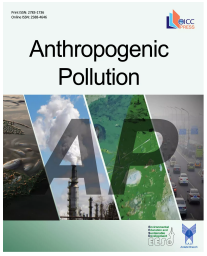

Volume 7, Issue 2, 072322 (1-11)

Anthropogenic Pollution (AP)

https://dx.doi.org/10.57647/j.jap.2023.0702.22

Investigating the effect of pulsed electric field (PEF) on *Escherichia coli* (*E. coli*) bacteria as an indicator of water contamination

Noushin Osouleddini[1]*, Amir Hesam Mahlooji[2], Mohammad Abdollahzadeh[3]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Chemistry, Islamic Azad University of Medical Sciences, Tehran, Iran.*
[2]*Department of Applied Chemistry, Faculty of Pharmaceutical Chemistry, Islamic Azad University of Medical Sciences, Tehran, Iran.*
[3]*East Tehran Management, Tehran Water and Wastewater Company, Tehran, Iran.*

***Corresponding author**: osouleddini.n@gmail.com

Received 02 April 2023; Accepted 01 November 2023; Published Online 30 November 2023 ORIGINAL RESEARCH

Abstract:
Preserving the health of food resources, including drinking water, is considered one of the most important issues in human usability. Therefore, all efforts and research are based on finding and utilizing the best methods to safeguard the hygiene of water and municipal wastewater, aiming to protect human health and improve the quality of treated sanitary sewage. Hence, the objective of this study is to investigate water contaminated with *Escherichia coli* bacteria (*E.coli*) and the removal of this microorganism as an indicator of drinking water contamination and the degree of treatment of sanitary wastewater using a Pulsed Electric Field (PEF). In this research, the PEF device was utilized within the range of 1 to 13 kV/cm with a frequency of 500 Hz for some time of 3 to 60 min. The investigation was conducted using the Most Probable Number (MPN/100 mL) test in a 9-tube format. The results of this assessment at a temperature of 20 ℃ showed that the minimum removal efficiency of total coliform bacteria was achieved at 1 kV voltage with a contact time of 1 min, yielding a 0% efficiency, while the highest removal efficiency was obtained at 13 kV voltage with a contact time of 30 min, resulting in a 99.8% efficiency.

Keywords: Pulsed Electric Field (PEF); *Escherichia coli* (*E. coli*); Wastewater; Microbial pollution; Microorganism removal

1. Introduction

Water is the primary source of life for humans. It covers over 70% of the Earth's surface, but only 3% of it is freshwater, and even from this 3%, more than 90% is frozen in polar regions and inaccessible to humans. Unfortunately, a significant portion of this freshwater is contaminated with various microorganisms, including bacteria, fungi, and viruses. These types of water sources are constantly at risk of different types of pollution, including pollution from municipal and industrial wastewater, polluted water bodies, agricultural and horticultural pollutants (such as fertilizers and pesticides), and radioactive and thermal substances. In recent years, researchers have proposed new methods to remove chemical pollutants from industrial wastewater. Among the water pollutants, we can mention organic compounds (Shirkavand et al. 2023; Rajaei 2023), dyes (Karimnezhad et al. 2023; Osouleddini et al. 2018; Osouleddini et al. 2020; Fekri et al. 2021), heavy metals (Moghimi et al. 2019; Abniki and Moghimi 2022), drugs (Abniki et al. 2023; Osouleddini et al. 2019; Nouri-Mashiran et al. 2022), and polymer industries (Abniki et al. 2022), etc (Osouleddini et al. 2023; Rastegar and Osouleddini 2017; Safarkar et al. 2020). Therefore, ensuring safe drinking water is one of the fundamental issues for human survival. One of the microorganisms that serve as an indicator of water contamina-

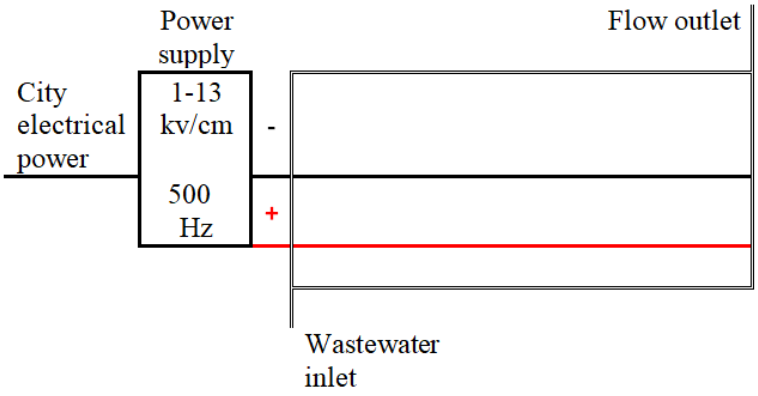

Figure 1. It shows the schematic of the PEF device.

tion from sewage and municipal wastewater is *Escherichia coli* (*E. coli*) bacteria (Herz et al. 2017; Mishra and Newhouse 2009; Filippa et al. 2013). *E. coli* is a gram-negative bacterium from the Enterobacteriaceae family that commonly exists in the intestines of warm-blooded animals. It is the most prevalent facultative anaerobic organism found in the colon and feces and is the most common cause of urinary tract infections. This bacterium is considered a potential indicator of sanitary sewage contamination in water (Yang et al. 2021; Chen et al. 2005; González-Mariño et al. 2020; Sardari and Osouleddini 2018). Its presence in water indicates that the water has been contaminated with human or animal feces, and there is a possibility of contamination by other bacteria and the occurrence of diseases such as cholera, typhoid, and hepatitis. There are various methods for removing contaminants from drinking water, such as ozone disinfection (O_3), ultraviolet (UV) radiation, and chlorination, which is a common method used in many countries (Sakamoto et al. 2009). However, the aim of this study is to investigate the presence of *Escherichia coli* bacteria in contaminated water and the effects of pulsed electric field (PEF) on reducing these bacteria and ultimately eliminating them as an indicator of contamination of drinking water with sewage using the PEF method.

Carlito delso et al. examined the resistance of Saccharomyces bayanus, Brettanomyces bruxellensis, Lactobacillus plantarum, and Oenococcus oeni to PEF in celery. The exact effect of PEF parameters on microbial inactivation and Monte Carlo simulation of the variability of factors and the hypothetical maximum microbial load in the treated final product was considered. The results showed that PEF treatments at 15 kV/cm and 129 or 153 kJ/kg guarantee microbial elimination in celery (Potter et al. 2018). Walter et al. studied the inactivation of a protease obtained from

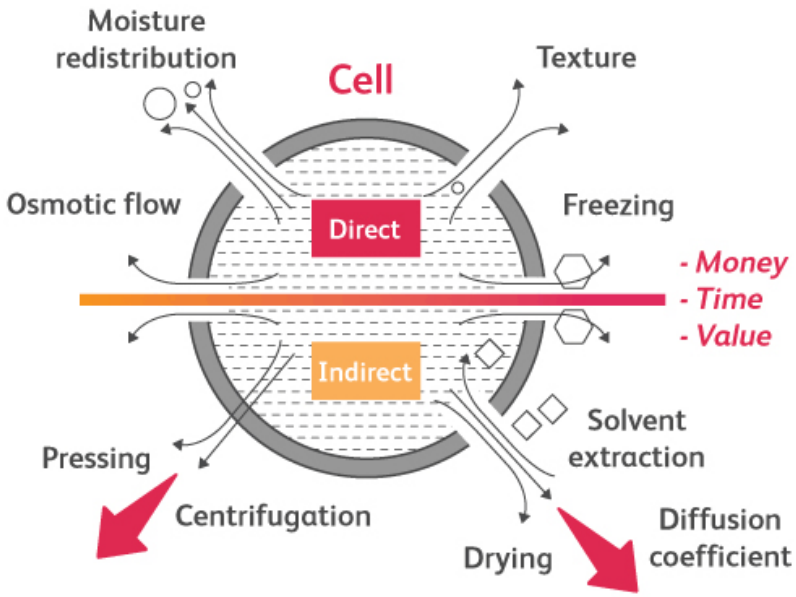

Figure 2. Mechanisms of effect of different factors on bacterial cell wall rupture.

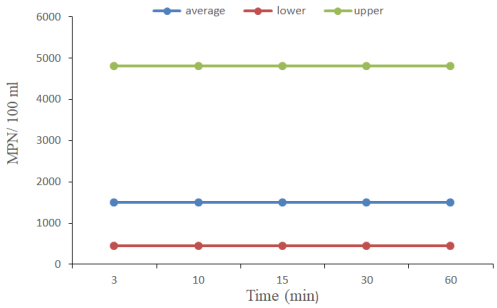

Figure 3. Sample examined in terms of the amount of coliform contamination by passing through a voltage of 1 kV and times from 3 to 60 min.

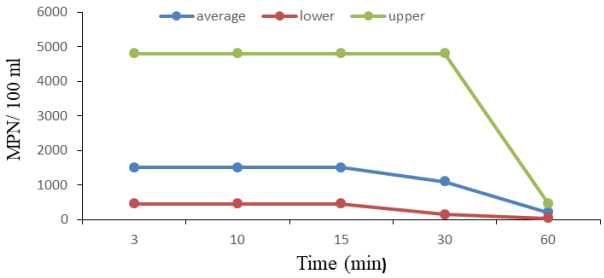

Figure 5. The sample examined in terms of the amount of coliform contamination by passing through a voltage of 3 kV and times from 3 to 60 min.

Pseudomonas fluorescens. The protease was inactivated after 20 pulses in an electric field with an intensity of 18 kV/cm and yeast extract up to 80%. However, the rate of inactivation was achieved after 98 pulses and an electric field intensity of 14 kV/cm in 60% whole milk. The activity of alkaline phosphatase decreased by 60% using 70 pulses with an electric field intensity of 8.18 kV/cm in raw milk, 2% in fat milk, and 65% in fat-free milk (Bevilacqua et al. 2018).

Nicolo et al. investigated how pulsed electric field (PEF) can alleviate metabolic stress responses in plant tissues as a function of the applied inducing conditions. Metabolic response is related directly to the quality of fresh fruits and vegetables, which affects the final product quality. The field of pulsed electric fields (PEF) and other emerging technologies can have desirable and effective effects on the tissue metabolism of products (Walter et al. 2016). Rivas Solar et al. conducted a study to investigate the nutritional properties (carbohydrates, proteins, pigments, and phycocyanins), total antioxidant capacity (TAC), and the ability to simulate the growth of Lactobacillus rhamnosus. Platanus was extracted using conventional water-based methods and pulsed electric field (PEF) technology. Significant improvements in the nutritional profile of Chlorella and Spirulina extracts obtained with pre-treatment using PEF technology at higher specific values were confirmed in terms of total carbohydrates, chlorophyll a, chlorophyll b, carotenoid content, and TAC. Furthermore, the Spirulina extract demonstrated the ability to stimulate the growth of a probiotic, Lactobacillus rhamnosus, with specific metabolic characteristics rich in biologically active short-chain fatty acids (SCFAs) and lactic acid (3-phenyllactic acid) fermentation. This study highlights the application of PEF extraction technology under optimized conditions for improving the nutritional and functional properties of microalgae and cyanobacteria-derived products (Ricós-Muñoz et al. 2023). Pulsed electric fields (PEF) can be used to improve the quality characteristics of meat, such as tenderness and mass transfer kinetics during dry aging. This study investigated the effect of PEF on the aging of mineral profiles, fatty acids, conjugated linoleic acid, and oxidative stability of venison. PEF treatments had no effect on the mineral content. The use of both PEF treatments (HPEF: 10 kV, 50 Hz, 5 microseconds; LPEF: 2.5 kV, 50 Hz, 5 microseconds) potentially improved the drying of venison with a risk of reducing oxidation (Alvarez et al. 2003). In a study conducted by Vezzaro et al., gram-positive pathogenic bacteria (*Monocytogenes*, *Staphylococcus aureus*, and *Listeria*) and a gram-negative bacterium (*Escherichia coli*) were subjected to pulsed electric fields (PEF) in milk, and their inactivation kinetics were studied using non-selective, selective, and PEF media (Mungure et al. 2023). In an experiment by Joshua et al., pulsed electric fields (PEF) were used to inactivate microorganisms and prevent loss of aroma and taste in liquid and beverage food products as an alternative to thermal pasteurization. Both Salmonella and EHEC were gradually inactivated at 55 ºC.

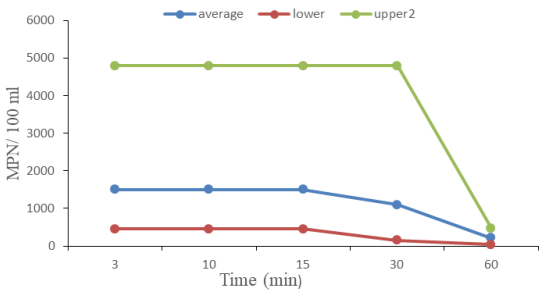

Figure 4. Sample examined in terms of the amount of coliform contamination by passing through a voltage of 2 kV and times of 3 to 60 min.

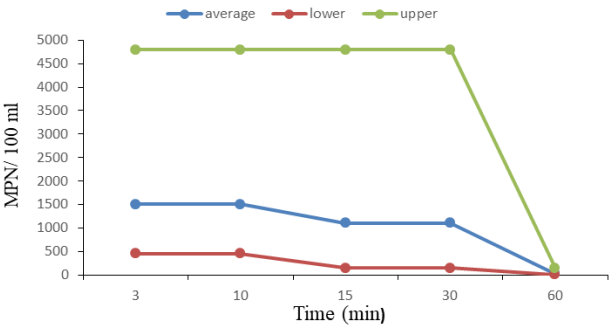

Figure 6. The sample tested for the amount of coliform contamination by passing through a voltage of 4 kV and times of 3 to 60 min.

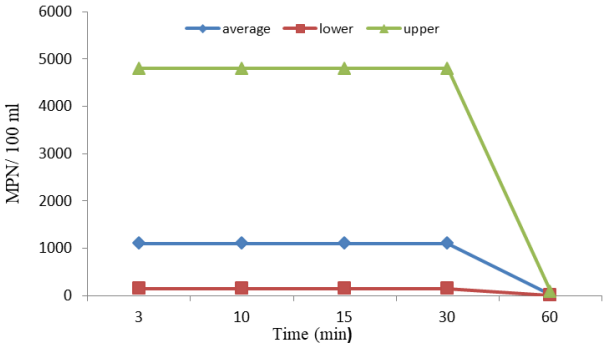

Figure 7. Sample examined in terms of the amount of coliform contamination by passing through a voltage of 5 kV and times from 3 to 60 min.

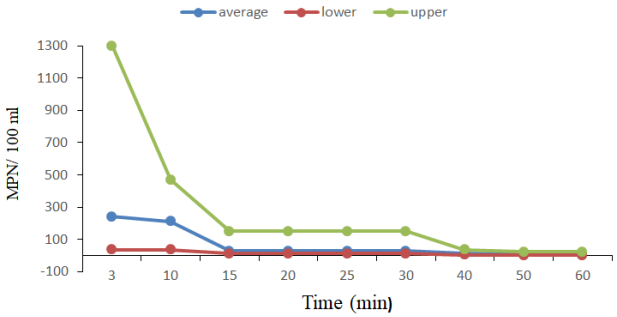

Figure 9. Sample examined in terms of the amount of coliform contamination by passing through a voltage of 7 kV and times from 3 to 60 min.

Under similar conditions, EHEC was inactivated slightly more than this temperature. NPEC was tested as a surrogate for EHEC in terms of inactivation kinetics at 45, 50, and 55 ºC using voltages between 7.86 and 32.5 kV/cm. In 2023, Garal et al. conducted a study using a pulsed electric field (PEF) with selected parameters to enrich the probiotic strain L. rhamnosus B442 with calcium ions. Six types of ice cream mixes were prepared and supplemented with 200 μg of calcium ions. The enrichment of three types of mixes, including untreated, lyophilized, and fermented mixes, involved adding bacteria exposed to PEF to enhance calcium bioaccumulation. Calcium levels were measured in bacterial cells and ice cream. After 24 h of ice cream production, its chemical composition, pH, melting rate, and texture were determined. Color parameters and total microbial counts were also analyzed. The highest accumulation of Ca^{2+} ions in cells was achieved when the pulsed electric field was applied at a field strength of 3.0 kV/cm and a calcium concentration of 200 μg/mL of medium. The use of PEF-modified L. rhamnosus B442 bacteria for milk fermentation resulted in ice cream with the highest levels of dry matter, fat, protein, and carbohydrates, as well as the lowest melting rate. No significant differences were found in the color parameters (a$*$ and ΔH). Calcium-enriched ice cream using PEF showed no significant difference in bacterial survival rates. In one of the studied methods, TiO_2 was used for the photocatalytic degradation of *E. coli* in natural pyrite. In this study, TiO_2 nanoparticles (TiO_2NP) were successfully prepared and characterized using FTIR, SEM-EDS, and XRD analysis. The operational parameters such as pH, catalyst dosage, catalyst ratio, light intensity, ultraviolet irradiation, aeration, mineral ions, and organic matter were investigated for their effect on bacterial inactivation. The results showed that aeration, UVA, and increased bacterial and mineral matter density contributed to bacterial activity reduction, and the inhibitory effect of mineral ions was in the order of $F^- > Ca^{2+} > NO^{3-} > SO_4^{2-} > Cl^-$. The effect of catalyst type was in the order of TiO_2NP > NP > TiO_2; however, TiO_2 generated OH. species in the photocatalytic degradation process and inactivated *E. coli* (Pankiewicz et al. 2020). This study confirmed the successful inactivation of *E. coli* in aqueous environments using TiO_2NP (Cebrián et al. 2016). Tony et al. used selenium-coated polymer feed for reverse osmosis membrane fouling control and feed spacers. Initial tests with feed spacers containing 0.55% selenium coating in the preservative materials showed antimicrobial activity of 72.2% and a 2.7% decrease in biofilm thickness against *Staphylococcus aureus*. At a 1% selenium coating concentration, the average antimicrobial activity against specific bacterial biomass concentration was 92.2% for each bacterium, and the average reduction in biofilm thickness was 3.9 to 2.9 for *Staphylococcus aureus* and *E. coli*, respectively (Anandan et al. 2021). In this study, the effect of electric field intensity with a frequency of 500 Hz in the range of 1 to 13 kV/cm and a treatment time of 3 to 60 min was investigated on the microbial pollutant *E. coli*

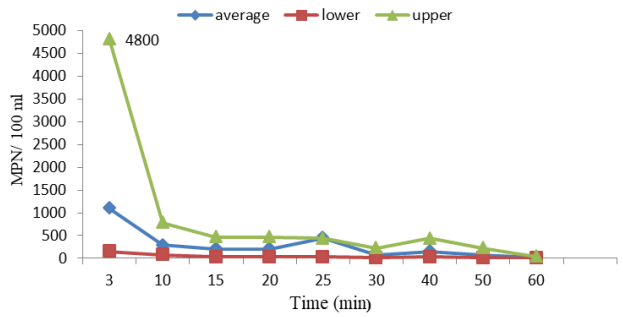

Figure 8. The sample examined in terms of the amount of coliform contamination by passing through a voltage of 6 kV and times from 3 to 60 min.

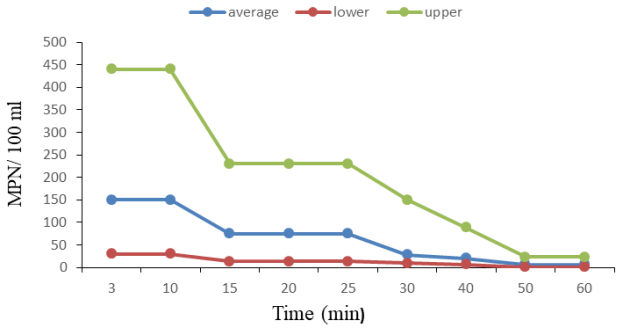

Figure 10. The sample tested for the amount of coliform contamination by passing through a voltage of 8 kV and times from 3 to 60 min.

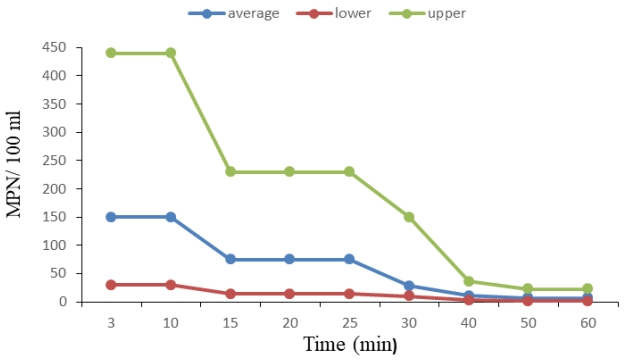

Figure 11. The sample examined in terms of the amount of coliform contamination by passing through a voltage of 9 kV and times from 3 to 60 min.

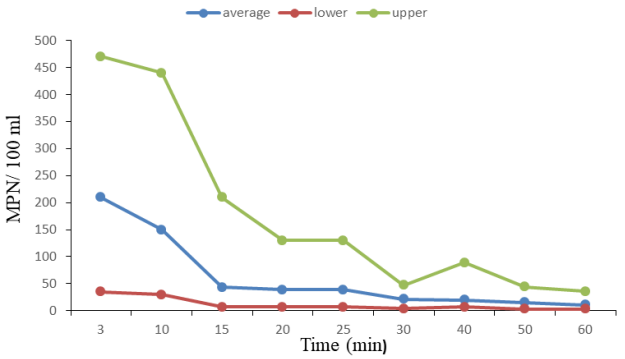

Figure 13. The sample examined in terms of the amount of coliform contamination by passing through the voltage of 11 kV and the time from 3 to 60 min.

in wastewater using the MPN/100 mL test.

2. Materials and methods

The devices used in this research include the pulsed electric field (PEF) device, which was designed and manufactured by us with a 13 to 1 kV/cm power supply with a frequency of 500 Hz by Metna. SHIN SAENG brand bacterial culture medium incubator shaker, MEMMERT brand desktop incubator made in Germany, SHIN SAENG heater and magnetic stirrer made in South Korea, GENERAL STEEL culture medium refrigerator made in Iran, LABORATORY healthy material refrigerator made in Iran, SARTORIUS digital scale made in Germany, autoclave 75-Liter RAHIMY MEDICAL INSTRUMENTS made in Iran, LAMINAIR FLOW hood made in Iran, Lactose broth culture medium made by Merck Germany, *E.Coli* special culture medium made by Merck Germany, Brilliant Green Bile Broth culture medium (BGB) brand Merck made in Germany, deionized water Produced by Aria Mehrgan Teb Tebhiz company, Metrohm brand pH meter device made in Switzerland, mercury thermometer, glass sampling bottles.

Sample preparation method

The solution prepared for testing the target sample was prepared using sterilized deionized water of Mehrgan Teb Tehiz Aria Company. If the water contains residual free chlorine or other halogens, a reducing agent sodium thiosulfate ($Na_2S_2O_3$) should be added. $Na_2S_2O_3$ is a suitable dechlorinator that can neutralize all remaining halogens and prevent the bactericidal effect of chlorine during the research (Bactericidal). If there is chlorine in the incoming raw water, enough thiosulfate should be used in a 120 mL sampling container or its equivalent for different volumes, 0.1 mL of 10% thiosulfate solution can produce a sample containing 15 mg Neutralize the residual free chlorine. In the case of drinking water, the amount of chlorine must be added to 0.1 mL of 3% thiosulfate solution in a 120 mL glass to neutralize the remaining chlorine concentration up to 5 mg/liter. Water or wastewater samples containing large amounts of copper or zinc or heavy metals should be collected in containers containing a chelating agent to reduce the toxicity of these substances. This is very important for samples that take more than 4 h to be transported to the laboratory. For this purpose, EDTA solution (sodium salt of ethylene diamine tetraacetic acid) with a concentration of 372 mgL is used. The pH of this solution should be adjusted to about 5.6 before use. 3/ to a 120 mL sampling bottle before sterilization. The 15% EDTA solution or a mixture of it and sodium thiosulfate is added. When sampling, it is necessary to pay attention to the following points. The sample should be a part of

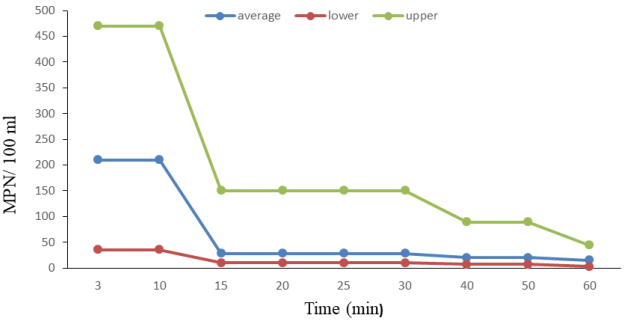

Figure 12. The sample examined in terms of the amount of coliform contamination by passing through a voltage of 10 kV and times from 3 to 60 min.

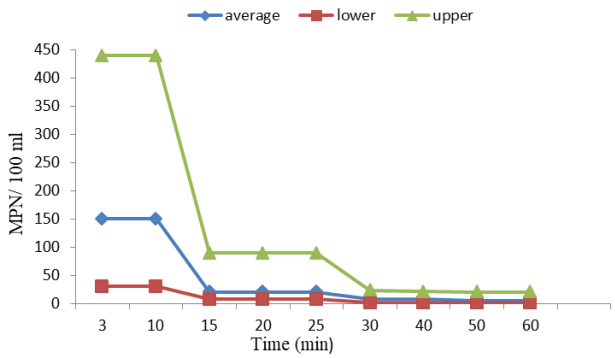

Figure 14. The sample examined in terms of the amount of coliform contamination by passing through a voltage of 12 kV and times from 3 to 60 min.

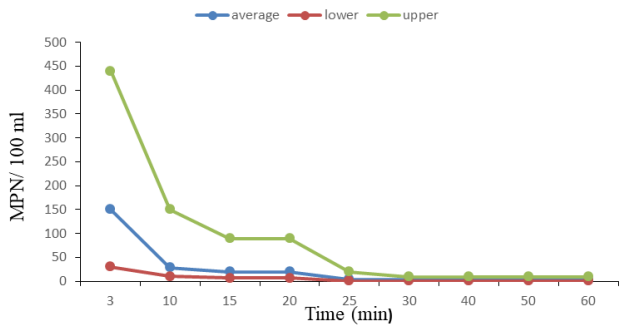

Figure 15. The sample examined in terms of the amount of coliform contamination by passing through the voltage of 13 kV and the time from 3 to 60 min.

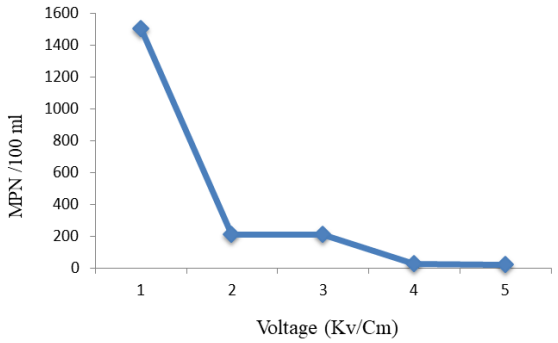

Figure 17. Total average Fermi pollution of voltages from 1 to 5 kV.

the whole. After washing and rinsing with city water, the sampling container is rinsed with distilled water and then sterilized. After sampling, the empty space at the top of the container (at least 2.5 cm) should be considered for stirring and mixing. In order to prevent contamination with samples containing pathogenic agents, hygiene and disinfection must be observed by the sampler. Sampling containers do not need to be rinsed with the sample first and are filled directly with the sample. Sodium hypochlorite solution can be used to disinfect sample collection valve. The volume of the sample on which all microbial tests can be performed should not be less than 100 mL. Microbiological tests should be done immediately after sampling. If the samples cannot be delivered to the laboratory within 1 hour. You should use the container containing the ice bags to store the samples until they reach the laboratory and bring them to the laboratory for up to 6 h. The temperature of all surface water, drinking water and sewage samples must be kept below 10 ºC during the maximum 6 h of transfer to the laboratory. After reaching the laboratory, the samples can be placed in the refrigerator for up to 2 h and then the test can be started. The time interval between sampling and testing should not exceed 24 h under any circumstances.

Data collection method

Information was collected by visiting libraries of higher education institutions, archival centers, government organizations, and private sectors. Discussions were held with some experts and stakeholders regarding the subject matter. Initial information was gathered, categorized, and classified accordingly. Partial sampling was conducted as a representative sample of the whole, and the experiments were carried out over a period of three months. The pulsed electric field (PEF) experiments were performed at the Chemistry Research Laboratory, and the overall contamination and total coliforms were determined using the MPN/100 mL method at the Microbiology Research Laboratory of the School of Pharmacy. The equipment used is in accordance with Table 1.

The pulsed electronic field (PEF) process

The components of this device include: 1- Primary tank. 2. Feeding pump. 3- High voltage power source (high voltage) in the range of 1 to 13 kV/cm with a frequency of 50 Hz. 4- Coaxial reaction chamber high voltage current was applied, which It has an entry and exit point for the sewage flow. In this research, first of all, an initial tank with a volume of 50 L was prepared as a homogenizing tank with a certain concentration of *E.Coli* bacteria. Different concentrations of *E.Coli* bacteria, which were previously in the raw water entering the device, were prepared by increasing a certain amount of urban raw sewage and the concentration was determined. Then, it was injected by the pump to the place and entrance chamber of the PEF device and the investigations were conducted at various voltages from 1 to 13 kV at various times. Inside the

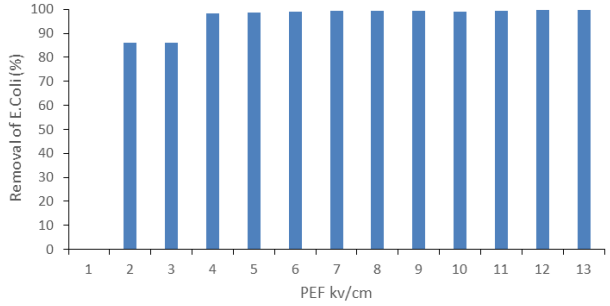

Figure 16. Comparison of coliform removal efficiency by PEF method at different voltages.

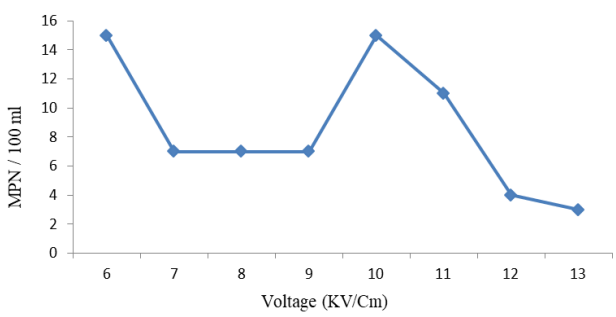

Figure 18. Total average Fermi pollution of 6 to 13 kV voltages.

Table 1. The examined sample in terms of the amount of coliform contamination without passing through the device is as described in the table.

Voltage (kV)	Time (Min)	number of tubes giving positive reaction out of			MPN index per 100 mL	95% confidence limits	
		3 of 10 mL each	3 of 1 mL each	3 of 0/1mL each		lower	upper
0	0	3	3	3	>1100	450	4800

PEF reaction chamber, which consists of two electrodes with positive and negative poles with a certain distance from each other inside a PVC tube with a diameter of 3 inches, and receives high voltage pulses. The desired samples are placed between the electrodes, which at regular intervals, usually every few milliseconds, pulses with high voltage in the range of 1 to 13 kV/cm are introduced to those electrodes, which creates a strong electric field on the samples, and this issue It causes the inactivation of bacteria. These electrodes are made of titanium. The system overview of this device is shown in Figure 1. This system destroys the bacteria by destroying the cell wall.

MPN method

MPN test is a method of calculating the maximum possible number of bacteria in a sample. To detect and compare the level of contamination in different water samples, this method is largely decisive, so if after 24 to 48 h the tubes in the incubator, the various signs are mentioned, such as the clouding of the culture medium and the accumulation of gas inside the Durham tube. They show themselves as a reason for the existence of pollution.

Test method

In the first step, the construction of the Broth Lactor culture medium was done. For this purpose, 2 Erlenmeyer flasks with a volume of 500 mL were used. In the first flask, we dissolved 3.9 g of lactose broth in 150 mL of distilled water, and in the second flask, we dissolved 3.9 g of lactose broth in 300 mL of distilled water. We used a 1000 mL beaker to easily add distilled water to the Erlenmeyer flask. In order for the added powder to dissolve well in the distilled water and a clear solution with a yellowish color to be obtained, put a magnet in both Erlen and place it on the heater device. It was taken to the pre-prepared tubes under the hood (for greater safety from possible contamination, then sterilized and cleaned the surfaces under the laminar hood using alcohol savolon and flame for cultivation).
Now, added the Erlenmeyer flasks with the culture medium to the test tubes, and then added a Durham tube to each of the tubes in reverse, and used cotton and foil to prevent possible contamination and spillage of the test tubes. Pack the prepared tubes so that they are ready to be in the autoclave.
Thus, by turning on the autoclave and placing the tubes and falcons for 60 min at a temperature of 121 ℃ with a pressure of 1 bar, the desired devices are sterilized.
After completing the sterilization operation by autoclave, the tubes containing the lactose broth culture medium were put in the refrigerator, and proceed to prepare water contaminated with *Escherichia coli*.
Using the voltmeter device to control the voltage, start from the first level of the device (1 kV) add some samples each time, and set the level to 1 kV by turning on the power switch and by setting the constant voltage in 5 different time intervals (3-10-15-30-60 min), and then added the samples to the pre-sterilized falcons.
Now the samples passed through the device are taken to the microbiology research test and added to the tubes containing the culture medium under the hood. In this experiment, the MPN method was used in the form of 9 tubes and put in an incubator with a culture medium for 48 h.
After this time, we remove the tubes from the device and check each one of them. The sign of the presence of general pollution is the turbidity of the cultivation environment and the accumulation of gas in the Durham pipe. If these signs are observed, we can separate the contaminated pipes and calculate the amount of contamination according to the MPN table or the Thomas formula.
The next step is to prepare the EMB culture medium and after making it, it is put in the autoclave to prepare the existing gel. After leaving the machine and after about 15 min, we put the 10 cm plates under the laminar hood and next to the flame and add EMB to the plates so that it closes (about 15 min) after closing the gels until adding the infected samples to the EMB culture medium by means of a loop, and after completing this step, we place all the plates inside the incubator with a temperature of 36 ℃. After 24 h, we take out the samples from the incubator and check them. The presence of green metallic polish indicates the presence of Escherichia coli bacteria in the sample.

3. Results

MPN method
The coliform group includes several genera of bacteria belonging to the Enterobacteriaceae family. The old definition of this group is not based on scientific bacteriology, but based on their identification method (lactose fermentation). Therefore, in relation to the fermentation method, this group is called aerobic and facultative anaerobic bacteria, gram negative, without spores. and rod-shaped, which ferment lactose within 48 h and produce gas and acid at a temperature of 35 ℃, are defined. Standard test to identify the coliform group based on the multi-tube fermentation (MT) method (in the form of three possible, confirmatory, supplementary stages). Multiple-tube fermentation technique for members of the coliform group is also performed with the Membrane Filter (MF) technique or by the Chromogenic substrate method. Each method has its own limitations and

can be used according to the objectives of the experiment. In order to obtain reliable results, it is necessary to implement serious and accurate work quality control methods. MPN test is a method of calculating the maximum possible number of bacteria in a sample. To detect and compare the level of contamination in different water samples, this method is largely decisive, so that if after 24 to 48 h in the incubator, the various signs mentioned such as cloudiness of the culture medium and gas accumulation inside the Durham tube. They show themselves as a reason for the existence of pollution. The number of volumes removed from the sample (or dilution factors for the samples) depends on the desired accuracy of the work results. The MPN table is prepared based on the assumption of Poisson distribution (random distribution). However, if the sample is not thoroughly stirred and mixed before removing the desired volume at each step, or if the bacterial cells are clumped together and condensed, the MPN value will be lower than the actual number of bacteria. Estimating the concentration or density of bacteria (MPN calculation). A-Accuracy of multi-tube fermentation method: The accuracy of this method is relatively low. Unless the number of prepared samples is large. For example, even when the sample contains 1 coliform per milliliter, considering the random distribution of bacteria in each tube, it can be expected that about 37% of the 1 mL tubes will give a negative response to the test. Now, if five tubes are considered for a 1 mL sample, under the same conditions, less than 1% of the results may give a completely negative answer.

Even in the five-tube method, the accuracy of the obtained results is not very high. As a result, care should be taken when interpreting the results of the health check of a source regarding the presence of coliform. Especially if the number of tubes prepared with different dilution factor is small or if the number of samples taken from the source is not enough.

B. Calculate and express MPN

To calculate the concentration or density of bacteria, the method of statistical expression of the most probable number or the largest probable number (MPN) is used. MPN values are calculated for many and varied tests that have been performed on various samples and are presented in several tables of the EPA water and wastewater method standard. This ratio is calculated considering the 95% confidence interval for each MPN. If the volume of the sample prepared in a test is the same as the values in the table, the MPN related to that test can be found and reported in terms of MPN/100mL or the presence or absence of total coliforms or faecal coliforms from the table. When the dilution rate or the dilution factor of the yield is different from the numbers 10, 1, 0.1, the MPN can be selected from the table for a combination of positive tubes and calculated and reported using the following Equation 1 for the test performed.

$$\frac{\text{MPN}}{100\ \text{mL}} = \frac{10}{V_{max}} \times \text{MPN} \tag{1}$$

when a combination of positive tubes is not found in the table, MPN can be calculated from the simple Thomas Equation 2 as follows:

$$\frac{\text{MPN}}{100\ \text{mL}} = \frac{\text{Number of positive tubes} \times 100}{\sqrt{(\text{volume (mL) in negative tubes} \times \text{volume (mL) in all tubes})}} \tag{2}$$

As can be seen in the above diagram, the voltage of 1 kV and increasing the contact time up to 60 min had no effect on the removal of coliform bacteria. (Fig. 3)

By increasing the voltage to 3 and 2 kV, the number of coliform bacteria decreases after 15 min. until the average number of MPN reaches 210 from 1500 in 60 min. This removal efficiency in 60 min is approximately equal to 86%. In total, the removal efficiency compared to the number of primary coliform bacteria (MPN/100mL = 1500) is 86% in 60 min. (FigS. 4, 5)

By increasing the voltage to 4 kV, the number of coliform bacteria decreases after 10 min. until the average number of MPN reaches 28 from 1500 in 60 min. This removal efficiency in 60 min is equal to 98.13%. In total, the removal efficiency compared to the number of primary coliform bacteria (MPN/100mL = 1500) is 98.13% in 60 min. (Fig. 6)

By increasing the voltage to 5 kV, after 30 min, while the average number of coliform bacteria decreases from 1500 to 1100, the average number of MPN reaches 20 in 60 min. This removal efficiency in 60 min is equal to 98.18%. In total, the removal efficiency compared to the number of primary coliform bacteria (MPN/100mL = 1500) is 98.66% in 60 min. (Fig. 7)

By increasing the voltage to 6 kV, after 10 min, while the average number of coliform bacteria decreases from 1100 to 290, the average number of MPN reaches 15 in 60 min. This removal efficiency in 60 min is equal to 98.63%. In total, the removal efficiency compared to the number of primary coliform bacteria (MPN/100mL = 1500) is 99% in 60 min. (Fig. 8)

By increasing the voltage to 7 kV after 10 min while reducing the average number of coliform bacteria from 240 to 28, the average number of MPN reaches 7 in 50 min. This removal efficiency in 60 min is equivalent to 89% compared to 3 min after PEF application.

In total, the removal efficiency compared to the number of primary coliform bacteria (MPN/100mL = 1500) is 99.53% in 60 min. (Fig. 9)

By increasing the voltage to 8 kV, after 10 min, the average number of coliform bacteria decreases from 150 to 75, and the average number of MPN reaches 7 in 50 min. This removal efficiency in 60 min is equal to 95.33% compared to 3 min after PEF application. In total, the removal efficiency compared to the number of primary coliform bacteria (MPN/100mL = 1500) is 99.53% in 60 min. (Fig. 10)

By increasing the voltage to 9 kV, after 10 min, while the average number of coliform bacteria decreases from 150 to 75, the average number of MPN reaches 7 in 50 min. This removal efficiency in 60 min is equal to 95.33% compared to 3 min after PEF application. In total, the removal efficiency compared to the number of primary coliform bacteria (MPN/100mL = 1500) is 99.53% in 60 min. (Fig. 11)

By increasing the voltage to 10 kV after 10 min while reducing the average number of coliform bacteria from 210 to 28, the average number of MPN reaches 15 in 60 min. This removal efficiency in 60 min is equal to 92.86% compared to 3 min after PEF application. In total, the removal efficiency compared to the number of primary coliform bacteria (MPN/100mL = 1500) is 99% in 60 min. (Fig. 12)
By increasing the voltage to 11 kV after 15 min while reducing the average number of coliform bacteria from 210 to 43, the average number of MPN reaches 11 in 60 min. This removal efficiency in 60 min is equivalent to 94.76% compared to 3 min after PEF application. In total, the removal efficiency compared to the number of primary coliform bacteria (MPN/100mL = 1500) is equal to 99.27% in 60 min. (Fig. 13) By increasing the voltage to 12 kV after 10 min while reducing the average number of coliform bacteria from 150 to 20, the average number of MPN reaches 4 in 50 min. This removal efficiency in 60 min is equal to 97.33% compared to 3 min after PEF application. In total, the removal efficiency compared to the number of primary coliform bacteria (MPN/100mL = 1500) is 99.73% in 60 min. (Fig. 14)
By increasing the voltage to 13 kV after 3 min while reducing the average number of coliform bacteria from 150 to 20, the average number of MPN reaches 3 in 30 min. This removal efficiency in 60 min is equivalent to 98% compared to 3 min after PEF application. In total, the removal efficiency compared to the number of primary coliform bacteria (MPN/100mL = 1500) is equal to 99.8% in 60 min. (Fig. 15)

4. Discussion

Due to its high efficiency, this method is suggested as the best method to reduce the microbial contamination of urban wastewater for disposal in receiving waters and irrigation of agricultural lands.
According to the predictions and investigations carried out regarding the performance and effects of the pulsed electric field (PEF) on the *Escherichia coli* microorganism during this period, it was concluded that the increase in voltage and time alone has a direct effect on the removal and reduction of the number of this bacterium. (Fig. 16)
Figures 17, and 18 show the trend of bacteria reduction (MPN/100ml) with increasing voltage from 1 to 13 kV.

5. Conclusion

The results showed that the lowest removal efficiency of coliform pollution in different voltages according to diagram 14 is related to the voltage of 1 kV, which is equal to zero, and the highest removal efficiency is related to the voltage of 13 kV in 30 min with a removal efficiency of 99.8%. The following are the advantages of the PEF method, reducing costs related to the purchase (storage, preparation, and injection) of disinfectants for water. It is easy to prepare, install, and use. Non-creation of by-products from this method for drinking water. High-quality and fresh production in beverages and liquids. New method, low cost or no operating cost (if it reaches the exploitation stage). Preserving compounds such as vitamins, minerals, pigments, and flavors in liquids and foods. saving in economy, heat, energy, and time. Due to the newness of the technology, this method will take time to replace other methods in society and needs to be advertised.

Conflict of interest statement
The authors declare that they have no conflict of interest.

Author Contributions
Noushin Osouleddini: Prposed the plan, conceived the experiments, analyzed the data, authored or revised drafts of the paper, approved the final draft.

Ethics Statement
Vice Chancellor for Research and Technology, Islamic Azad University of Medical Sciences, Tehran, Iran.

References

Abniki M, Azizi Z, Poorebrahim S, Moniri E (2023) Development of p-amino acetanilide functionalized multi-walled carbon nanotubes as an effective carrier for lansoprazole release. *Biomedical Physics & Engineering Express* 9:045021.

Abniki M, Hadavand B Shirkavand, Najafi F, Ghasedi I (2022) Synthesis of the effective flame retardant via modification of epoxy resin with phenylboronic acid. *Journal of Macromolecular Science* 59:411–420.

Abniki M, Moghimi A (2022) Removal of Cd (II) ions from water solutions using dispersive solid-phase extraction method with 2-aminopyridine/graphene oxide nanoplates. *Current Analytical Chemistry* 18:1070–1085.

Alvarez I, Virto R, Raso J, Condon S (2003) Comparing predicting models for the Escherichia coli inactivation by pulsed electric fields. *Innovative Food Science & Emerging Technologies* 4:195–202.

Anandan M, Saraswathi V, Rubeshkumar P, Ponnaianh M, Jesudoss P, Karumanagounder K, Murhekar M (2021) Outbreak of acute diarrhoeal disease attributed to consumption of faecal contaminated water supplied through damaged pipelines in Thiruper, Tiruvallur district, Tamil Nadu, India, 2016. *Clinical Epidemiology and Global Health* 10:100701.

Bevilacqua A, Petruzzi L, Perricone M, Speranza B, Campaniello D, Sinigaglia M, Corbo MR (2018) Nonthermal technologies for fruit and vegetable juices and beverages: Overview and advances. *Comprehensive Reviews in Food Science and Food Safety* 17:2–62.

Cebrián G, Condón S, Mañas P (2016) Influence of growth and treatment temperature on Staphylococcus aureus resistance to pulsed electric fields: relationship with membrane fluidity. *Innovative Food Science & Emerging Technologies* 37:161–169.

Chen J, Tang C, Sakura Y, Yu J, Fukushima Y (2005) Nitrate pollution from agriculture in different hydrogeological zones of the regional groundwater flow system in the North China Plain. *Hydrogeology Journal* 13:481–492.

Fekri R, Mirbagheri S-A, Fataei E, Rajaei G Ebrahimzadeh, Taghavi L (2021) Organic compound removal from textile wastewater by photocatalytic and sonocatalytic processes in the presence of copper oxide nanoparticles. *Anthropogenic Pollution* 5:93–103.

Filippa N, Carricajo A, Grattard F, Fascia P, Sayed F EL, Defilipps JP, Berthelot P, Auberd G (2013) Outbreak of multidrug-resistant Klebsiella pneumoniae carrying qnrB1 and bla CTX-M15 in a French intensive care unit. *Annals of Intensive Care* 3:1–4.

González-Mariño I, Baz-Lomba JA, Alygizakis NA, Andres-Costa MJ, Bade R, Bannwarth A, Barron LP, Been F, Benaglia L, Berset JD (2020) Spatio-temporal assessment of illicit drug use at large scale: evidence from 7 years of international wastewater monitoring. *Addiction* 115:109–120.

Herz E, Antonovsky N, Bar-On Y, Ddvidi D, Gleizer S, Prywes N, Noda-Garcia L, Frisch K Lyn, Zohar Y, Wernick DG (2017) The genetic basis for the adaptation of E. coli to sugar synthesis from CO_2. *Nature Communications* 8:1705.

Karimnezhad K, Moghimi A, Adnan R, Abniki M (2023) An alternative method of dispersive solid-phase extracting Hg (II) from environmental aqueous solutions using carboxylic functionalized carbon nanotubes. *Micro & Nano Letters* 18:e12150.

Mishra P, Newhouse D (2009) Does health aid matter? *Journal of Health Economics* 28:855–872.

Moghimi A, Qomi M, Yari M, Abniki M (2019) Solid phase extraction of Hg (Π) in water samples by nano-Fe. *Int. J. Bio-Inorg. Hybr. Nanomater* 8:163–172.

Mungure TE, Farouk MM, Carne A, Staincliffe M, Stewart I, Jowett T, Bhat ZF, Bekhit AEDA (2023) Understanding the influence of PEF treatment on minerals and lipid oxidation of wet-and dry-aged venison M. longissimus dorsi muscle. *Innovative Food Science & Emerging Technologies* 83:103238.

Nouri-Mashiran M, Taghavi L, Fataei E, Rajaei G Ebrahimzadeh, Ramezani M (2022) Green synthesis of ZnO nanoparticles and comparison of 2,4-dinitrophenol removal efficiency using photocatalytic, sonocatalytic, and adsorption processes. *Main Group Chemistry* 21:559–575.

Osouleddini N, Heydari M, Motevalli MD, Khosravi T (2020) Application of artificial neural networks and response surface methodology for analysis of malachite green removal from aqueous solution using phosphoric acid-modified pumice powder: kinetic and isotherm studies. *Desalin Water Treat* 178:296–311.

Osouleddini N, Moradi M, Khosravi T, Khamotian R, Sharafi H (2018) The iron modification effect on performance of natural adsorbent scoria for malachite green dye removal from aquatic environments: modeling, optimization, isotherms, and kinetic evaluation. *Desalin Water Treat* 123:348–357.

Osouleddini N, Noei M, Rahimianshahreza N (2023) Employing metal-doped carbon nanocones for the drug delivery of purinethol anticancer: Insights from DFT calculations. *Diamond and Related Materials* 132:109641.

Osouleddini N, Tajik L, Moradi M (2019) Degradation of amoxicillin by persulfate activated with Fe_3O_4/GO nanocomposite in aqueous solution.. *Desalination and Water Treatment* 153:392–401.

Pankiewicz U, Góral M, Kozłowicz K, Góral D (2020) Application of pulsed electric field in production of ice cream enriched with probiotic bacteria (L. rhamnosus B 442) containing intracellular calcium ions. *Journal of Food Engineering* 275:109876.

Potter RF, D'souza AW, Wallace MA, Shupe A, Patel S, Gul D, Kwon JH, Beatty W, Andleeb S, Burnham C AD (2018) Superficieibacter electus gen. nov., sp. nov., an extended-spectrum β-lactamase possessing member of the Enterobacteriaceae family, isolated from intensive care unit surfaces. *Frontiers in Microbiology* 9:1629.

Rajaei G Ebrahimzadeh (2023) Removal of reactive red-P2B from aqueous solutions by montmorillonite clay; Kinetics, thermodynamic and isotherm studies. *Inorganic Chemistry Communications* 149:110386.

Rastegar SF, Osouleddini N (2017) TCNE-modified graphene as an adsorbent for N_2O molecule: a DFT study. *Journal of Molecular Modeling* 23:1–6.

Ricós-Muñoz N, Soler AR, Castagnini JM, Moral R, Barba FJ, Pina-Perez MC (2023) Improvement of the probiotic growth-stimulating capacity of microalgae extracts by pulsed electric fields treatment. *Innovative Food Science & Emerging Technologies* 83:103256.

Safarkar R, Rajaei G Ebrahimzadeh, Arjagi S Khalili (2020) The study of antibacterial properties of iron oxide nanoparticles synthesized using the extract of lichen Ramalina sinensis. *Asian Journal of Nanoscience and Materials* 3:157–166.

Sakamoto CM, Johnson KS, Coletti LJ (2009) Improved algorithm for the computation of nitrate concentrations in seawater using an in situ ultraviolet spectrophotometer. *Limnology and Oceanography: Methods* 7:132–143.

Sardari R, Osouleddini N (2018) The data on the removal of turbidity and biological agents in spent filter backwash by bed ceramic in water treatment process. *Data in Brief* 19:1794–1798.

Shirkavand B, Abnili M, Rahmani Z, Salmi R, Behbahani P Rezaei (2023) The process of removing paratoleic acid by advanced oxidation process from the effluent of terephthalic acid production in textile fiber production industries. *Journal of Textile Science and Technology* 12:17–26.

Walter L, Knight G, Ng SY, Buckow R (2016) Kinetic models for pulsed electric field and thermal inactivation of Escherichia coli and Pseudomonas fluorescens in whole milk. *International Dairy Journal* 57:7–14.

Yang X, Zhuo L, Xie P, Huang H, Feng B, Wu P (2021) Physical versus economic water footprints in crop production: a spatial and temporal analysis for China. *Hydrology and Earth System Sciences* 25:169–191.