EVALUATION OF PROTECTIVE PROPERTIES OF BIOTEXTILE WITH INCORPORATED AMBER NANO/MICRO PARTICLES AGAINST THE LOW-FREQUENCY ELECTROMAGNETIC FIELDS (ELF-EMF)

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Abstract: The impact of extremely low frequency electromagnetic field (ELF-EMF) on living organisms and the identification of their threshold levels remain uncertain. Amber nanoparticles and microparticles are being explored as potential raw materials used for production of innovative biotextiles. This study aimed to develop methods for assessing the protective properties of biotextiles against LF-EMF including testing the influence of a novel biotextile with incorporated amber particles on growth parameters of model bacteria and fungi species.

The assessment of biotextile properties is based on comparison of mycelial growth in Petri dishes of fungi *Chaetomium globosum* Kunze: Fries (ATCC^{*} 6205TM), and the growth rates of bacteria *Enterococcus faecalis* (Andrewes and Horder 1906) Schleifer and Kilpper-Bälz 1984 (NCTC 12697) and *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919 (AL) (NCTC 12241), grown in flasks wrapped and unwrapped in fabrics with incorporated amber particles under LF-EMF exposure. While no significant differences were observed in the growth rate of *E. faecalis* under LF-EMF exposure, regardless of fabric wrapping, the biotextile with amber particles demonstrated a statistically significant protective effect (P < 0.01) inducing growth rate changes in *E. coli*.

Key words: succinate, mycelial growth, *Chaetomium globosum*, bacterial growth, optical density, flow cytometry, *Escherichia coli* and *Enterococcus faecalis*

Introduction

The general aim of the research projects "Innovative multifunctional bio-textile, integrated with silica dioxide and succinate development, and its impact on biosystems" and "3D Biotextile with Technological Composition of nano particles to enhance

the protecting properties" was to determine protective properties of newly developed innovative biotextile with incorporated amber nano and micro particles.

Modern cities present a growing concern: a complex of environmental factors cause negative impact on human health. Increasing ultraviolet radiation, amplified by contemporary architecture, enhansment of electromagnetic radiation including low-frequency electromagnetic fields, and a multitude of chemical pollutants all contribute to this global social problem (Duhaini, 2016). Ubiquitous electrical devices, from power lines to everyday appliances, create electromagnetic fields documented to potentially influence biological processes (Strasak et al., 1998; Panagopoulos et al., 2002; Grassi et al., 2004). Public anxieties have risen around low-frequency electromagnetic fields (LF-EMF), becoming the most common reasons increasing magnetic fields density in our environment (caused by alternating current below 300 Hz), due to their potential health risks (Feyyaz & Kargi, 2011). Research on the effects of these fields on living organisms, including bacteria (Strašák et al., 2002; Strašák, 2005; Cellini et al., 2008; Masood et al., 2020; Salmen et al., 2018), has seen a recent surge.

One promising solution to mitigate these negative urban environmental impacts lies in the development of innovative biotextiles with protective properties (Grauda, et al., 2023). Researchers are exploring the potential of textiles to shield against extremely low frequency electromagnetic field (ELF-EMF), thereby contributing to improved human health (Ziaja et al., 2008). Baltic Amber (*succinite*, CAS 9000-02-6), a natural polymer resin material with potential protective properties, is under investigation as a raw material that could be applied for designation of novel biotextiles. This study focuses on the assessment of protective properties of such biotextiles incorporating amber nano and microparticles aiming to establish convenient methods for evaluation of shielding effects against LF-EMF. The research employs microscopic cellulose degrading fungi (*Chaetomium globosum*) and bacteria (*Escherichia coli* and *Enterococcus faecalis*) as test organisms.

The aim of this study was to adapt methods and techniques for determination of protective properties of biotextiles against electromagnetic field looking for the ways to make evaluation of the protective properties of innovative biotextiles most relevant and cost-effective. Following these considerations microscopic cellulose degrading fungi *Chametomium globosum* and bacteria *Esherichia coli* and *Enterococcus faecalis* were chosen as test objects.

Materials and methods

Designation of the System for Assessment of the Effects of Magnetic Field on Test Objects Exposured to Low Frequency Electromagnetic Field

A Helmholtz coil was employed to generate a constant 50 Hz LF-EMF (Figure 1, 2). The coil's parameters were R = 25 cm, distance between coils = 25 cm, number of turns = 189. This configuration ensures uniform magnetic field (MF) intensity at the coil's center. The regulation of magnetic field intensity (0-725 μ T) generated by LF-EMF was

controlled by adjusting the electric current and mesaured using. Narda model 8532-60 device. For this study, a moderately high MF intensity of 518 μ T was selected, considering existing MF intensity standards and potential health impacts.

Description of textiles

Two fabric types were used in the experiments:

- Control: Twill Code T564, warp Linen yarn 28 Tex, weft Cotton yarn 20 Tex (11.02.20), 320±60 g/m²; weaving textile material was developed by A Grupe, JSC (Jonava, LT).
- Biotextile: Twill Code T561, warp Linen yarn 28 Tex, weft Cotton yarn 20 Tex, Amber fiber 7.8 Tex (11.02.20), 330±60 g/m²; weaving textile material was developed by A Grupe, JSC (Jonava, LT).

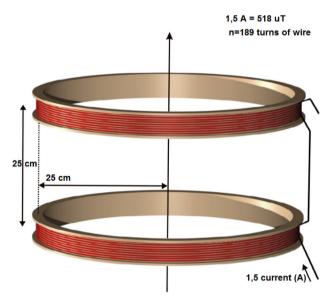


Figure 1. Helmholtz coil scheme.



Figure 2. Helmholtz coil and MF intensity measuring equipment – Narda, model 8532-60.

Microorganism cultures used in expirements

The strain *Chaetomium globosum* Kunze: Fries (ATCC* 6205^{TM}), obtained from the company Microbiologics (US) in form of Kwik Stick, stored at 5 ± 3 °C were used in experiments. The *C. globosum* strain ATCC* 6205^{TM} is the licensed derivative of original stock cultures obtained exclusively from the American Type Culture Collection (ATCC*). The *C. globosum* was originally isolated from cotton fabric in 1933 and is therefore suitable for testing biotextiles as representative of a common strain environment (Government of Canada 2018). Selected fungal strain were maintained on potato dextrose agar (PDA) medium (SIFIN, Berlin) and were grown on PDA for ten days before being used in the study.

Bacterial strains gram – negative *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919 (AL) (NCTC 12241) and gram – positive *Enterococcus faecalis* (Andrewes and Horder 1906) Schleifer and Kilpper-Bälz 1984 (NCTC 12697), were obtained from TCS Biosciences Ltd, in form of culture discs. These cultures are the first-generation derivative of original stock cultures obtained exclusively from the National Collection of Type Cultures (NCTC^{*}). Culture discs were stored at 5 ± 3 °C. Before use in experiments, culture discs were removed from vials by sterile forceps, then culture disc inoculate on the Tryptic soy agar (TSA) and incubate under optimum conditions for 3 h.

Determination of fabric's ability to retain electromagnetic field impact on mycelial growth

The radial growth method was used to assess the impact of EMF on fungal mycelial growth. Agar plugs (5×5 mm) from pure fungal cultures were placed in the center of 90 mm Petri dishes with PDA.

Six experimental variants were set up:

- **Control:** Uncovered, wrapped in linen/cotton fabric, or wrapped in biotextile No CL561, all non-exposed to EMF.
- **EMF-Treated:** Uncovered, wrapped in linen fabric, or wrapped in biotextile No CL561, all exposed to EMF growing test organisms in Petri dishes placed in the center of the Helmholtz coil.

Each variant was replicated six times. Inoculated Petri dishes were incubated at 24 ± 2 °C. Mycelial growth was measured daily for ten days or until the fungus reached the dish edge. Mycelial growth was compared between control and EMF-treated groups and expressed as a percentage in comparison to control growth.

Bacterial Growth Assay

Bacterial cultures were re-inoculated onto fresh TSA and incubated at 37 °C for 18 hours. A single colony was sub-cultured in 100 ml of TSB and incubated at 37 °C for 2 hours. The optical density was adjusted to 0.1 MFa at 565 nm to obtain 1×10^{6} CFU/ml.

Two sets of experiments were conducted, each with three variants: uncovered tubes, tubes wrapped in linen/cotton fabric, and tubes wrapped in biotextile encompassing amber particles. Each variant was replicated six times. One set was exposed to the magnetic field, while the other served as a control. Experiments were performed at room temperature $(24 \pm 2 \text{ °C})$.

Bacterial growth rate was determined by measuring optical density at 565 nm after 16, 24, and 48 hours of EMF exposure. Morphological changes of bacteria have been studied using light microscopy; the smear method and stained with MGG Quick Stain kit (Bio-Optica Milano s.p.a).

Flow Cytometry

Flow cytometry was employed to assess cell count and reaction changes. Bacterial suspensions were filtered and stained with propidium iodide. A BD FACSJazz[®] cell sorter was used to measure relative fluorescence.

Following the methods outlined by Grauda et al. (2015), 2 mL of lysis buffer was added to cytometer tubes. After 16, 24, and 48 hours of incubation, bacterial cultures were homogenized by vortexing. 100 μ L of the bacterial suspension was filtered through a 40 μ m flow cytometry filter and added to the lysis buffer. To induce cell fluorescence, 10 μ L of propidium iodide was added.

A BD FACSJazz^{*} cell sorter (BD Biosciences, USA) was used to measure the relative fluorescence of bacterial cells. The device was equipped with a 100 µm nozzle and used phosphate-buffered saline (PBS) as a sheath fluid. Cell counting events were triggered by forward-scattered light. A 488 nm blue laser excited cell fluorescence, and emission was measured at 585 nm (bandwidth 29 nm). Before measurements, the flow cytometer was calibrated using Sphero[™] rainbow calibration particles (3.0 µm, BD Biosciences, USA) in PBS. A coefficient of variation (CV) below 3 % was considered successful calibration.

The intensity of bacterial relative fluorescence was expressed in arbitrary logarithmic units. For each subsample, events were recorded over a fixed 5-minute period.

Statistical methods

The means of colony diameters were separated using Least Significance Different Test, at P < 0.05. Analytical statistical methods were performed with the R program version 4.2.0.

Results and discussion

In vitro mycelial growth assay

We assessed the impact of ELF-EMF exposure on the mycelial growth of *C. globosum* by comparing growth between control (non-exposed) and exposed variants. Two days after ELF-EMF exposure, a significant (P < 0.01) stimulation of mycelial growth was observed in the unwrapped variant, with a 119.51 % increase compared to the control (Table 1). In all variants where Petri dishes with *C. globosum* were wrapped in linen fabric or biotextile with amber particles, mycelial growth stimulation was limited to 9.72 % and 7.81 %, respectively.

As shown in the unwrapped variant, the growth stimulating effect of ELF-EMF is most pronounced two days post-exposure, followed by a decline on the effect at third day. Five days after ELF-EMF exposure, a slight growth increase (103.52 % to 104.92 %) was observed in unwrapped group, but this was not significantly different (P < 0.05) from

the fabric-wrapped variants. After seven days, mycelial growth was indistinguishable from the control. These findings align with those of Gao et al. (2011), who reported that EMF initially stimulates metabolite production.

Table 1. Evolution of protective properties of linen/cotton fabric and biotextile with amber particles on *Chaetomium globosum* mycelial growth after inoculation on PDA, fabric wrapping, and 2–7-day ELF-EMF Exposure.

Variant	Colony diameter in variants exposed to ELF- EMF expressed in percentage comparing to control (%) (mean ± SE)					
	Days of exposition in ELF-EMF					
	2 days	3 days	4 days	5 days	6 days	7 days
Unwrapped	119.51 ± 0.00 a	114.06 ± 0.37 a	110.04 ± 1.08 a	104.70 ± 1.63 a	104.92 ± 2.27 a	103.52 ± 0.90 a
Wrapped in linen/cotton fabric	106.83 ± 1.08 b	107.50 ± 0.30 b	109.72 ± 0.77 a	105.13 ± 0.70 a	104.04 ± 1.98 a	104.32 ± 1.70 a
Wrapped in biotextile with amber particles	101.72 ± 1.22 c	106.81 ± 1.28 b	104.26 ± 1.03 b	106.32 ± 2.06 a	105.78 ± 0.88 a	103.96 ± 0.60 a
Means with the same letters within columns are not significantly different at $P < 0.01$.						

Bacterial Growth Assay Results

Bacteria have coexisted with electric and geomagnetic fields throughout their evolutionary history. Numerous studies have investigated the effects of extremely low-frequency electromagnetic field (ELF-EMF) exposure on various bacterial strains (Strašák, 2005; El-Sayed et al., 2006; Cellini et al., 2008; Aslanimehr et al., 2013; Ibraheim et al., 2013; Martirosyan et al., 2013; Bayır et al., 2015). These studies have shown that EMFs can exert either negative (Strašák et al., 2002; Fojt et al., 2004; Justo et al., 2006) or positive (Gaafar et al., 2006; Inhan-Garip et al., 2011) effects on cell growth and viability, depending on factors such as frequency, exposure duration, magnetic field intensity, and the specific bacterial strain. The results of our experiment indicate that ELF-EMF exposure has a variable impact on the growth of different bacterial cultures and depends on the duration of exposure. We observed that 16-hour exposure to EMF stimulated the growth of *E. coli* compared to the control (Figure 3A). The optical density of the unwrapped, exposed variant after 16 hours was 8.86 McF, which is significantly different from the control (unwrapped, non-exposed) with an optical density of 5.33 McF (P < 0.01). However, after 24 and 48 hours of exposure, bacterial growth reached a stationary phase, and no significant differences were observed between the control and exposed variants.

Properties of Biotextile Affecting Bacterial Growth

In the variant where *E. coli* was wrapped in biotextile and exposed to ELF-EMF for 16 hours, the growth rate, measured as optical density, differed significantly from the unwrapped, exposed variant but was not significantly different from the unwrapped, non-exposed control. This indicates that the biotextile with amber particles can protect

E. coli from the detrimental effects of EMF, preventing both growth inhibition and stimulation. After 48 hours, bacterial growth reached a stationary phase, and no significant differences were observed between control and exposed variants.

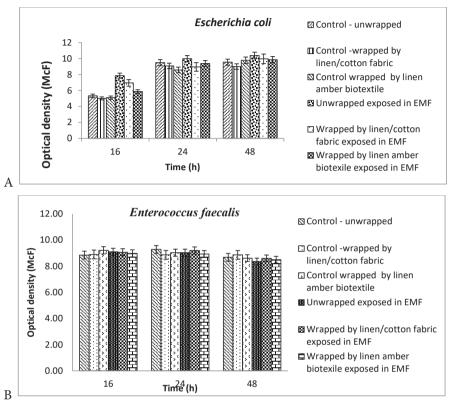


Figure 3. Impact of ELF EMF (exposing time 16, 24 and 48h) on bacteria growth (optical density in McFarland units) dependent on presence/absence of used protective wrapping. A – *Escherichia coli*; B – *Enterococcus faecalis*.

In contrast to *E. coli*, no significant differences were found in the growth of exposed *E. faecalis* (Figure 3B), regardless of whether it was wrapped in fabric or not.

While we observed a growth stimulating effect of EMF on *E. coli* after 16 hours of exposure, the optical density measurement method does not provide information about changes in bacterial viability. Therefore, we conducted additional morphological examinations and fluorescence studies.

Light microscopy of bacterial smears after 48 hours of incubation revealed no visible differences between non-treated controls and ELF-EMF-treated samples (Figure 4 A, B, C, D).

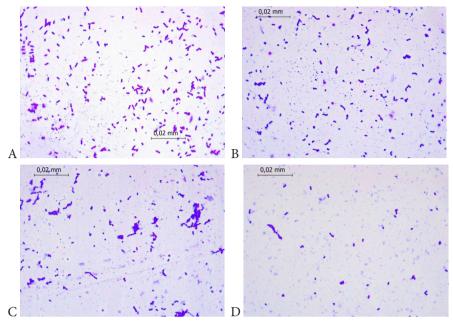


Figure 4. Light microscopy of bacterial smears of bacterial cultures wrapped in biotextile, both non exposed and exposed to ELF-EML 50 Hz (518 μ T) 48 h. A – *E. coli* wrapped in biotextile and non-exposed, B – *E. coli* wrapped in biotextile and exposed to ELF-EML; C – *E. faecalis* wrapped in biotextile and nonexposed; D – *E. faecalis* wrapped in biotextile and exposed to ELF-EML.

We employed flow cytometry to assess cell reaction changes in test bacterial cultures wrapped in textiles, both non-exposed and exposed to ELF-EMF (518 μ T) after 16, 24, and 48 hours of incubation (Figures 5 and 6). Propidium iodide, a stain that penetrates only bacteria with damaged membranes (Cellini et al., 2008), was used.

Our results showed that the number of *E. coli* bacteria with damaged membranes (fluorescent) after 16 hours of incubation ranged from 153 ± 6 to 568 ± 23 (Figure 5A). Notably, the number of damaged *E. coli* bacteria was significantly higher in control variants than in exposed variants. After 24 and 48 hours of exposure to ELF-EMF (518 µT), the number of fluorescent *E. coli* (damaged bacteria) increased in exposed variants compared to controls.

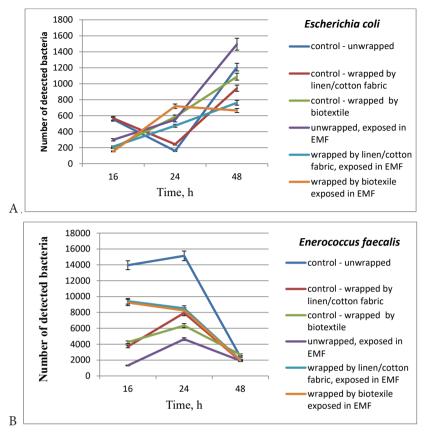


Figure 5. Number of fluorescent bacteria counted by FaxJaz flow cell sorter, depending on used protective wrapping, incubation time (16, 24 and 48 h) and presence of ELF – EML treatment. A – *Escherichia coli*; B – *Enterococcus faecalis*. The error bars indicate the confidence interval, at P < 0.05.

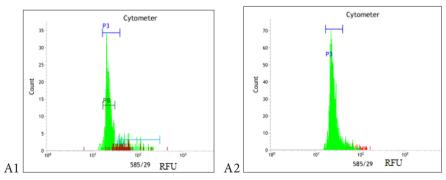


Figure 6. Count of Escherichia coli wrapped by linen/cotton amber biotextile depending on exposition to ELF-EML (518 μ T). A1 – non exposed, after 24 h; A2 – exposed to ELF-EML (518 μ T) for 24 h.

The relative fluorescence of *E. coli* cells after 16 h ranges from 46 ± 2 to 80 ± 3 RFU and in control variants are significantly lower (P < 0.01). than in exposed variants (Fig. 7A). In unwrapped variant exposed to ELF-EML (518 µT) for 16 h *E. coli* relative fluorescence is significantly higher (P < 0.01), than in wrapped exposed variants. After 48 h relative fluorescence decreases in both control and exposed variants.

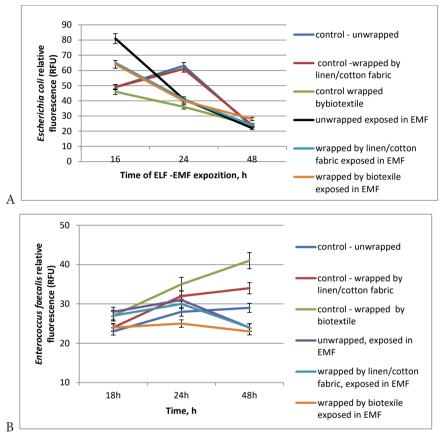


Figure 7. Impact of ELF-EML (exposing time 16, 24 and 48h) on bacteria relative fluorescence depending on used protective wrapping. A – *Escherichia coli*; B – *Enterococcus faecalis*.

The relative fluorescence of *Enterococcus faecalis* cells after 48 h ranges from 23 ± 3 to 41 ± 4 RFU and in control variants are significantly higher (P < 0.01), than in exposed variants (Fig. 7B). In unwrapped variant exposed to ELF-EML (518μ T) for 48 h *Enterococcus faecalis* cells relative fluorescence are significantly higher (P < 0.01), than in wrapped exposed variants. After 48 h relative fluorescence decreases in all exposed variants.

Conclusions

This study successfully adapted methods to determine the protective properties of biotextiles against extremely low-frequency electromagnetic fields (ELF-EMF). The research employed two bacterial strains (*Escherichia coli* and *Enterococcus faecalis*) and a microscopic cellulose degrading fungus (*Chaetomium globosum*) as test organisms. Here are the key findings:

Unwrapped *C. globosum* exhibited stimulated mycelial growth after exposure to LF-EMF compared to the control. However, wrapping the fungus in either linen fabric or the biotextile with amber particles significantly reduced this stimulation.

Short-term (16 hours) exposure to ELF-EMF increased *E. coli* growth compared to the control. The biotextile with amber particles appeared to protect *E. coli* from the detrimental effects of prolonged exposure, maintaining growth similar to the non-exposed control.

Bacterial Growth (*Enterococcus faecalis*): No significant differences in *E. coli* growth were observed under LF-EMF exposure, regardless of fabric wrapping.

The Flow Cytometry analysis of the biotextile with amber particles seemed to offer some protection for *E. coli* by reducing the number of bacteria with damaged membranes after short-term exposure (16 hours) compared to the control.

These findings suggest that the biotextile with amber particles holds promise for mitigating the potential negative effects of LF-EMF on *E. coli*. Further research is needed to explore the long-term effects of LF-EMF exposure on various bacterial strains and to optimize the protective properties of the biotextile.

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