and the current density (J) are related by Ohm's laws and given by

$$J = \sigma E_{in}, \quad (2.1)$$

where E_{in} is the induced electric field inside the body. First, we must calculate the induced charge density on the surface of a sphere. From electrodynamics it is known that the electric potential outside sphere [16] is,

$$V(r, \theta) = \sum_{l=0}^{\infty} \left(A_l r^l + \frac{B_l}{r^{l+1}} \right) P_l(\cos \theta), \quad (2.2)$$

where A_l and B_l are constants to be found from the boundary conditions and $P_l(\cos \theta)$ is a spherical Legendre function. At the moment explained in Figure 2.1 the negative charges will be collected on the left and positive charges on the right hemi-spherical region. Hence the sphere is at equipotential and we may set it to zero (by grounding the sphere).

Then by symmetry the entire yz -plane is at potential zero. Therefore the appropriate boundary conditions [15] for this problem,

$$V = 0 \quad \text{When} \quad r = R, \quad (2.3)$$

$$V \rightarrow -rE_0 \cos \omega t \cos \theta \quad \text{When} \quad r \gg R. \quad (2.4)$$

Applying the first boundary condition (2.3) in equation (2.2) yields,

$$A_l r^l + \frac{B_l}{r^{l+1}} = 0 \Rightarrow B_l = -A_l R^{2l+1}, \quad (2.5)$$

Then we have,

$$V(r, \theta) = \sum_{l=0}^{\infty} A_l \left(r^l - \frac{R^{2l+1}}{r^{l+1}} \right) P_l(\cos \theta), \quad (2.6)$$

for $r \gg R$, the second term in parenthesis of the equation (2.6) is negligible and therefore the second boundary condition (2.4) requires that,

$$\sum_{l=0}^{\infty} A_l r^l P_l(\cos \theta) = -rE_0 \cos \omega t \cos \theta. \quad (2.7)$$

Hence only one term is present and that term is for $l=1$ since $P_1(\cos\theta) = \cos\theta$.

Therefore, we can read off immediately,

$$A_l = -E_0 \cos \omega t . \quad (2.8)$$

As a result the electric potential outside the sphere is,

$$V(r, \theta) = -E_0 \cos \omega t \left(r - \frac{R^3}{r^2} \right) \cos \theta . \quad (2.9)$$

The first term ($-rE_0 \cos \omega t \cos \theta$) is due to the external field, the contribution for the induced charge is definitely,

$$E_0 \frac{R^3}{r^2} \cos \omega t \cos \theta . \quad (2.10)$$

The induced charge density can be calculated as [16],

$$\sigma(\theta) = -\varepsilon \left. \frac{\partial V}{\partial r} \right|_{r=R} = \varepsilon E_0 \cos \omega t \cos \theta \left(1 + 2 \frac{R^3}{r^3} \right) \Big|_{r=R} , \quad (2.11)$$

which yields,

$$\sigma(\theta) = 3\varepsilon E_0 \cos \omega t \cos \theta , \quad (2.12)$$

where $\varepsilon = \varepsilon_0 \varepsilon_r$ is the permittivity of the human body. Here ε_r denotes the relative

permittivity and $\varepsilon_0 = 8.85 \times 10^{-12} \left(\frac{C^2}{Nm^2} \right)$ is the free space permittivity. The net charge

on one of the hemisphere is evaluated by integrating this induced charge (2.12) over

the hemisphere in which $0 \leq \theta \leq \frac{\pi}{2}$. Hence,

$$q = \int \sigma dA = \int_0^{\frac{\pi}{2}} \int_0^{2\pi} (3\varepsilon E_0 \cos \omega t \cos \theta) R^2 \sin \theta d\phi d\theta . \quad (2.13)$$

After integration the net charge on one of the hemisphere is,

$$q = 3\varepsilon \pi R^2 E_0 \cos \omega t . \quad (2.14)$$

In order to find the current we take the time derivative of both sides,

$$I = \frac{dq}{dt} = -3\varepsilon \pi R^2 E_0 \omega \sin \omega t . \quad (2.15)$$

We ignore the negative sign and take $I = JA$ where J the current density is and A is the surface area of the hemisphere. We have,

$$J 2\pi R^2 = 3\varepsilon \pi R^2 \omega E_0 \sin \omega t . \quad (2.16)$$

After simplification of (2.16) we have,

$$J = \frac{3}{2} \varepsilon \omega E_0 \sin \omega t , \quad (2.17)$$

Using this in equation (2.1) we finally have,

$$E_{in} = \frac{3}{2} \varepsilon \omega \rho E_0 \sin \omega t . \quad (2.18)$$

Since $\varepsilon = \varepsilon_0 \varepsilon_r$, the relative permittivity ε_r can be as much as 100. In terms of numerical values,

$$E_{in} \approx (10^{-7} \times E_0) . \quad (2.19)$$

Consequently, even for the very large external field of 10 kVm^{-1} , induced electric field inside the body is bounded to,

$$E_{in} \leq 1 \text{ mVm}^{-1} . \quad (2.20)$$

Thus, external electric field effectively is shielded by the human body and it does not cause any health problem.

Now let us consider the magnetic field itself. ELF magnetic fields are not shielded by the electric properties of the body. From electrodynamics we have learnt that changing magnetic field generates internal electric fields through Faraday's law,

$$-\oint_c \vec{E} \cdot d\vec{r} = \frac{d}{dt} \left(\int_S \vec{B} \cdot d\vec{A} \right), \quad (2.21)$$

Where c is the contour bounding the area S . This generated electric field is usually larger than the one induced by the external electric field. If we consider a typical effective human body area as that of a circle with radius $r = 10\text{cm}$, we estimate a mean amplitude of electric field generated by a 50 Hz oscillating magnetic field with an amplitude $B_0 = 50\mu\text{T}$ field acting over the body as;

$$|\vec{E}_B|(2\pi r) = \frac{d}{dt} \left(\int_A (B_0 \sin \omega t) dA \right) = B_0 \omega \pi r^2 \cos \omega t, \quad (2.22)$$

which simplifies to,

$$|\vec{E}_B| = \frac{B_0 \omega r}{2} \approx 10^{-3} \text{Vm}^{-1}. \quad (2.23)$$

As reported in reference [15], investigated ELF magnetic fields less than $50\mu\text{T}$ are too small to cause biological effects through their interaction with magnetic materials inside the body.

2.4 Calculation of the Magnetic Field of Helmholtz Coil

In order to produce low frequency magnetic fields with intensities ranging from 1–10mT , we construct Helmholtz coil system. The theoretical formula which gives the intensity of the magnetic fields at the midpoint between the coils is obtained from Biot-Savart law as below.

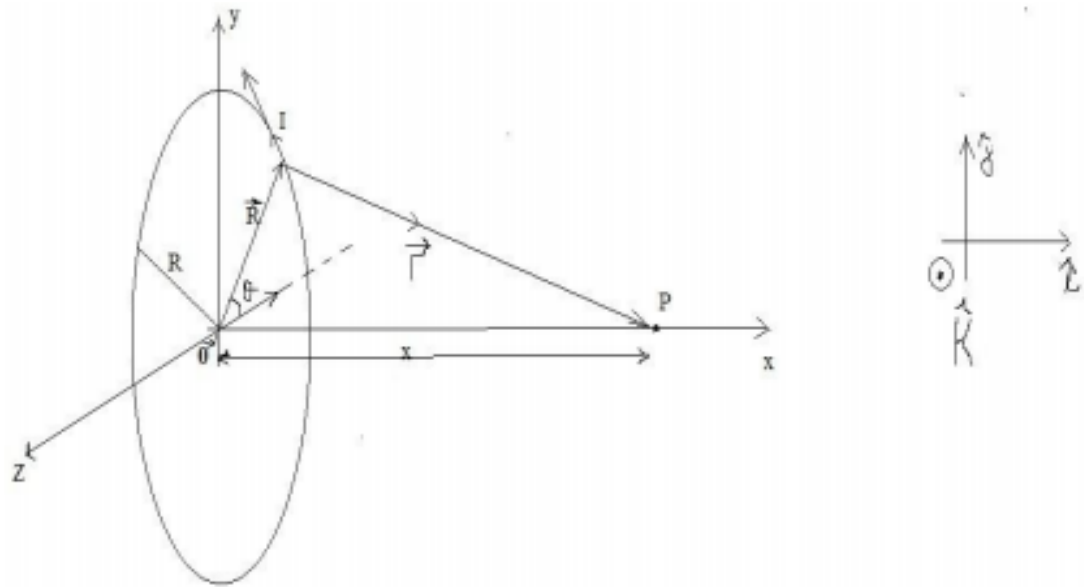


Figure 2.2. The magnetic field of single turn wire loop on the x-axis at point P which passes through its axis is obtained from the Biot-Savart law

The Biot – Savart law is given by,

$$\vec{B} = \frac{\mu_0 I}{4\pi} \int \frac{d\vec{l} \times \hat{r}}{r^2}, \quad (2.24)$$

where $\mu_0 = 4\pi \times 10^{-7} T.m/A$, is the permeability constant, \hat{r} is the unit vector and

r is the distance between the current element $d\vec{l}$ and the point at which the magnetic

field will be calculated. By using the Figure 2.2, we have,

$$\vec{R} + \vec{r} = \vec{x} = x\hat{i}, \quad \text{and} \quad \vec{R} = -R \cos \theta \hat{k} + R \sin \theta \hat{j}, \quad (2.25)$$

Then, the unit vector is found to be,

$$\hat{r} = \frac{\vec{r}}{|\vec{r}|} = \frac{x\hat{i} - R \sin \theta \hat{j} + R \cos \theta \hat{k}}{\sqrt{X^2 + R^2}}, \quad (2.26)$$

and square of the distance is,

$$r^2 = (X^2 + R^2), \quad (2.27)$$

$$d\vec{l} = R d\theta \{ \sin \theta \hat{k} + \cos \theta \hat{j} \}. \quad (2.28)$$

By substituting Eq (2.26), Eq (2.27), and Eq (2.28) into Eq (2.24) yields,

$$\vec{B} = \frac{\mu_0 I}{4\pi} \int \frac{R d\theta [\sin \theta \hat{k} + \cos \theta \hat{j}]}{(X^2 + R^2)} \times \left\{ \frac{x\hat{i} - R \sin \theta \hat{j} + R \cos \theta \hat{k}}{\sqrt{X^2 + R^2}} \right\}, \quad (2.39)$$

$$\vec{B} = \frac{\mu_0 I R}{4\pi (X^2 + R^2)^{3/2}} \left\{ x \int_0^{2\pi} \sin \theta d\theta \hat{j} - x \int_0^{2\pi} \cos \theta d\theta \hat{k} + R \int_0^{2\pi} d\theta \hat{i} \right\}, \quad (2.30)$$

after taking the above integral the following formula is obtained for a magnetic field of a single wire loop,

$$\vec{B} = \frac{\mu_0 I R^2}{2(X^2 + R^2)^{3/2}} \hat{i}. \quad (2.31)$$

For N turns coil the above formula becomes,

$$\vec{B} = \frac{\mu_0 N I R^2}{2(X^2 + R^2)^{3/2}} \hat{i}. \quad (2.32)$$

Helmholtz coil system consists of two N-turns coil oriented parallel to each other as in Figure 2.3.

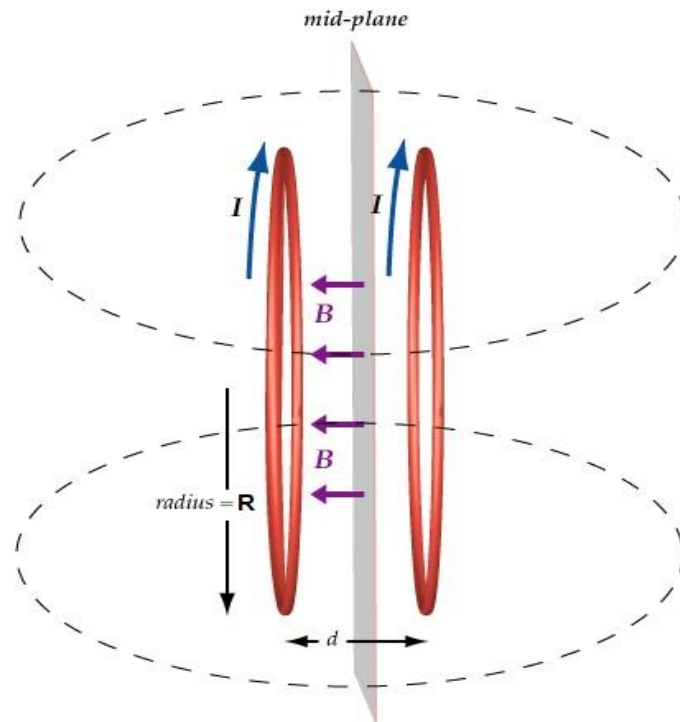


Figure 2.3. Helmholtz coil system made of two N -turns oriented coils

As a result, the net magnetic field generated by the coils is obtained by using the principle of superposition which leads to the following formula.

$$\vec{B} = \frac{\mu_0 N I R^2}{(X^2 + R^2)^{3/2}} \hat{i}. \quad (2.33)$$

Chapter 3

PREPARATION OF THE EXPERIMENTAL MATERIAL

3.1 Construction of the Helmholtz System

One of the main elements of the experimental setup is to construct the Helmholtz coil system as shown in Figure 2.3. To generate a uniform time varying magnetic fields at the frequency of 50 Hz and magnetic field intensity of 0.5 – 12 mT. Helmholtz coil system consists of two coils oriented parallel to each other as in Figure 3.1. When the time varying current passes through coils, a time varying uniform magnetic field is produced at the midpoint between the coils on the axis passing through the centre of the coils.

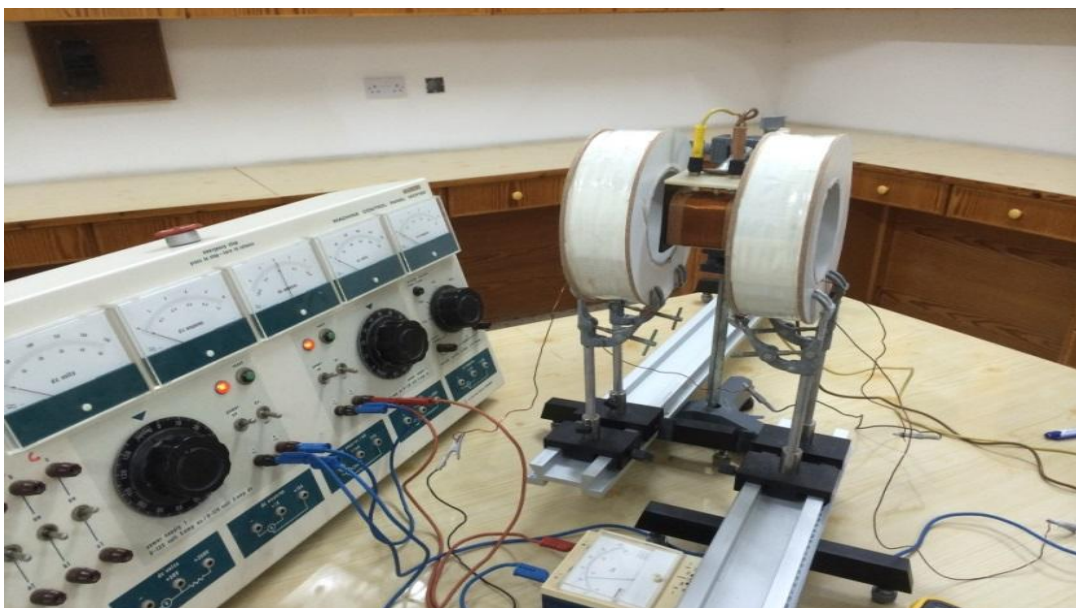


Figure 3.1. Experimental devices by placing induction coil at the midpoint of the two Helmholtz coil and connecting voltmeter to the end of the induction coil

To serve the purpose of our experiment, the number of turns in each coil is specified as 1200 turns. The mean radius of each coil is taken to be 8 cm, and the mean midpoint where the magnetic field is assumed to be uniform is taken to be 6.5 cm. The theoretical formula which was derived from the Biot-Savart law in the previous chapter for N-turns coil system is found to be,

$$B = \frac{\mu_0 INR^2}{(R^2 + X^2)^{3/2}} . \quad (3.1)$$

$$B_{\text{Theoretical}} = 8.8 I \text{ mT}. \quad (3.2)$$

Experimentally measured magnetic field $B_{\text{practical}}$ values for specific values of current are compared with the above formula and the results are tabulated in Table 3.1.

Table 3.1. Theoretical and practical results of the magnetic field measurement

I (A)	V (V)	$B_{\text{Theoretical}}$ (mT)	$B_{\text{practical}}$ (mT)
0.11	0.94	0.97	1
0.24	1.88	2.11	2
0.37	2.82	3.25	3
0.49	3.76	4.31	4
0.62	4.66	5.45	5
0.75	5.65	6.60	6
0.87	6.51	7.74	7
0.99	7.53	8.71	8
0.12	8.47	9.85	9
1.25	9.42	11.11	10
1.37	10.36	12.06	11
1.50	11.30	13.20	12

It is clear to see that, the experimentally measured values and theoretically calculated values are close to each other.

3.2 Measurement of the Magnetic Field Intensity $B_{\text{practical}}$

The proper method to measure the time varying magnetic field at the midpoint of the Helmholtz coil system is to use a suitable Teslameter which is a device used for measuring magnetic field intensities. However, the available Teslameter in our physics lab is more suitable to measure magnetic field intensity produced by DC currents.

To overcome this problem, we have used another method which involves the use of Faraday's law of induction. This is achieved by placing $N' = 1200$ turns induction coil at the midpoint of the Helmholtz coil system and connecting voltmeter to the ends of the induction coil. The mean dimensions of the 1200-turns induction coil are $5 \text{ cm} \times 5 \text{ cm}$. When a time-varying current passes through the Helmholtz coil system, the produced time-varying magnetic field at the centre is assumed to be,

$$B(t) = B_0 \sin \omega t , \quad (3.3)$$

in which $\omega = 2\pi f$ is the angular velocity and B_0 is the intensity of the magnetic field. This magnetic field produces time-varying flux across the cross-sectional area of the induction coil which is given by,

$$\Phi(t) = \int \vec{B}(t) \cdot d\vec{A} = B_0 A \sin \omega t , \quad (3.4)$$

where $A = 25 \times 10^{-4} \text{ m}^2$. The magnitude of the induced EMF across the induction coil is calculated from the Faraday's law,

$$|V| = \left| N' \frac{d\Phi}{dt} \right| = N B_0 A \omega \cos \omega t . \quad (3.5)$$

And the maximum value of the induced voltage obtained when $\cos \omega t = 1$ is given by,

$$|V| = N B_0 A \omega , \quad (3.6)$$

from this relation, the intensity of the magnetic field is obtained as,

$$B_0 = \frac{|V|}{N A \omega} , \quad (3.7)$$

$$|V| = 942 B_0 . \quad (3.8)$$

For specific values of current, the induced EMF $|V|$ is measured with the voltmeter and the results are tabulated in the Table 3.1.

It should be noted that the time-varying current supplied to the Helmholtz coil is provided by the power supply named as variac. Variac is a device which uses ordinary AC-currents from the mains supply current and using step down transformers to reduce it to desired value.

3.3 Faraday's Cage

The main goal of this project is to investigate the low frequency magnetic fields on a living microorganism. For this purpose, two sets of same sample are prepared. One set will be exposed to magnetic field and the other set will be the control set which must be placed in a region in which no magnetic field exists.

This is provided by the use of Faraday cage. Faraday cage is constructed from aluminum foil, as in Figure 3.2. Aluminum foil shields all the external magnetic fields and leaves the inside magnetic free region.



Figure 3.2. Faraday cage is constructed from aluminum foil, electric bulb was used to keep the temperature at 37°C

3.4 Temperature Control

Temperature is an important parameter in this experiment. The bacteria used, *Escherichia coli* best grows 37°C. Therefore throughout the experiment the exposed and control samples must be kept at a temperature of 37°C. This is provided by split unit system and supported by bulbs to keep the temperature at $37 \pm 0.5^\circ\text{C}$. The temperature is continuously measured by a digital Phywe thermometer as shown in Figure 3.3.



Figure 3.3. Digital Phywe thermometer

Chapter 4

ESCHERICHIA COLI

4.1 The Effect of Extremely Low Frequency Electromagnetic Field on the Growth Rate of Prokaryote *Escherichia coli*

The effect of low frequency magnetic field on the biological system of living organisms is an important research field as far as human health is concerned. During the last two decades, many studies have been published to prove the direct effects of low frequency magnetic fields on small biological objects. The primary research in this field is published by Moore, reported the effect of magnetic fields on microorganisms and showed that the stimulation of five bacterial species and yeast was dependent on the strength, frequency and types of bacteria [17].

In another study, Fojt et al. reported that an exposure of 50 Hz, 10 mT magnetic field for a period of 30 minutes causes decrease in the colony forming units of *Escherichia coli*, *Leclercia adecaboxylata* and *Staphylococcus aureus* [5]. Similarly, Strasak et al. explored the effect of 50 Hz, 10 mT magnetic field exposed for a period of 24 hours on *Escherichia coli* and *Staphylococcus aureus*, the result showed decrease in the growth rate [18].

In this thesis, we are aiming to investigate the growth rate of *Escherichia coli* when exposed to 50 Hz low frequency magnetic field for different field intensities 1 mT, 5 mT and 10 mT. The reason that we chose this bacteria to work is that, it is not pathogenic and is easy to obtain.

4.2 Bacterial Growth

4.2.1 Preparation of LB Broth (Luria Broth)

One liter of LB Broth was prepared by melting 10 grams of tryptone, 5 grams of yeast and 10 grams of sodium chloride in 800 ml of distilled deionized water in a measuring cylinder. The mixture was topped up to 1 liter. The mixture was then autoclaved at 121°C for 20 minutes. The broth was kept at 4°C until further use.

4.2.2 Preparation of LB Agar Plates

LB agar was prepared by adding 10 grams of tryptone, 5 grams of yeast extract, and 10 grams of sodium chloride and 15 grams of agar in 1 liter of distilled deionized water. The mixture was autoclaved at 121°C for 20 minutes. After cooling down, the agar was poured into the plates. The plates were labeled and kept at 4°C until further use.

4.2.3 Preparation of Fresh *Escherichia coli* suspension

Escherichia coli were used for the magnetic field experiment. The bacterial samples were grown in a sterile falcon tube containing LB broth. They were incubated in a shaker, shaking at 250 revolutions per minute (rpm) at 37°C for 12-15 hours.

4.2.4 Serial dilution of *E. coli* Samples and Plating

Freshly prepared *E. coli* samples were serially diluted in LB broth from 10^{-1} to 10^{-7} . For this purpose, bacterial colonies were serially diluted by adding 1ml of the original LB suspension containing *E. coli* to 9 ml of LB broth. The diluted sample was then vortexed and used to carry out another dilution until the desired dilution

was reached. The diluted samples were then plated on LB agar plate and incubated 37°C overnight. The schematic diagram indicating the dilution process. is shown in Figure 4.1.

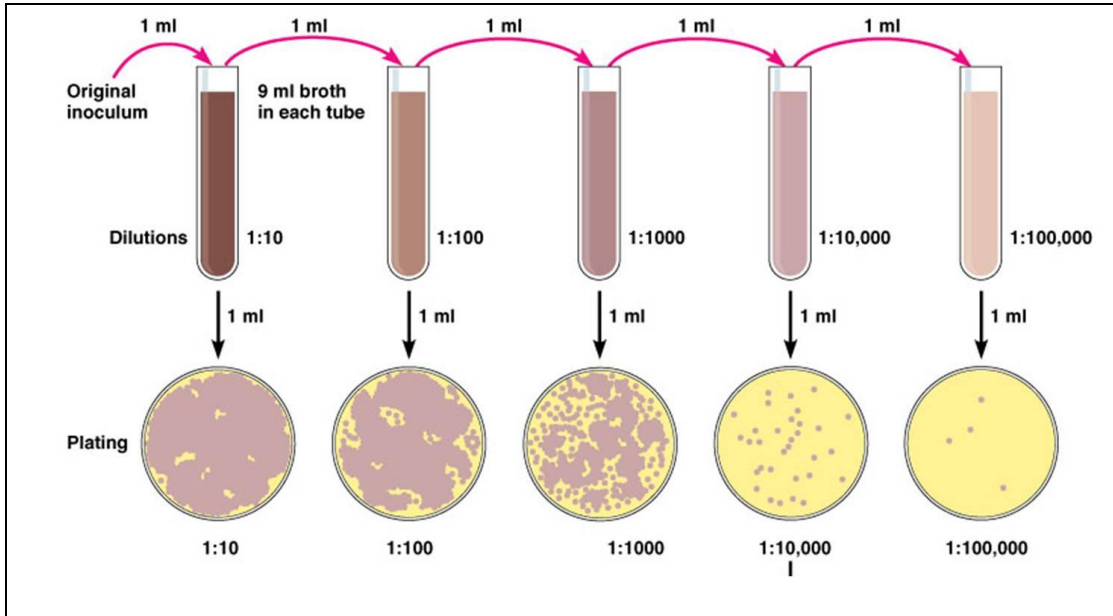


Figure 4.1. A representative figure of a serial dilution and plating. Bacterial colonies are serially diluted by adding 1 ml of the original samples to 9 ml of broth, the diluted sample is mixed well until the desired dilution is reached and incubated at 37°C

4.3 Experimental Plan

The experiment bacteria *E.coli* were placed in the middle of the two Helmholtz coil at different range of magnetic field 1 mT, 5 mT and 10mT. The magnetic field was time-varying and has a frequency of 50 Hz. The corresponding currents for these magnetic field intensities were 0.11 A, 0.62 A and 1.25 A respectively. The temperature inside the coil was kept at 37°C, which is the optimum temperature of the *E.coli* growth. The heat was provided by split unit plus electric light bulb and the temperature was measured using a digital thermometer, as in Figure 4.2. Similarly, another petridish of unexposed bacteria *E.coli* (control) is placed in Faradays' cage (Figure 3.2).

Experimental *E.coli* was exposed to magnetic field for a period time of 12 hours and the growth in the diameter of the selected colonies were recorded every two hours, as in Figure 4.3. The readings were made by taking three diameter measurements for each colony and the average of these three readings was recorded for the selected colony, as in Figure 4.4. In order to minimize the reading errors, six colonies were selected and after measuring the average diameter of each colony, the general average of diameter was calculated for six colonies.



Figure 4.2. Electromagnetic field exposure system the sample was placed at the midpoint of the two Helmholtz coil. Electric bulbs was used to keep the temperature at 37°C

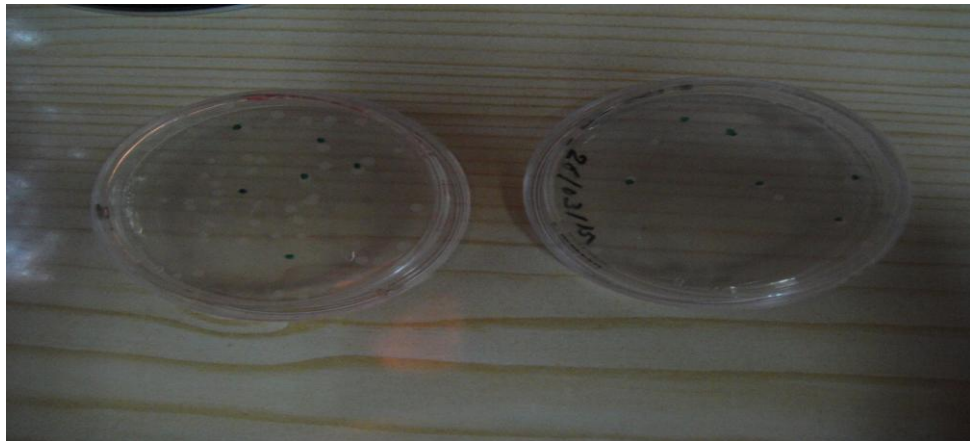


Figure 4.3. Selected colonies bacteria in two petridishes one was exposing to magnetic field and another to control, the *E.coli* dilution was (10^{-6}) cfu/ml

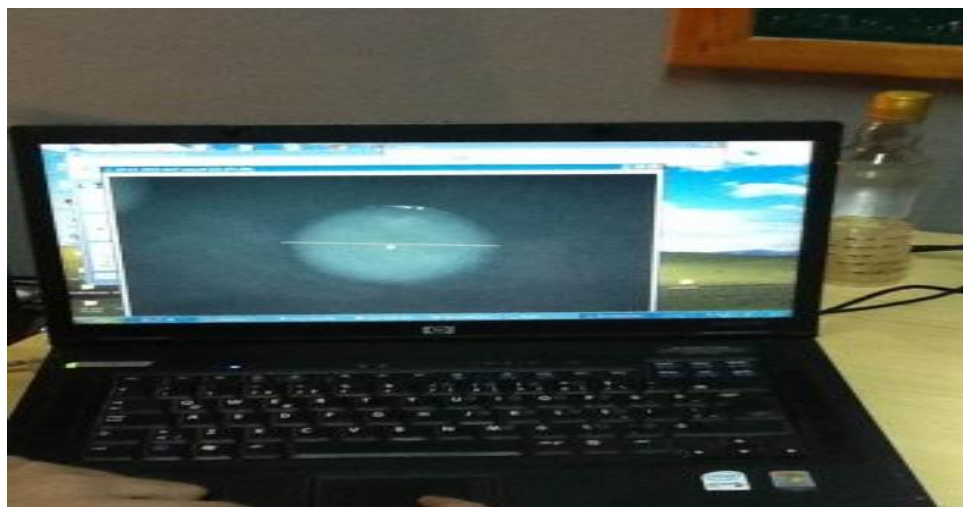


Figure 4.4. Measuring the growth in the diameter of selected colonies

4.4 Statistical Analysis

The measurement of the diameters of the experimental bacterial colonies has shown that; the samples to low frequency magnetic field were affected negatively in the sense that, their growth rate decreased in comparison to control samples. In order to evaluate statistical significance of differences recorded in the experiments performed with and without low frequency magnetic field, statistical significance level was evaluated by using **t- test** which given by

$$t = \frac{\bar{d} - \mu_D}{S_d / \sqrt{n}}, \quad (4.1)$$

where \bar{d} is the deviation means between control sample data and their corresponding EMF data; \sqrt{n} is the square root of the sample size; S_d is the standard deviation, μ_D is the test hypothesis. The statistical test where carried using different value of the magnetic field and the results are tabulated in the followings tables below.

In order to see the changes in the diameters, the measured diameters are normalized. This is done by dividing the diameter recorded every two hour D_i ($i= 0, 2, 4, 6, 8, 10, 12$) to the diameter recorded at the beginning of the experiment, where D_0 , is the diameter before starting the experiments.

Table 4.1. Results of experiment 1 were B=1 mT and I=0.11 A

N	EMF Di/ Do	Control Di/ Do	d_i	d_i^2
0	1	1	0	0
2	1.14	1.16	-0.02	0.4×10^{-3}
4	1.22	1.26	-0.04	1.6×10^{-3}
6	1.29	1.37	-0.08	6.4×10^{-3}
8	1.36	1.43	-0.07	4.9×10^{-3}
10	1.38	1.47	-0.09	8.1×10^{-3}
12	1.38	1.48	-0.1	10×10^{-3}

In order to compare the significance of the difference due to the two conditions under which the experiment was carried, the difference also called deviation between the value recorded for the control sample and the EMF sample was calculated for corresponding index (i) and denoted as d_i . The statistical test was then carried out based on d_i . The mean was,

$$\bar{d} = \frac{\sum_{i=1}^7 d_i}{n} = -0.057, \quad (4.2)$$

the variance was,

$$S_d^2 = \frac{1}{n(n-1)} \left[n \sum_{i=1}^7 d_i^2 - \left(\sum_{i=1}^7 d_i \right)^2 \right] = 1.432 \times 10^{-3}, \quad (4.3)$$

it follows that the standard deviation was $S_d = 0.0377$. The test statistical was then done using the formula (4.1) where $\mu_D = 0$ using the previous computed value, $t = -4$.

From the statistical **t**-distribution table, with sample size (n=7) and degrees of freedom $\nu=6$, the P-value corresponding to the computed **t** was a value in between 0.005 and 0.025, i.e.: $0.005 < \text{P-value} < 0.025$. By a simply linear interpolation, the exact value was P-value = 0.015. This P-value showed the no significance difference between the two conditions of the experiment is acceptable [19].

Table 4.2. Results of experiment 2 were B=5 mT and I=0.62 A

N	EMF Di/ Do	Control Di/ Do	d_i	d_i^2
0	1	1	0	0
2	1.06	1.09	-0.03	$0.9 \cdot 10^{-3}$
4	1.21	1.23	-0.02	$0.4 \cdot 10^{-3}$
6	1.26	1.35	-0.09	$8.1 \cdot 10^{-3}$
8	1.31	1.56	-0.25	$62.5 \cdot 10^{-3}$
12	1.3	1.56	-0.26	$67.6 \cdot 10^{-3}$

In order to compare the significance of the difference due to the two conditions under which the experiment was carried, the difference also called deviation between the value recorded for the control sample and the EMF sample, was computed for corresponding index (i) and denoted as d_i . The statistical test is then carried out based on d_i . The mean was,

$$\bar{d} = \frac{\sum_{i=1}^6 d_i}{n} = -0.1083, \quad (4.4)$$

the variance was,

$$S_d^2 = \frac{1}{n(n-1)} \left[n \sum_{i=1}^6 d_i^2 - \left(\sum_{i=1}^6 d_i \right)^2 \right] = 0.01380, \quad (4.5)$$

it follows that the standard deviation was $S_d = 0.1175$. The test statistical was then done using the formula (4.1) where $\mu_D = 0$ using the previous computed value, $t = -2.25$.

From the statistical t-distribution table, with sample size ($n=6$) and degrees of freedom $v=5$, the P-value corresponding to the computed t is a value in between 0.25 and 0.4, i.e.: $0.025 < \text{P-value} < 0.05$. By a simply linear interpolation, the exact value was $\text{P-value} = 0.039$. This P-value shows the no significance difference between the two conditions of the experiment is acceptable [19].

Table 4.3. Results of Experiment 3 were B=10 mT and I=1.25 A

N	EMF Di/ Do	Control Di/ Do	d_i	d_i^2
0	1	1	0	0
2	1.15	1.19	0.04	$1.6*10^{-3}$
4	1.36	1.41	0.05	$2.5*10^{-3}$
6	1.48	1.59	0.11	$12.1*10^{-3}$
8	1.58	1.71	0.13	$16.9*10^{-3}$
10	1.54	1.84	0.3	$90*10^{-3}$
12	1.55	1.92	0.37	$370*10^{-3}$

In order to compare the significance of the difference due to the two conditions under which the experiment was carried, the difference also called deviation between the value recorded for the control sample and the EMF sample was calculated for corresponding index (i) and denoted as d_i . The statistical test was then carried out based on d_i . The mean was,

$$\bar{d} = \frac{\sum_{i=1}^7 d_i}{n} = -0.142, \quad (4.6)$$

the variance was,

$$S_d^2 = \frac{1}{n(n-1)} \left[n \sum_{i=1}^7 d_i^2 - \left(\sum_{i=1}^7 d_i \right)^2 \right] = 0.05835, \quad (4.7)$$

it follows that the standard deviation was $S_d = 0.2415$.The test statistical was then done using the formula (4.1) where $\mu_D = 0$ using the previous computed value, $t = -1.55$,

From the statistical **t**-distribution table, with sample size ($n=7$) and degrees of freedom $\nu=6$, the P-value corresponding to the computed **t** was a value in between 0.01 and 0.025, i.e.: $0.05 < \text{P-value} < 0.1$. By a simply linear interpolation, the exact value is $\text{P-value} = 0.08$. This P-value showed the no significance difference between the two conditions of the experiment is acceptable [19].

The calculations revealed that the probability level is found to be ($p < 0.1$), which is meant that the probability levels of our experiment is acceptable [19].

4.5 Results and Discussion

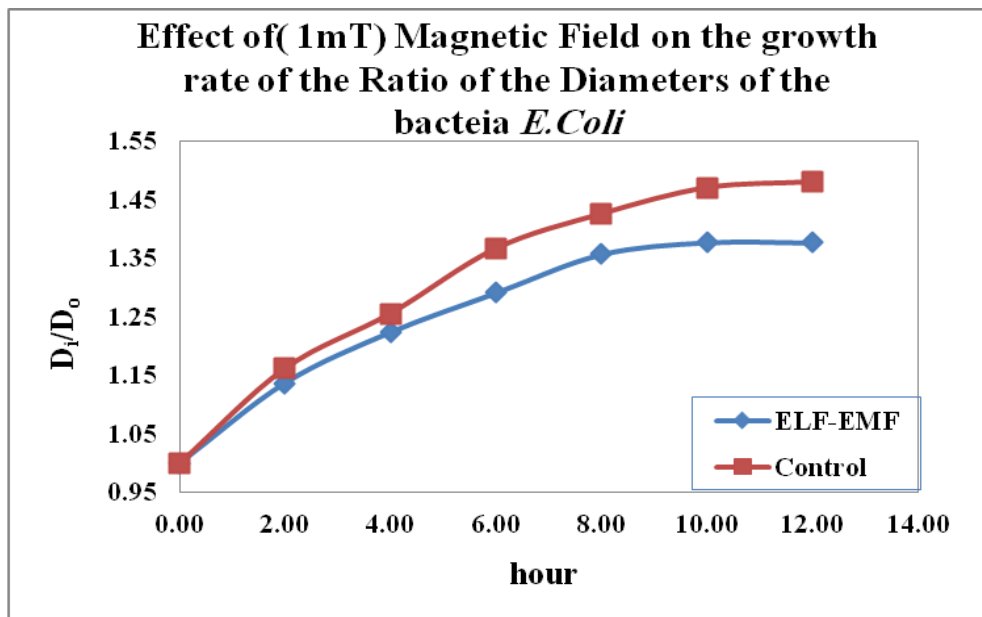


Figure 4.5. Effect of 1mT magnetic fields on the growth rate of the ratio of the diameters of the bacteria *E.coli*, it is clear from the graph that the growth rate of the exposed sample to low frequency magnetic field affected negatively

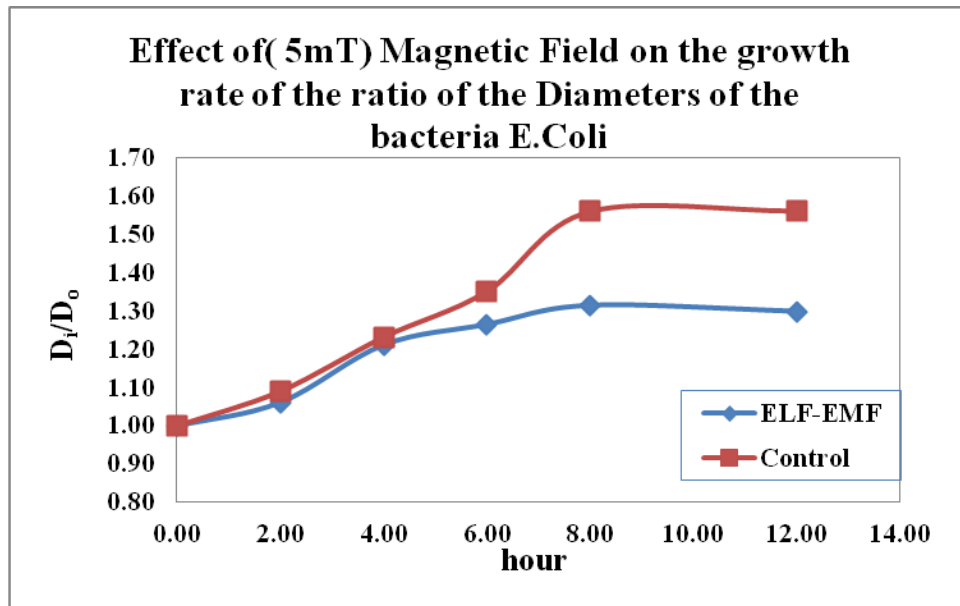


Figure 4.6. Effect of 5mT magnetic field on the growth rate of the ratio of the diameters of the bacteria *E.coli*, it is clear from the graph that the growth rate of the exposed sample to low frequency magnetic field affected negatively

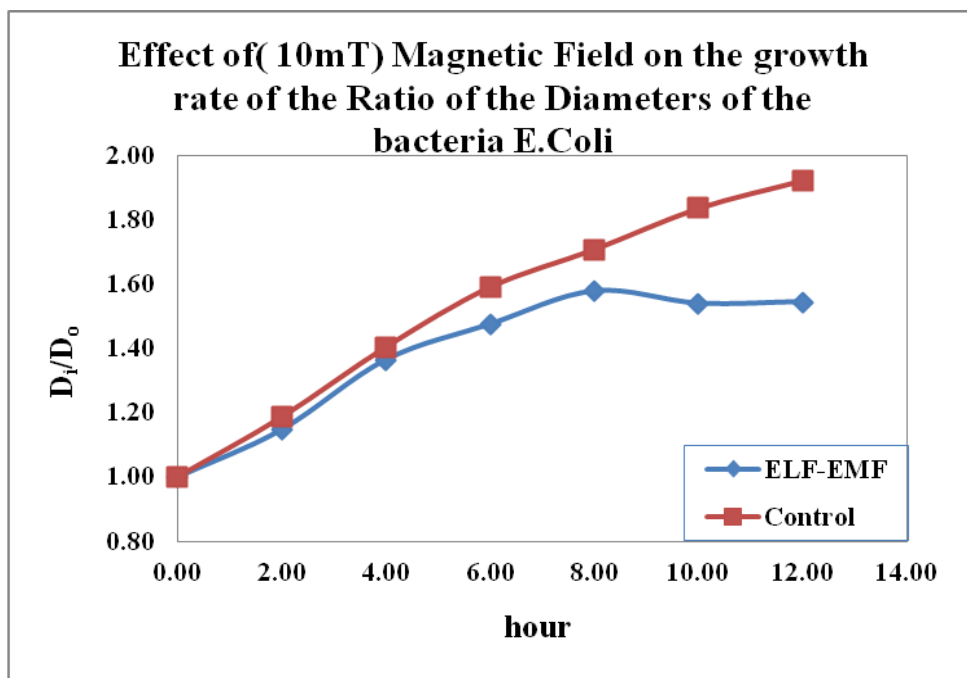


Figure 4.7. Effect of 10mT magnetic field on the growth rate of the ratio of the diameters of the bacteria *E.coli*, it is clear from the graph that the growth rate of the exposed sample to low frequency magnetic field affected negatively

From the figures we observed that for the first 4 hours, there is no significant change in the growth rate. Irrespective of the intensity of the magnetic field, the growth rate is almost parallel for the control samples and the samples exposed to low frequency magnetic field. However, between 4-8 hours period, there is a dramatic change in the growth rate. For the remaining periods 8-12 hours again no significant change is observed on the growth rate. In some research results, it has been reported that when the living organism like *E.coli* is exposed to low frequency magnetic field, the bacteria gets stressed and tries to adopt itself to this unusual condition. During this period the bacteria response is in the form of an increasing heat shock protein (hsp). This conclusion is first reported in Del et al [20].

In this thesis, we can explain the decrease in the growth rate of the bacteria as a consequence of an increasing heat shock protein, as was reported in [20].

Chapter 5

DROSOPHILA MELANOGASTER

5.1 The Effect of Extremely Low Frequency Electromagnetic Fields on the Fecundity of *Drosophila Flies*

Many epidemiological studies performed to explain the health hazards may have result from exposure to extremely low frequency electromagnetic fields [1]. Previous studies on *Drosophila melanogaster* flies have mainly concerned the response of insects to low frequency magnetic fields and different result have been obtained, Ramirez et al. observed that the egg production of *Drosophila* decreased by exposure to pulsed extremely low frequency 100 Hz, 1.76 mT and sinusoidal fields 50 Hz, 1 mT [21]. In comparisons, Tipping et al. was reported that 50 Hz 8 mT electromagnetic fields exposure did not have any effect on egg production when exposed to the third instars larvae of *Drosophila melanogaster* [22]. In a similar work, Walters and Carstensen accounted that 60 Hz magnetic field did not affect eggs production of *Drosophila* flies [23].

In this thesis, we have investigated reproductive capacity of the *Drosophila* flies, as this fly is so small and including a short life cycle, we separated the flies into two groups, the exposed group 1, two of the tubes contained 10 virgin females and 10 males with 10 ml standard medium food at the bottom of each tube. The tubes were closed with cotton. Similarly, the unexposed group 2, two tubes contained 10 virgin

females and 10 males with 10 ml standard medium food at the bottom of each tube, the tubes were closed with cotton, as shown in Figure 5.1.

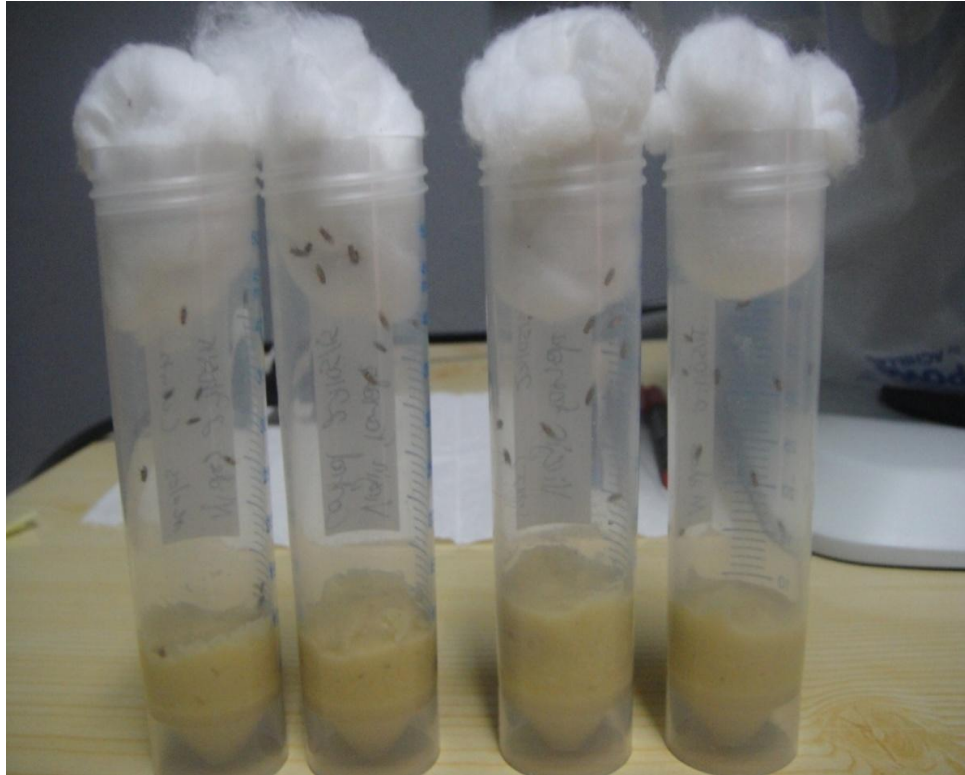


Figure 5.1. Four tubes of *Drosophila melanogaster* flies, right two tubes subjected to low frequency magnetic field 10mT and another two tubes were put in control

5.2 Preparation *Drosophila* Medium Food

The medium food consists of 4 gram agar, 13 gram yeast, 16 gram sugar, 25 gram tomato pulp, 32 g rice flour, and 450 ml distilled water. This mixture was sterilized for over 10 minutes boiling procedure. This was preserved by the addition of 2 ml of prop ionic acid and 2 ml ethanol. The food had been prepared and kept in room temperature conditions before being added into sterilized tubes. The food formed a 10 ml at the bottom of each tube.

Finally, all the tubes with food and *drosophila* flies were kept at room temperatures $22 \pm 2^\circ\text{C}$.

5.3 Experimental Plan

The two tubes of the exposed *Drosophila melanogaster* were put in the middle of the two Helmholtz coil at intensity field range of 10 mT and single frequency 50 Hz, the corresponding currents were 1.25 A at 22°C room temperature. The *Drosophila* flies were exposed to magnetic field for a period of 4 hours.

At the same time, two tubes of the unexposed *Drosophila* flies were put in the Faradays cage in the same temperature conditions. After 4 hours. Flies were (passive voice) very lightly with ether and mixed exposed 9 females (one dead) with exposed 10 male's flies and put them in other tube with fresh food. Similarly the unexposed 8 females (one escaped and one dead) with unexposed 10 male's flies, we put together in other tube with fresh food .After an exposure period time of 24 hours, eggs were laid kept in the rearing room, after the end 7 days they can be clearly seen and easily to count pupa.

5.4 Statistical Analysis

In the Table 5.1 indicate that the difference between exposed groups and control group was determined during the experiment by statistically significant percentage at the end of the experiment was about 20% [19]. The computed percentage based on the ratio between the magnetic field exposed *Drosophila* flies and the control *Drosophila* flies.

Table 5.1. Effect of magnetic field at single frequency 50Hz and intensity 10 mT follows of exposed group and unexposed group at 4 hours on fecundity *Drosophila* flies

Name of system	At 0 hours		After 4 hours		After 7day
	Number of females	Number of males	Number of females	Number of males	Number of Pupa
ELF-EMF	10	10	9	10	120
Control	10	10	8	10	151

5.5 Result and Discussion

The number of pupa was counted and compared the number of pupa obtained of exposed flies group decreased with respect to the number of pupa was obtained of unexposed flies group (control), as shown in Figure 5.2. This results indicates that magnetic field causes stress in *Drosophila* flies cells and create electric current inside the body, this current may cause a damage to the cellular DNA of *Drosophila melanogaster* flies and the number of pupa decreased in magnetic field compared with control ones. As well, previous experiments had shown that a few minutes of daily exposure were enough to produce a significant effect on the fecundity *Drosophila* flies, [9, 21, and 24]. Finally, many factors affect of *Drosophila* reproductive capacity such as (temperature, dryness, food, population density, prior anesthesia etc.) and internal factors (genetic structure, age etc) [25, 26, and 27].

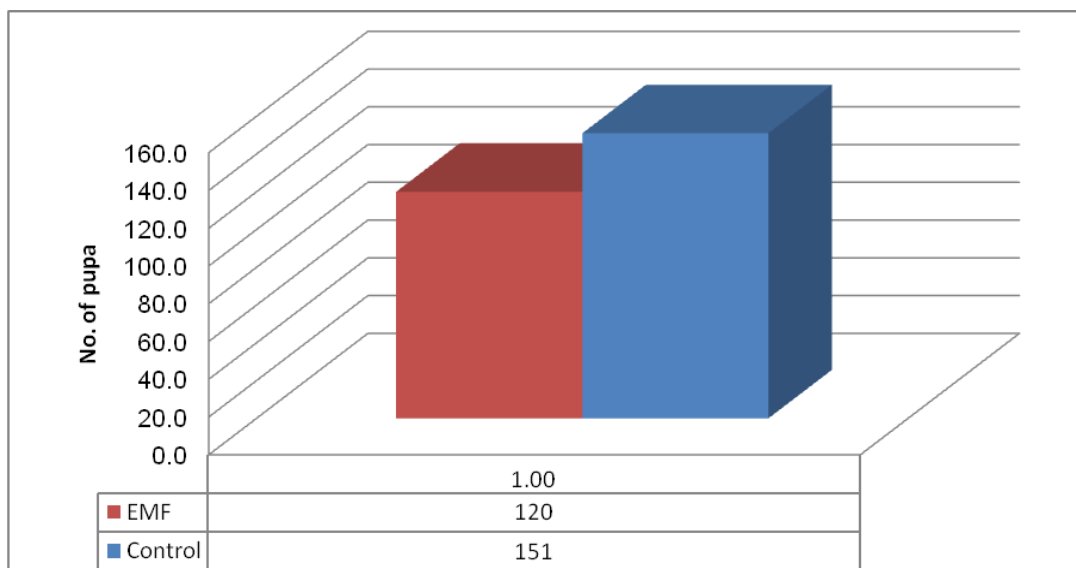


Figure 5.2. Number of pupa under EMF and control

Chapter 6

CONCLUSION

In this thesis, we have investigated the effect of low frequency magnetic fields when it is exposed to structurally different cell types namely prokaryotic and eukaryotic species. As a prokaryote cell type, the bacteria *E.coli* (ATCC 25922) was used on the other hand, *Drosophila melanogaster* (fruit flies) was used as a eukaryote sample.

We have observed from the graphs that, for the first 4 hours the exposure samples and the control samples grow parallel. However, after 4 hours the growth rate decrease in the exposed samples as compared to control, suggesting that when the bacteria is exposed to low frequency magnetic field, bacteria gets stressed and the bacteria tries to adopt itself to this new situations by increasing the rate of the heat shock proteins (hsp). This affects its growth rate.

The second experiment in this thesis, is to use *Drosophila melanogaster* as a eukaryote sample, and investigate the effects of the 10 mT magnetic field intensity on the fecundity of *Drosophila melanogaster*. Under the experimental conditions, it was determined that there was a general decreased in pupa production of the exposed *Drosophila* flies groups compared to control *Drosophila* flies groups.

This means that exposure to magnetic field directly or indirectly causes stress and may a damaged to cellular DNA of the exposed *Drosophila* flies. Consequently, if a biological system is exposed to low frequency magnetic field for a long period of time, it may experience some hazards on its physiology.

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