Influence of tornado plasma gliding arc discharge on the bacteria

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Abstract
Using tornado plasma gliding arc discharge system of non-thermal atmospheric pressure that ran frequency 9.1(kHz), AC power supply high voltage 8 kV, argon gas flow rate of two liters per minute and use magnetic coil contains 500 turns and two electrodes. The current study aimed to detect the effect of tornado plasma gliding arc discharge on the bacteria. Use three types of bacteria is Staphylococcus aurous (Gram-positive), While salmonella and Escherichia coli (Gram-negative). Each type of bacteria is subdivided into six groups one control and five treated with tornado plasma gliding arc discharge for times (10,15,20,25, and 30 min). The results of the study revealed a large rise ($P<0.05$) in the rate of killing of bacteria treated with tornado plasma compared with control group. It can be noted that increasing the exposure time leads to a decrease in the number of bacteria colonies.
Keywords: Tornado plasma gliding arc discharge, Salmonella, Escherichia coli, Staphylococcus aurous.

1- Introduction

The use of non-thermal plasma, particularly gliding arc (GA) discharge, has caught the interest of engineers andscientists because it blends large energies, to maximize reactor efficiency, with ahigh level ofimbalance, for supporting of specific chemical processes [1]. Commonly, plasma is an ionized gas. It involves the merging of molecules, ions, and electrons with charges [2]. For the purpose of reducing pollution, it has been designed to create non-thermal plasma using gliding arc discharges at atmospheric pressure [3]. The plasma column is extended by a gas flow when the discharge ignites at; the narrowest distance between (2) diverging electrodes. [4, 5]. The plasma discharge alternate between the imbalanced phase and the quasi-equilibrium phase, both of which are steady discharges connected to the thermal ionization effect [6, 7, and 8]. Numerous plasma processing techniques, such as surface modification of various materials, air treatment and water treatment, heavily rely on gliding discharge [9]. A big plasma zone, less heat loss, and lots of active species are benefits of GA reactors [10].

In this paper study the effect of the gliding arc tornado plasma on the bacteria. The technique of plasma sterilization is difficult because multiple bactericidal agents generated by plasma can engage in interactions with the bacteria being treated and may lead to its death, for example, species which includes reactive species that produce electron-impact excitation and dissociation in non-thermal plasma. [11]

Escherichia coli is a bacteria that can be found in both human and animal digestive tracts. E. coli bacteria come in over 700 different strains, many of which are unharmful. However, some strains of E. coli, known as enterohemorrhagic E. coli (EHEC), can result in death due to kidney

failure, severe anemia, bloody diarrhea, or urinary tract infections. [12].The bacteria Escherichia coli is regarded as a pathogen that is resistant to multiple drugs. Which has resulted in the development of new strains and is accompanied in Iraq by a lack of molecular studies that could aid in our understanding of the disease's mechanism, its causes, and strategies for containing it [13, 14]. Staphylococcus aureus is a spherical, gram-positive bacteria with a diameter of roughly 1 m. As cell division occurs in multiple planes, its cells group into clusters resembling grapes. Numerous studies have linked commensal bacteria to skin, skin glands, and mucous membranes, particularly in healthy people's noses. Twenty to thirty percent of the population are thought to be S. aureus carriers. [15,16]. These bacteria are regarded as one of the major hospital and community pathogens that can cause a variety of infectious disorders, including bacteremia, soft tissue infections, minor skin infections, infective endocarditis, osteomyelitis, and deadly pneumonia [17,18]. The Salmonella is a type of rod-shaped, gram-negative, facultative anaerobic bacillus that belongs to the Enterobacteriaceae family. According to estimates, this genus split off from Escherichia coli (E. coli) between 100 and 150 million years ago [15]. Only Gram-negative microbes, that have a thin murein layer and external thin membranes, can function via this technique. Gram-positive microbes have a murein layer that is noticeably thicker despite not having an outer membrane.which gives these bacteria greater strength and stiffness, will not experience the situation. [19]. In contrast, in plasma sterilization procedures, no appreciable bacterial cells are expected to experience temperature impacts. Low temperatures (room temperature) are required for the nonthermal plasmas to operate. Thus making the temperature of the samples under treatment close to the ambient temperature or at least below known value which causes cell damage [20].

Materials and Methods

1- Experimental part

The gliding arc tornado plasma discharge system which designed and constructed locally was used to treat a different various bacteria such as Salmonella; Escherichia coli & Staphylococcus aureus.

The tornado plasma which generated from this system was used to treat types of bacteria that shows in figure (1). The system works with frequency 9.1 kHz, Ac power supply with high voltage 8 kV, argon gas flow rate of two liters per minute and it contains magnetic coil with 500 turns. The two electrodes of coil were connected with DC power supply.

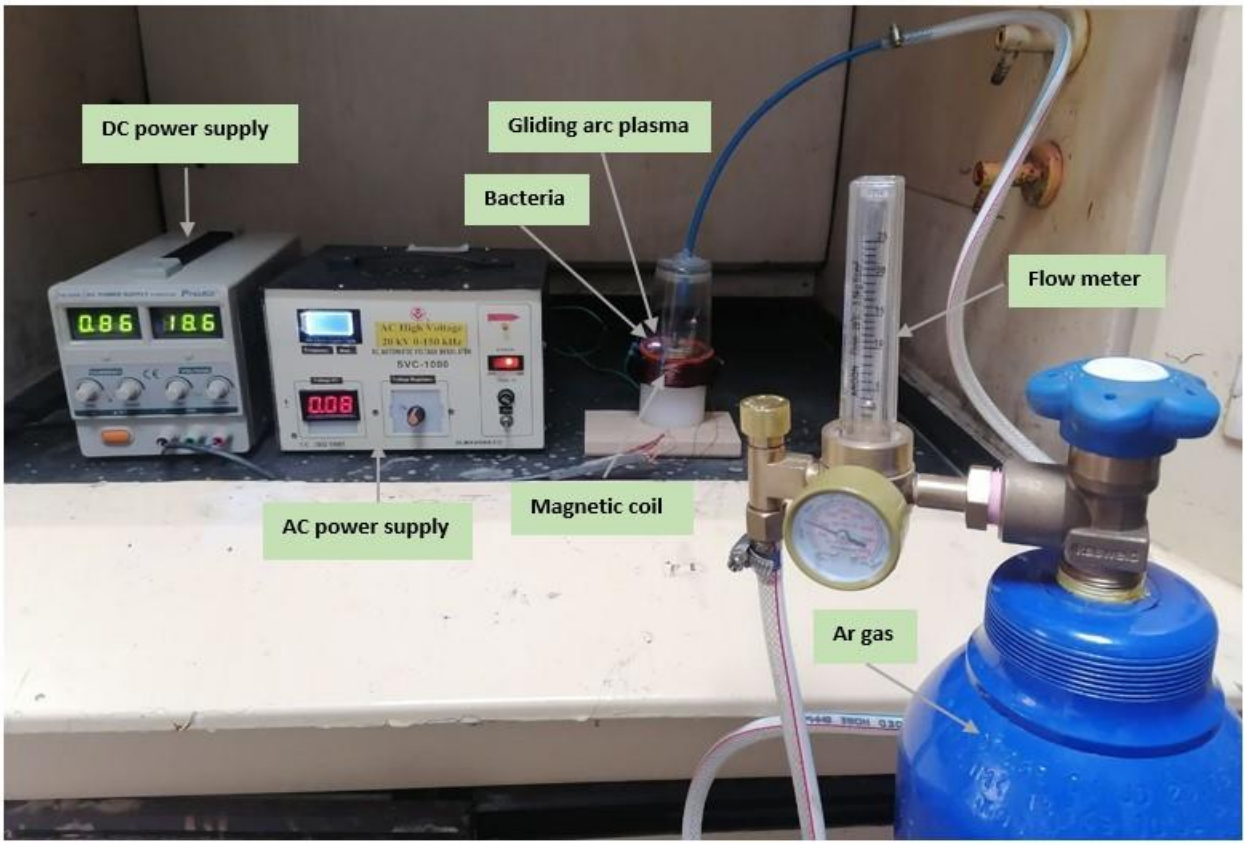

Fig .1 a photograph of the tornado plasma gliding arc discharge system.

2- Sample Preparation

This study included three different kinds of microorganisms. Salmonella and Escherichia coli are both Gram-negative, while Staphylococcus aureus is a Gram-positive organism. The bacteria were obtained from the laboratories biology department; College Of Science;University Of Baghdad. The pure colony of each microbe was isolated using the Viable Count technique.

3-Viable Count

The viable plate count is the method used the most frequently to count the number of bacteria. This technique involves plating successive dilutions of a live-microorganism sample onto a suitable growing medium. Spreading the suspension on agar plates' surface is known as the "spread plate method," whereas the "pour plate method" involves pouring the mixture onto plates after combining the suspension with molten agar, and letting it set. After that, the plates are incubated under microbial reproduction-friendly conditions so that colonies grow after mixing the suspension with molten agar, pouring the mixture on to plates As a result, the quantity of bacteria in the initial sample can be determined through counting the number of colonies and taking into consideration the dilution factor [21].

4- Viable Plate Count

1. There were labels on each of the four 9.9 ml saline tubes (10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8}).
2. Vortex the sample to ensure that the bacteria are distributed evenly. Using a sterile pipette, aseptically remove 0.1 ml of the sample and transfer it to the 10^{-2} dilution tube (as illustrated in Fig. (2)).
3. Following the 10^{-2} tube's vortexing, transfer 0.1 ml to the 10^{-4} tube.

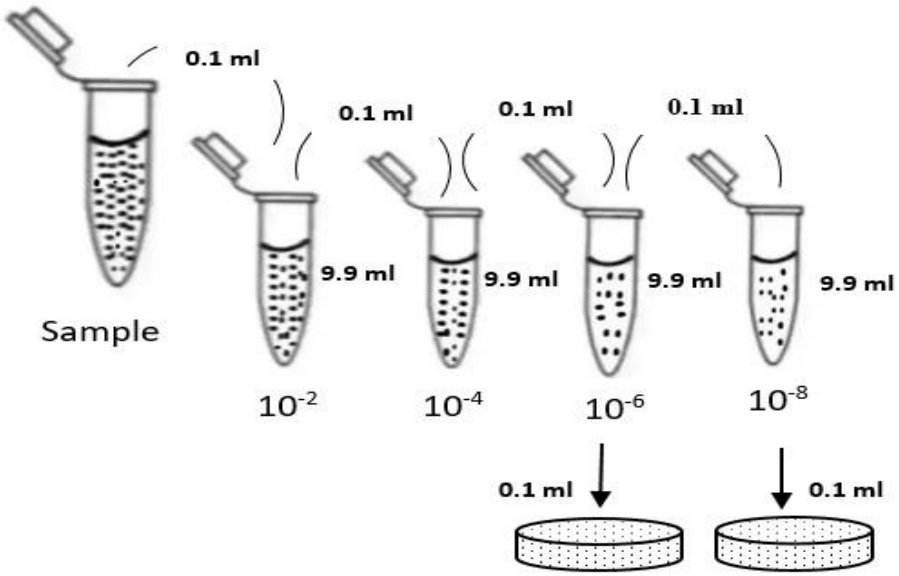

Fig. 2 diagram the serial of dilution

4. After a second vortexing of the 10^{-4} dilution tube, add 0.1 ml to the 10^{-6} tube. Well, this last tube is a vortex.
5. Centrifuge the 10^{-6} tube once more after adding 0.1 ml to the 10^{-8} tube.
6. Using anew, sterile pipette, aseptically transfer 1.0 ml from the 10^{-4} dilution tube to the plate marked 10^{-4} and 0.1 ml to the plate marked 10^{-5}. Utilizing a metal spreader that has been flamed and dipped in alcohol, spread the inoculum over the agar surface in each plate. After each spreading, quickly pass the spreader through the flame while dipping it into the alcohol jar. Let the alcohol burn off. Keep the spreader from getting too hot. The spreader should never be left in the flame for longer than a second.
7. Before you move or invert the plates, agar's surface should have time to dry. Plates were kept at 37 °C for two days.
8. Dilutions are used (10^{-6}, 10^{-8}) to calculate the percentage of killing bacteria.

5- Spread Plate Technique

The spread plate method can be used to distribute and measure every bacterium in a culture mix equally. The technique makes it easier to determine how many bacteria are present in a solution.

6- Principle of Spread Plate Technique

The method known as spread plate is using a sterilized spreading with a smooth metal or glass surface to evenly distribute a small amount of germs floating in a solution on a plate. The surface of the plate needs to be dry and at room temperature in order for the agar to further readily absorb the bacteria. If an identifiable amount of separate colonies of bacteria are evenly distributed on the spread plate, it will be good [22].

7- Statistical Analysis

The analysis of all data was performed using one-way analysis of variance (ANOVA-test), and the results were provided as means standard deviation means ± SD [23].

Result and discussion

The contaminated water was subjected to plasma exposure with a gas rate of flow (2 L/min) for different exposure times of (10, 15, 20, 25 and 30 min). Figures (4, 6 and 8) demonstrate how the plasma generated by a tornado's gliding arc discharge has affected on Staphylococcus aureus,Escherichia coli & Salmonella bacteriacolonies; respectively. The data show that the killing rates have increased with increased during the period when the plasma was exposed to contaminated water.

When exposed to gliding arc discharge plasma, the number of Salmonella bacteria colonies start at 12.00 ± 0.00 at control and this value decreases to 8.53 ± 0.20 at 10 min and at 15,20 min the value was 3.84 ± 0.10, 5.40 ± 0.00 respectively and reached to zero at (25-30) min. the number of Staphylococcus aureus bacteria colonies at control the value was 11.00 ± 0.00 and this value decreases to 7.87 ± 0.02 at 10 min and at 15,20,25 min the value was 4.38 ± 0.01, 1.44 ± 0.00, 0.53± 0.00 respectively and reach to zero at 30 min. while the number of E-coli bacteria colonies was 10.00 ± 0.00 at control and at 10,15 min this value decreases to 4.90 ± 0.01, 1.57 ± 0.17 respectively and reach to zero at 20,25,30 min). Compared to other forms of bacteria, Staphylococcus aureus is more resistant; within 25 minutes, just a few colonies were removed. When compared to other forms of bacteria, E. coli bacteria were the most affected. After 20 minutes, no sign of E. coli colonies is visible. As the exposure duration increases, the colonies' density gradually declines these agree with T A Hameed et al [24].

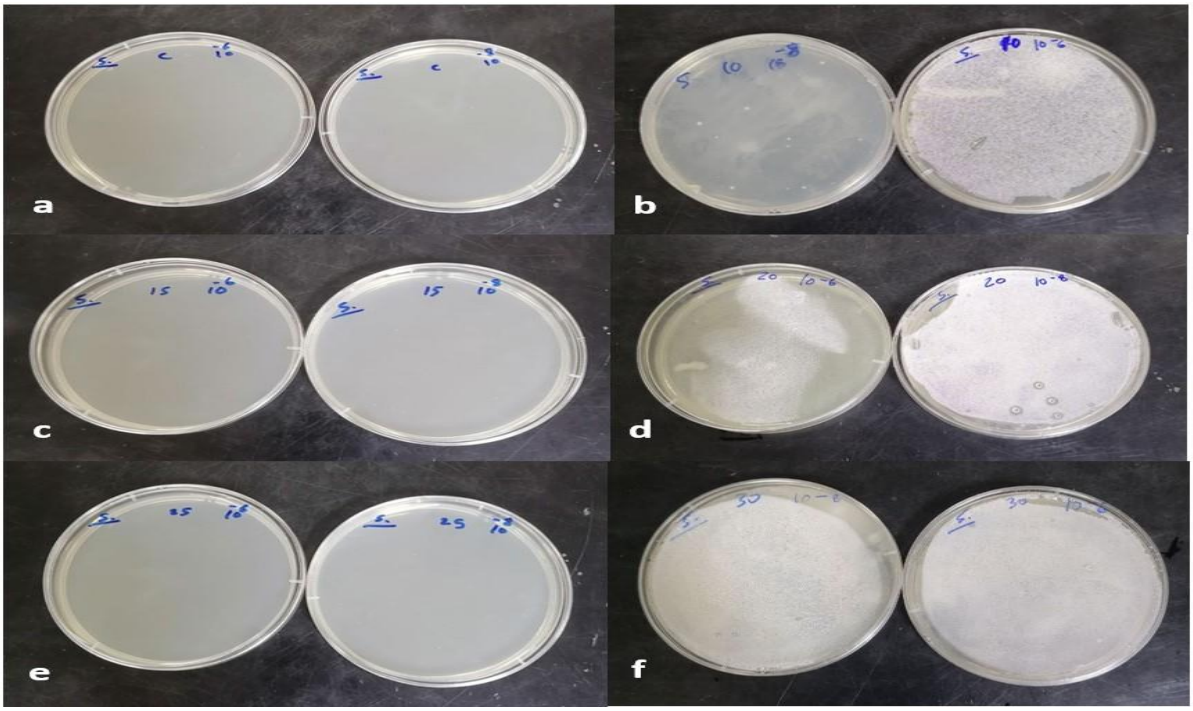

Fig.3 The picture showing the inactivation of the Salmonella bacteria at various times: a) control, b) 10min, c) 15min, d) 20min, e) 25min, and f) 30 min

Mean and standard deviation of the reduction in the growth rate of Salmonella in the suspension media after exposing to the gliding arc plasma at various time points was shown in table (1).

Table 1. Influence gliding arc plasma tornado of various times in Salmonella cell NO.

Time (min)	No	Cell viability ± SE x 10^4 (cell/ml)
Control	3	12.00 ± 0.00
10	3	8.53 ± 0.20
15	3	5.40 ± 0.00
20	3	3.84 ± 0.10
25	3	0.00± 0.00
30	3	0.00± 0.00
LSD value	---	0.330 **
** (P<0.01).		

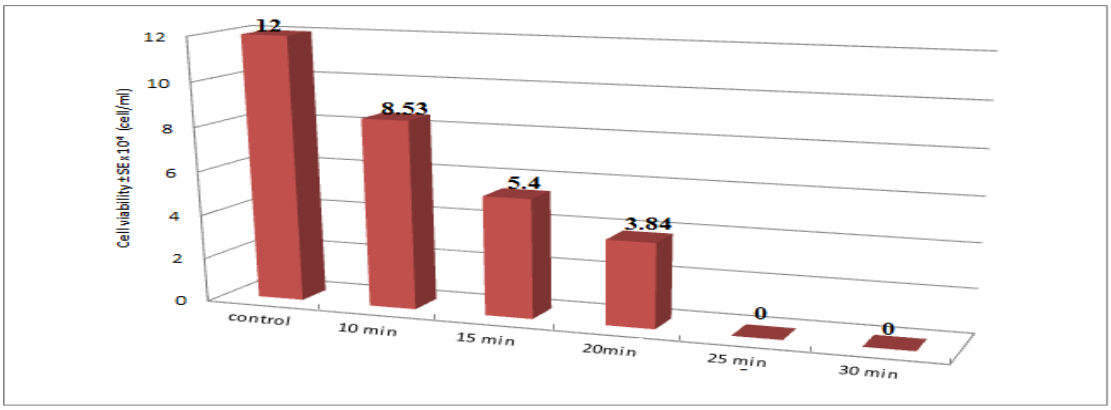

Fig.4 Histogram of the impact of gliding arc plasma at various times on Salmonella viability in cell Number.

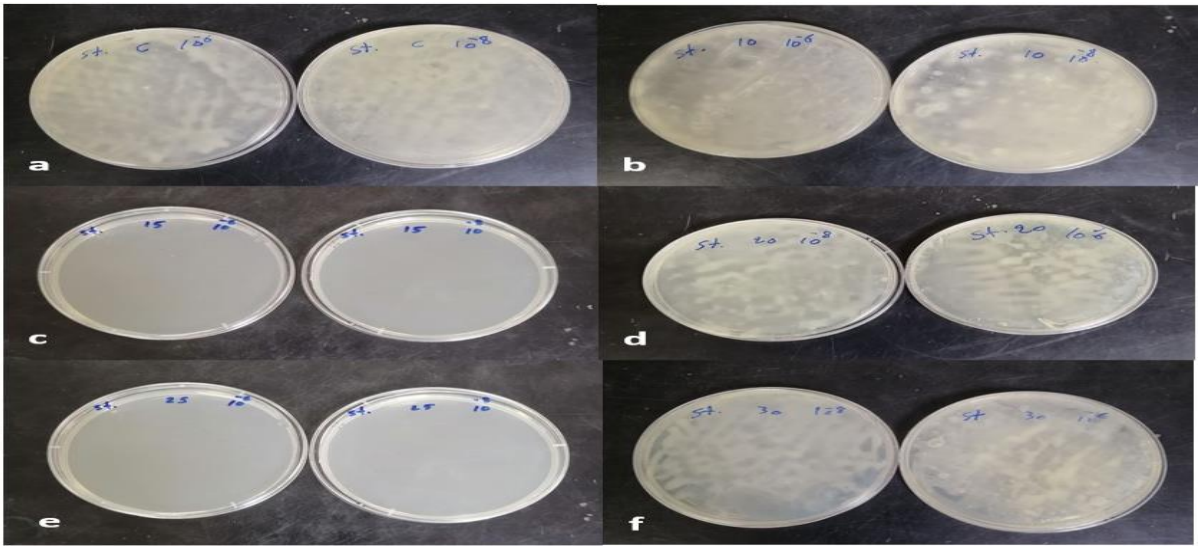

Fig .5 Image showing the inactivation of Staphylococcus aureus at various times**;** a) control; b) 10 min; c) 15 min; d) 20min; e) 25 min and f) 30 min.

Mean and standard deviation of the reduction in the growth rate of *Staphylococcus aureus* in the suspension media after exposing to the gliding arc plasma at various time points was shown in table (2).

Table 2. Influence gliding arc plasma tornado at various times in Staphylococcus aureus cell NO.

Time (min)	No	Cell viability ± SE x 10^4 (cell/ml)
Control	3	11.00 ± 0.00
10	3	7.87 ± 0.02
15	3	4.38 ± 0.01
20	3	1.44 ± 0.00
25	3	0.53± 0.00
30	3	0.00± 0.00
LSD value	---	0.035 **
** (P<0.01).		

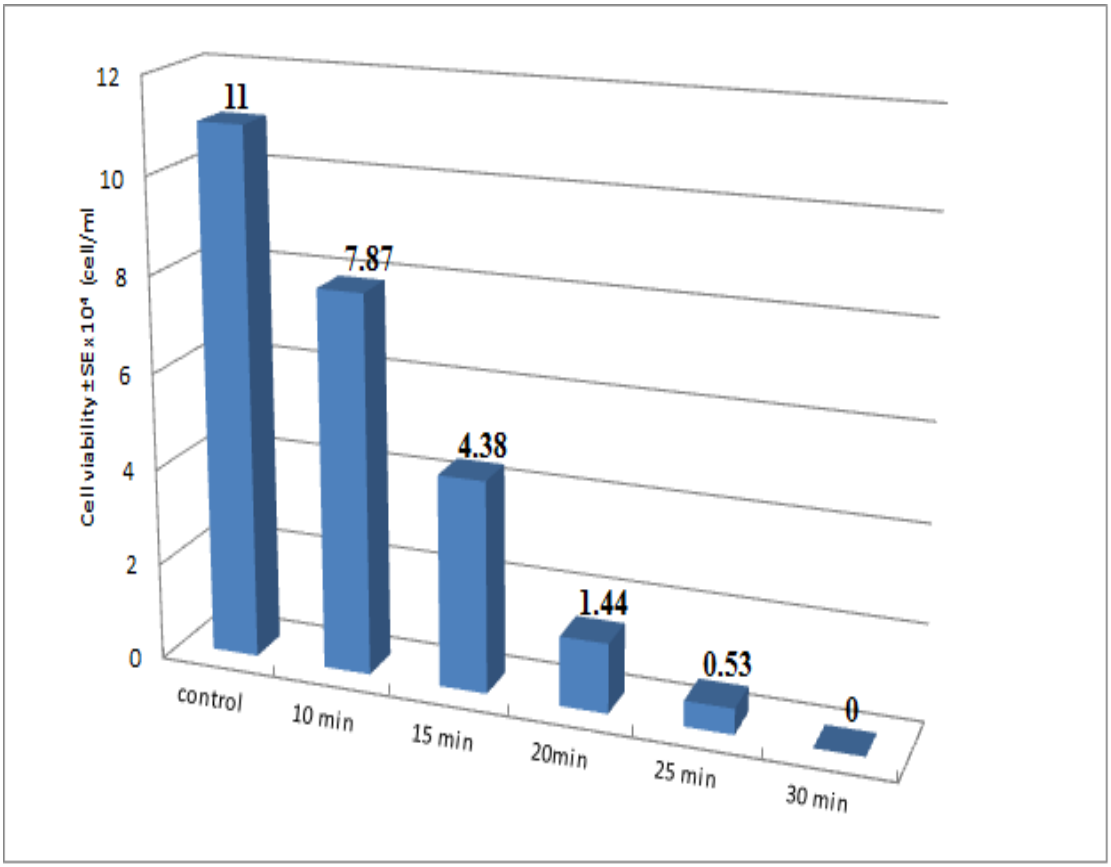

Fig.6 Histogram of the impact of gliding arc plasma at various times on Staphylococcus aureus vitality in cell Number.

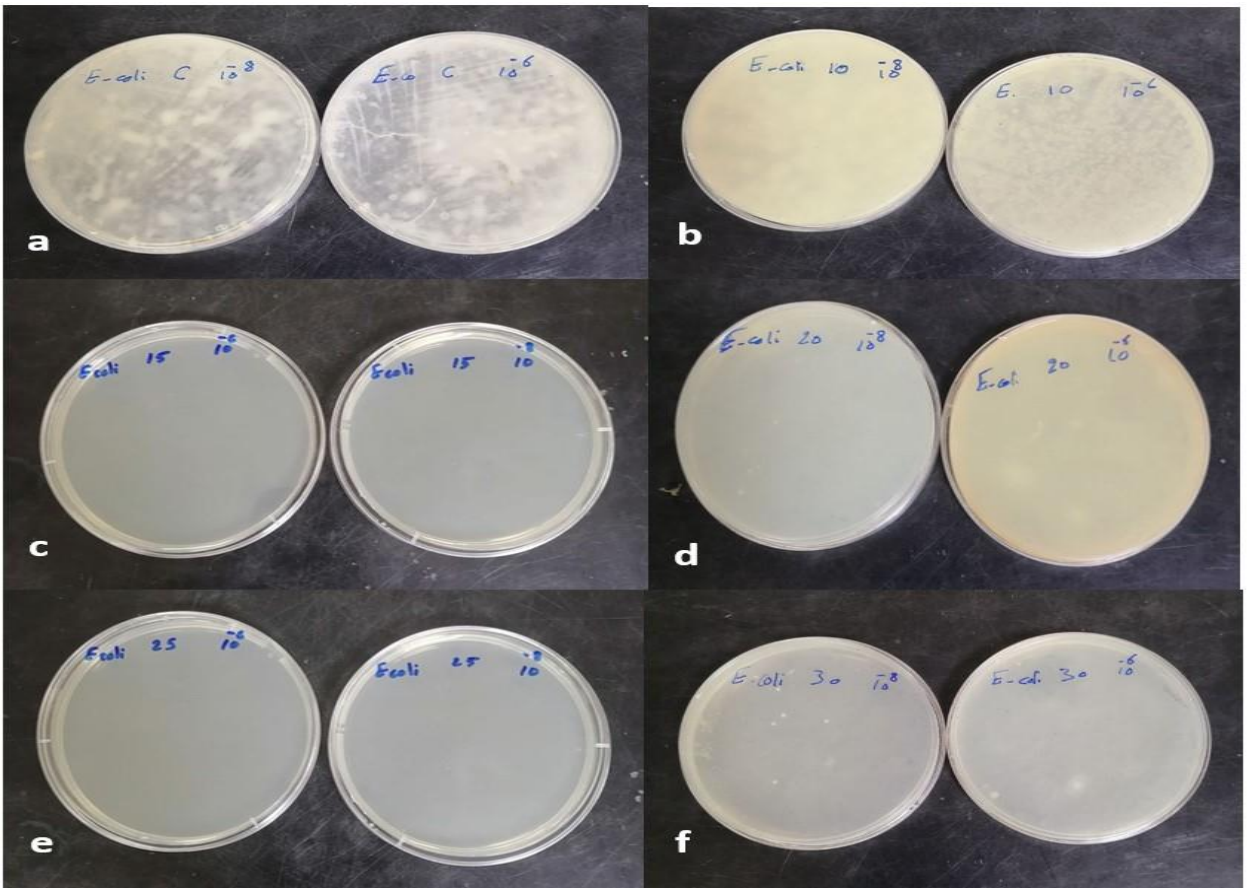

Fig .7 The Picture showing the inactivation of the Escherichia coli bacteria at various times : a) control; b) 10 min; c) 15 min; d) 20 min; e) 25 min and f) 30 min.

Mean and standard deviation of the reduction in the growth rate of Escherichia coli in the suspension media after exposing to the gliding arc plasma at different time points was shown in table (3).

Table 3. Influence gliding arc plasma tornado at various times in Escherichia coli cell number.

Time (min)	No	Cell viability ± SE x 10^4 (cell/ml)
Control	3	10.00 ± 0.00
10	3	4.90 ± 0.01
15	3	1.57 ± 0.17
20	3	0.00 ± 0.00
25	3	0.00± 0.00
30	3	0.00± 0.00
LSD value	---	0.288 **
** (P<0.01).		

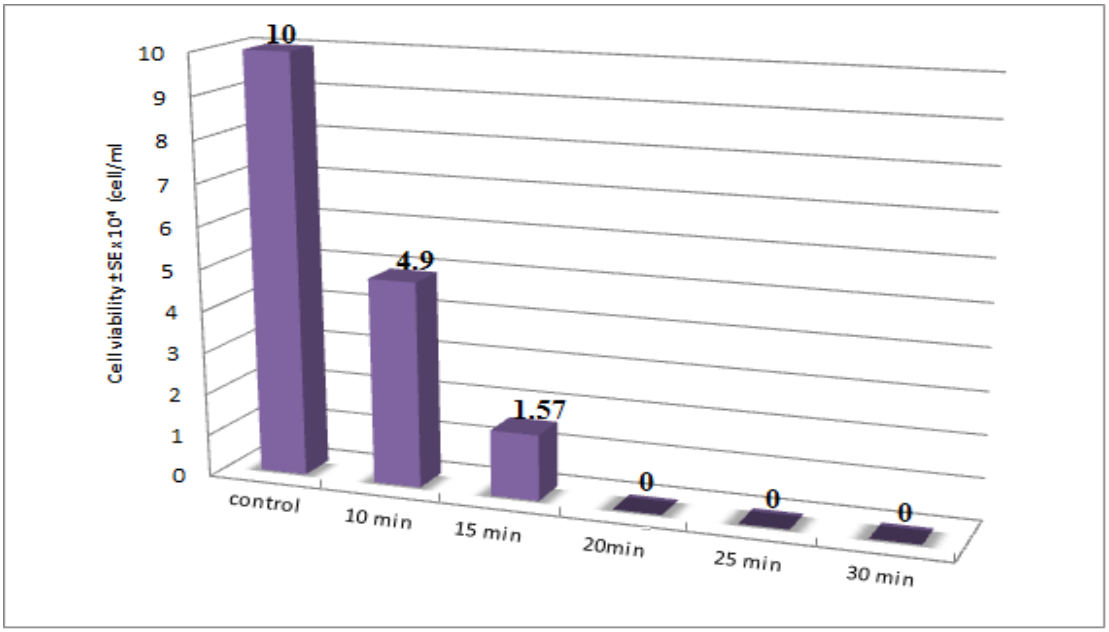

Fig. 8 Histogram Influence of gliding arc plasma at various times on Escherichia coli cell viability.

Conclusion

The treatment of biologically polluted water using the gliding arc discharge method is quite effective. This technique can treat a lot of water. The effect tornado plasma gliding arc discharge on the bacteria caused increase in the rate of killing with time exposure to reach zero at (30 min in Staphylococcus aureus, 25, 30 min in Salmonella and 20, 25, 30 min in Escherichia coli. When the time exposure increase the number of bacteria colonies decreased.

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