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PHYSICOCHEMICAL A PRELIMINARY STUDY OF CONTROL ESCHERICHIA COLI GROWTH VIA THE EXTREMELY LOW - FREQUENCY ELECTROMAGNETIC FIELDS.

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ABSTRACT: *Effects of low frequency electromagnetic fields (LF-EMFs) on Escherichia coli (E.coli) in the river water and riverbank filtration (RBF). The column of the sample is in each of coil with the number of turn was 100 of 1.5mm copper wires induced the magnetic fields with inductions 1-10mT. Duration of exposure varied up to 4 hour and exposure took place at laboratory temperature (28–30 °C) at the place of the sample. This study focused on the removal of most probable number (MPN) of E.coli in river water after using the LF-EMF column model. Water samples used for this study were collected from Sungai Kerian and tube well, Lubok Buntar Perak (Coordinates: 5° 7'37.60"N, 100°35'42.97"E) during dry and wet season. From this study, the concentrations of E.coli growth has shown significant decrease of 49.77% when the samples were exposed to an LF-EMF with column waveform of 10 mT amplitude with a frequency of 50 Hz. Thus, the results from this study proved that the utilization of LF-EMF is able to decrease the concentrations of E.coli, and contributes in slowing down their growth.*

Keywords: Electromagnetic field, extremely low-frequency, E.coli

1. INTRODUCTION

Electromagnetic fields (EMF) become an important to find out the environmental health impact in recently last years. Much attention has been paid to the influence of EMF on biological and bacterial cells effect. The extremely-low frequency electromagnetic fields (ELE-EMFs) are accepted can exert influence on biological functions of cells and bacteria morphology. A different type of EMF from microwaves to static magnetic field effects has been carried out concerning in many of several studies. The different of biological effects has been detect in such as morphological effect on two different bacterial strains after low frequency magnetic field exposure [1-3] or microwave exposure [4-6].

An electromagnetic technology is currently implemented in various ways through the application of either permanent magnets or ELF-EMF in combination with magnetic seeding, magnetic adsorption, or an electromagnetic device [7]. The different approaches to EMF applications have significantly different effects on the performance of each system and the permanent EMF use is normally creates a uniform EMF [8]. However, the field can be varied by changing the arrangement and orientation of the EMF. According to Zaidi et al., (2014) the different shape of permanent magnet can also exhibit different magnetic field and if an EMF device is used, the dynamic magnetic field is generally obtained [9]. Hence, this article also presents evidence of how various implementations of ELF-EMF field effect to *E.coli* growth.

The implementation of magnetic fields currently is a method of supplementary therapy and has gained extensive attraction in medicine and other fields [10]. EMF was recently acknowledge as a stimulator in biological wastewater treatment [11]. However, despite the extensive review there is still insufficient information related to the mechanisms and biological effects of EMF [12[10]-14]. Magnetic fields can cause biological effects and are accordingly classified as weak (<1 mT), moderate (1 mT–1 T), strong (1–5 T) and ultra-strong (>5 T) [12].

At present, there are many information regarding to possible effects of ELF-EMFs on *E.coli*. The effects of electromagnetic field 50 Hz frequency and strength 2 mT on each of growth characteristics and pathogenicity of *E.coli* cells are decrease in the cell growth and mortality rate recorded after injection with *E.coli* suspension exposed to 6 hour was 40%, but after an exposure period of 16 hour it was 80% [13]. Segatore et al., (2012) found that the exposure of E. coli to EMF-EMF (2mT; 50Hz) at 4, 6, and 8 hours of incubation the number of cells was significantly decreased in bacteria exposed to EMF when compared with the control [14].

Future more, it has been observed that *E.coli* exposure to a constant intensity EMF (0.5-2mT) for 20min exposure time has decrease in the cell growth and viability [15]. The aim of the present work to investigate the removal of the most probable number (MPN) of *E.coli* in river water exposed to an ELF-EMF with a column waveform of 2mT amplitude and frequency of 50 Hz.

2. MATERIALS AND METHODS

Study Area. Sungai Kerian is a main tributary of Kerian river basin in Perak. The river is providing potable water for residents of this area. This river originates from the hilly headwaters in Mahang, Kedah, and flows through Lubok Buntar area and down to Kerian Valley in Parit Buntar, Perak, then continues westward to Malacca Strait [16]. This river is also the main body of water that acts as a boundary between the states of Perak, Kedah, and Penang. On this study, this area is situated in Lubok Buntar, Kedah and the area is surrounded by oil palm and pineapple plantations. The villages and the housing communities are also located around this area. The location of study area is shows in Fig (1).

Fig (1) Study area. Location of Sungai Kerian, Lubok Buntar, Perak.

Electromagnetic. Magnetic fields were generated in a column coil. A magnetic field power generating system is transforming the magnetic field in the coil. The maximal effective current was 2A, the frequency 50 Hz and the other parameters given in Table (1). The exposure system consisted of an apparatus containing a pair of solenoid coils, a waveform generator, and a current amplifier. For exposure, the samples were placed in the column where uniformity of the magnetic field is optimal. The magnetic field distribution inside the coil for different current values see Fig. 2. Magnetic for the magnetic field generation pair of solenoid coils is employed, with a mean radius of 80mm. In each coil the number of turns was 100 with a 2 mm copper wire giving a resulting resistance of 1 Ω and an inductance of 39 ± 1 mH. The arrangement of the experiment is shown in Fig (2).

Table (1) The parameter of ELF-EMF treatment system Generator.

Diameter of column	80mm
Length of column	500mm
Number of turns	100
Diameter of wires	2mm

The sample of E.coli is taken from the river water and that sample is retained in the column with maximum time 2 hour expose (8cm in diameter and 50cm of length the column). The generator was able to generate an effective magnetic field in the range 0–2 mT, with a sinusoidal wave of frequency of 50 Hz. The magnetic flux density (B) at the center of the column coils is measured with magnetic sensor and B was adjusted by varying the coil current. The calculation of magnetic field density at the center of the column is according to Faraday law and Newton's law. The ELF_EMF treatment system shown in Figure 1, it is connected to Data Acquisition Box (DAQ) with USB connection to computer for monitoring and measuring the EMF. The current was supplied to the coli and magnetic field was induced on the column. The frequency and flux density of the sinusoidal EMF were maintained at 50 Hz and 2 mT, respectively. The intensity of magnetic field is calculated by the Biot-Savart Law.

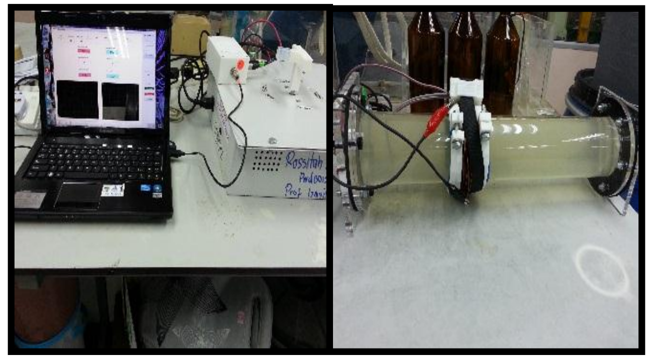

Fig (2) The ELF-EMF treatment system of E. coli (50 Hz of frequency and 0-2mTof intensities). The system is connected to the computer by a data acquisition box (DAQ) to measuring the EMF. The sample of water retained in the column with maximum time 2-hour expose in 80mm diameter and 500mm of length.

Sampling. Water samples were taken from Kerian River twice per week (Monday and Tuesday) start on June 20, 2016, to July 25, 2016. Water samples were collected, transported, and stored in strict accordance with the guide - lines described by Standard Methods [17]. Sterile glass flasks were used to collect the samples [18]. These samples were preserved in accordance with water and wastewater standards and then stored at a temperature of less than 4 °C. The laboratory apparatus used in this study were prewashed with 5% nitric acid (HNO_3) and rinsed with deionized water prior to testing.

IDEXX Colilert-18® /Quanti-Tray 2000. The IDEXX most probable number (MPN) test was performed according to procedures recommended in the IDEXX package insert. The chemical reaction of this test is based on Defined Substrate Technology™ (DST). Chemical sub- state containing 4-methyl-umbelliferyl β-D-glucuronide (MUG) was added to a 100-ml sample of Kerian River water, mixed, poured into a multi-well tray, and sealed. Quality control was run daily to validate test performance and included both positive (E. coli ATCC 25922) and negative (Pseudomonas aeruginosin ATC 27853) controls. *E.coli* possesses the enzyme β-glucuronidase, which metabolizes MUG, releasing the 4-methyl-umbelliferyl dye. This dye has the ability to fluoresce under long wave ultra-violet light (365 nm). After incubating for 24 hours at 35°C, the samples were examined for fluorescence. An MPN table provided with the kit converts the number of wells producing fluorescence to MPN/100 ml. This experiment was repeated three times over different samples and the results are expressed as means ± standard error at a given time point. The mean values and the variances obtained for the control and the treated samples at each time point were compared by using the ANOVA method. The result was considered with a statistical significance at $P<0.05$.

3. RESULTS AND DISCUSSION

Growth curves — dependence on the duration of exposure. In the first set of experiments, sample *E.coli* of the river water was exposed on the column to alternating magnetic fields with an amplitude of 2 mT and oscillating at a frequency of 50 Hz. At vertical column EMF exposed point investigated (Table 2), no significant variation was observed in the *E.coli* concentration. The Number of MPN/100ml decreases only 1% with 2 hours ELF-EMF exposure as shown in Fig (3).

Table (2) *E. coli* count discrepancies noted IDEXX Colilert-18® for vertical column of ELF-EMF.

Sample	Expose Time (min)	Colilert-18® MPN/100 ml
Raw water	0	1732
V_1	20	1413
V_2	40	1732
V_3	60	1986
V_4	80	1299
V_5	100	1299
V_6	120	1299
Prcentages of removal		**1%**

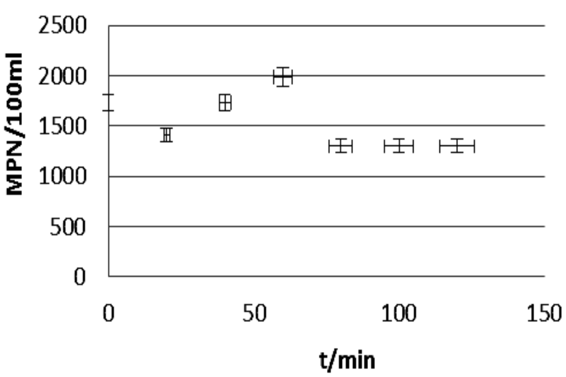

Fig (3) The dependence of vertical sample for the most probable number on the time of exposure. The magnetic fields were turn off in the t= 120 min.

In the second set of experiments a sample was exposed in the horizontal column (50 Hz; 2 mT). The duration of exposure varied in the range of 0 – 2 hour. In 7 of the sample, the IDEXX method exceeded the threshold (E. coli 866.4 MPN/100 ml in the raw water sample) and would have resulted in a treated sample H1 to H6. From the observation, a decrease of *E.coli* most probable number (MPN/100ml) for exposed samples has been found and presented in Table 3. Exposed samples are to higher of concentration values in raw water from Kerian River. In this case, after 2 h of EMF expose from the beginning of the treatment, a significant decrease in the percentage of concentration MPN/100ml. Moreover, after 2 h of exposure to the magnetic field, the percentage of concentration was significantly 49.77% decreased than that of the control (Fig. 4).

Table (3) E. coli count discrepancies noted IDEXX Colilert-18® for horizontal column of ELF-EMF.

Sample	Time Expose	Colilert-18® MPN/100 ml
Raw Water	0	866.4
H1	20	816.4
H2	40	686.7
H3	60	579.4
H4	80	488.4
H5	100	461.1
H6	120	435.2
Percentage of removal		**49.77%**

Hence, a significant decreased in the *E.coli* removal was also recorded during the ELF-EMF treatment. In order to examine a possible long-term effect of ELF-EMFs on E. coli, samples were diluted in 1:10 of distilling water. The decision to dilute was based on historical water quality data and the necessity to reduce the concentration of *E.coli* in raw water to achieve a countable result within the confines of this method.

From ANOVA analysis, it was proved that the ELF-EMF significantly remove the concentrations of E.coli ($p<0.05$). As shown in Figure 4, the concentration of E.coli resulted significantly decrease with an 866.4 MPN and a 435.2 MPN resulted significantly remove the treated sample with respect to the raw water. In order to investigate if 2mT is the minimum intensity value of the EMF wave able to effects on *E.coli* growth. Therefore, the ELF-EMFs can affect the *E.coli* growth by decreasing the percentage of concentration and control the growth rate of E.coli but these effects depend on waveform and amplitude of the applied magnetic field. The sine and square waves are similar since the amplitude (2 mT) and the frequency (50 Hz) are the same. According to the literature study, the oscillating magnetic field affects different bacterial strains in lag-phase of their growth [2]. This fact was found using CFU counting technique [19], different biological effects on bacterial cell proliferation [20] and growth rate and morphological changes for both Gram-negative and Gram-positive bacteria [21]. Morphology of *E.coli* are not evaluated in this preliminary study but in future investigations, it will be interesting to study the magnetic field morphological effect on E.coli. Fojt et al., (2009) had reported that bacteria that no any changes in bacterial morphology after magnetic field treatment. Therefore, more focuses on possible mechanisms of the magnetic field acting in the next experiments. There are a lot of theories that try to explain acting of magnetic fields on E.coli [22].

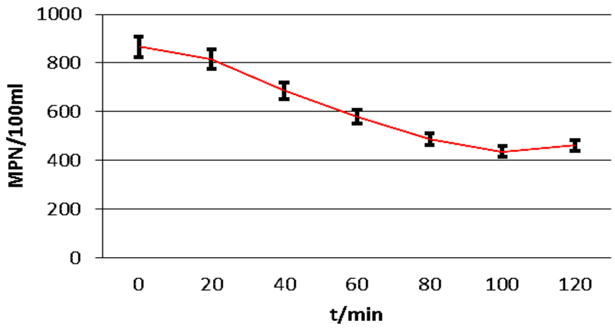

Fig (4) The dependence of horizontal sample for the raw water and treated sample. The magnetic fields were turn off in the t= 120 min. MPN/100ml of concentrations of *E.coli* versus time expose: *P<0.05 (one-way Anova, Student-Newman-Keulstest).

4. CONCLUSIONS

In this research, the measurable effects of magnetic on *E.coli* in the river water has identity. The ELF-EMF decreasing the concentrations of *E.coli*, and slow down their growth. It was demonstrated that the ELF-EMF have affected the *E.coli* growth after 2h, 50 Hz, and 2mT magnetic field exposure. From the results, it can be confirm that the magnetic fields kill a part of *E.coli* exposed in the river water.

5. ACKNOWLEDGEMENTS

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