



## INFLUENCE OF COLD PLASMA JET CONSTRUCTION IRRADIATION ONTO GROUPS OF STAPHYLOCOCCUS AND ESCHERICHIA COLI FOR HUMAN SKIN

Kadhim A. Aadim ,Ghaith H. Jihad

Department of Physics, College of Science, University of Baghdad, Iraq

E-mail: [kadhim\\_adem@scbaghdad.edu.iq](mailto:kadhim_adem@scbaghdad.edu.iq) , [ghaith.jihad1104@sc.uobaghdad.edu.iq](mailto:ghaith.jihad1104@sc.uobaghdad.edu.iq)

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<p><b>Received:</b> September 30<sup>th</sup> 2021 <b>Accepted:</b> November 1<sup>st</sup> 2021 <b>Published:</b> December 7<sup>th</sup> 2021</p>	<p>Cold atmospheric pressure plasma jets were capable of generating cold plasma plumes that were not confined by electrodes, which make them very enticing for biological, medical and polymer applications. A direct-current, cold atmospheric-pressure Argon plasma microjet (PMJ) was performed to inactivate <i>Staphylococcus aureus</i> (<i>S. aureus</i>) and <i>Escherichia coli</i> (<i>E. coli</i>) in air. The process of sterilization and morphology of bacteria was observed. We wish to know the possible inactivation mechanisms of PMJ and explore a potential application in dental and other temperature sensitive treatment. In this study, we employed a direct current, atmospheric pressure, cold Argon-gas to inactivate bacterias .electronic image was employed to evaluate the morphology of <i>S. aureus</i> and showed rupture of groups after the plasma treatment.</p> <p>During this work, experimental study of a low frequency, atmospheric plasma-jet discharge in argon has been presented. The experimental operation of this device was conducted with commercially Argon power supply. The discharge process operated by using Argon as input gas with different Voltages and different metals (Sn , Cu, Pb). The inactivation rates could reach 100% in 5 min. When the distance between the exit nozzle of the PMJ device and Petri dish was about 1.5cm , effective inactivation was also observed with a similar inactivation curve.</p> <p>The inactivation of bacteria is attributed to the abundant reactive oxygen and nitrogen species, as well as ultraviolet radiation in the plasma. Different life spans and defensibilities of these killing agents may hold the key to understanding the different inactivation curves at different treatment distances.</p>

**Keywords:** Atmospheric plasma jet, free radicals, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*).

### INTRODUCTION

LOW-TEMPERATURE plasma jets that are emitted in surrounding air proved to be very useful for biomedical applications [1],[2]. One of the great advantages of these plasma sources is the fact that they can generate a stable and controllable thin column of plasma outside the confinement of electrodes and into the surrounding environment. This is crucial in the case of medical applications since the ultimate goal is to treat actual patients. In addition, because the plasma propagates away from the high-voltage region and into a region where there is no externally applied electric field it is electrically safe and does not cause electrical shock/damage to the treated sample or exposed person. This property in combination with the low gas temperature renders plasma jets the devices of choice when it comes to

exposing biological cells or tissues [3]. The temperature of the neutral particles and ions is in about room temperature and suitably can interact with living biological cell without damaging the cell [4]. Important properties of this type of plasma are that it operates near room temperature, allows treatment of irregular surfaces and has a small penetration depth. These characteristics give the needle great potential for use in the biomedical field[5]. Several experiments have shown that the plasma needle is capable of bacterial decontamination [6] and of localized cell removal without causing necrosis to the treated or neighbouring cells [7]. In this study, we employed a direct current, atmospheric pressure, cold Argon-gas to inactivate bacterias .electronic image was employed to evaluate the morphology of *S. aureus* and showed rupture of groups after the plasma treatment.Different

inactivation rates were observed when the distance between the PMJ and Petri dish was extended from 1 cm to 3 cm. Electronic image and concentration of **METHODOLOGY**

groups were used to understand the possible inactivation mechanisms of PMJ.

Figure 1 illustrates the work steps for this research.

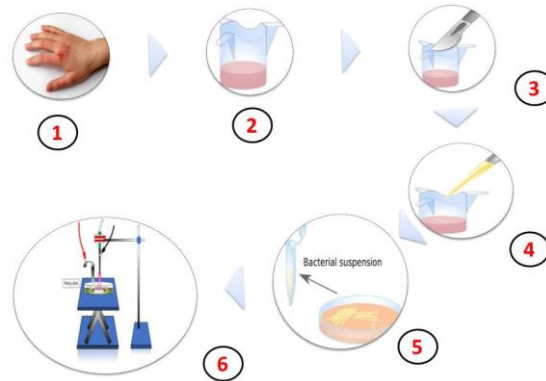


Figure 1: Diagram of the experimental frame work of the plasma jet

- 1- A sample of the hand of an adult human with second-degree burns.
- 2- Preparation of bacterial medium for growth.
- 3- Taking a sample of bacteria from the surface of the affected hand.
- 4- Cultivation of these bacteria in the prepared medium for growth.
- 5- A sample was taken from the medium and placed in Petri dish to be treated later.
- 6- Then the samples are exposed to jetting plasma of different voltages and using several metals to eliminate bacterial aggregates.

### PLASMA JET DEVICE

The plasma jet setup is consisting of two electrode one act as anode and the second act as cathode which immersed inside the petri dish. The high-voltage electrode is completely embedded in the device and powered by a DC power supply with varying voltages. The outer electrode is grounded for safety considerations. A schematic diagram of the device can be founded in Figure (2). For detailed plasma device operation, please refer to references

[2],[3]. We used compressed Argon gas as the working gas at a flow rate of approximately 4 L/min. The discharge sustaining voltage was range about (630, 860 and 1360)V with an operating current of 0.3 mA. Under these operating conditions, a PMJ of  $\leq 1.5$  cm visible length was generated. The gas temperature 1 cm away from the nozzle was measured to be around 38°C using a thermometer. Since the gas temperature decreased when the distance became longer.

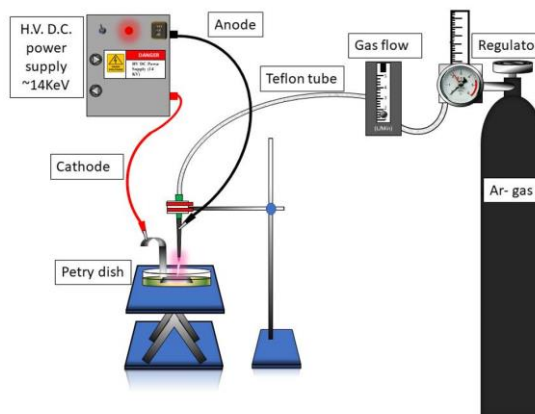


Figure 2 A schematic diagram of the device of PMJ sustained in Argon gas.

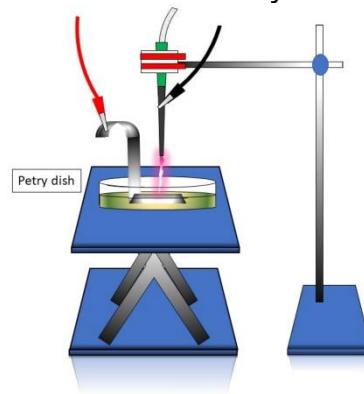
### MEDICAL APPLICATION (BACTERIA FOR HUMAN SKIN)

*S. aureus* and *E. coli* were cultured in Luria-Bertani (LB) medium for 14-20 h until logarithmic growth phase. The suspensions were then diluted to a concentration of  $10^{10}$  CFU (colony forming units) per mL, of which 150  $\mu$ L suspensions was spread uniformly on a LB agar culture medium in a Petri dish (90 mm in diameter) for plasma treatment and analysis.

After the treatment, plates were sealed and cultured in the incubator for 18-21 h at 37°C (*S. aureus*) or 30°C (*E. coli*). Subsequently, a CFU count was obtained on the Petri dish. The inactivation rate of the bacteria was defined as the percentage decrease in CFU counts of the plasma treated sample to that of the control.

### PLASMA TREATMENT

A diagram of the treatment setup is shown in Figure (3). Treating distance was defined as the distance from the exit nozzle of the PMJ device to the surface of the Petri dish. Three voltages (630, 860 and 1360)V were used in this study with different metals (Sn, Cu, Pb) distinct treating at constant distance 1.5 cm. The plasma treatment was limited to a 2 cm $\times$ 2 cm square area in the center of the Petri dish (referred to as the "treated area") (Figure (2)). For each bacteria sample, the Petri dish was moved under the plasma torch with a constant speed of gas flow 4 L/min along the gridlines indicated in Figure (2) for a period of 30 s for *S. aureus* and *E. coli*. The total treating time ranged from 30 s to 1 min. The experiment was repeated at least three times to obtain an average inactivation rate and a measure of variance. The control group was subjected only to gas flow at the same flow rate.

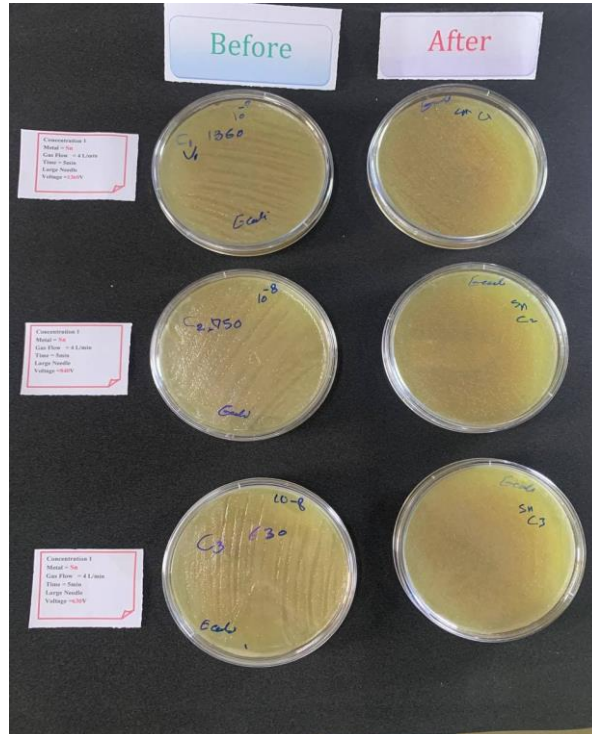


**Figure 3** Schematic diagram of the PMJ treatment of a Petri dish

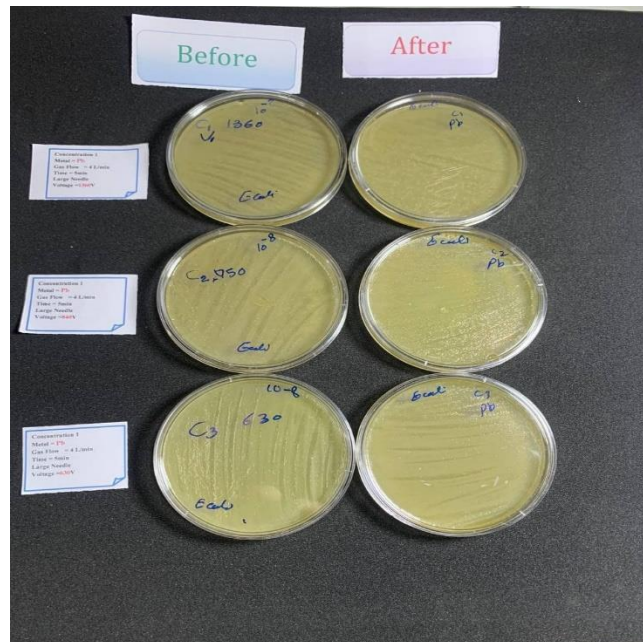
### ELECTRONIC CAMERA IMAGING

Bacterial suspensions (20 mL) before and after treatment for different voltage and different metals. The supernatant was discarded and the remaining film was treated by plasma (treated group) or Argon gas

(control group). The sample Lead have more efficient effect as shown in the following figures (4-a,b,c and 5-a,b,c):-

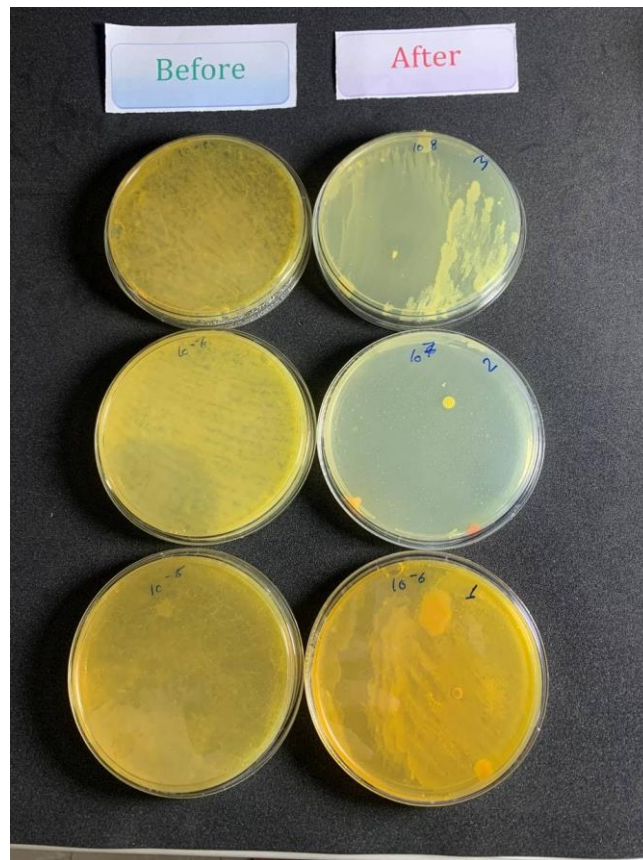


**Figure 4-a** image of E.coil before and after treatment plasma using Sn metal with different voltages

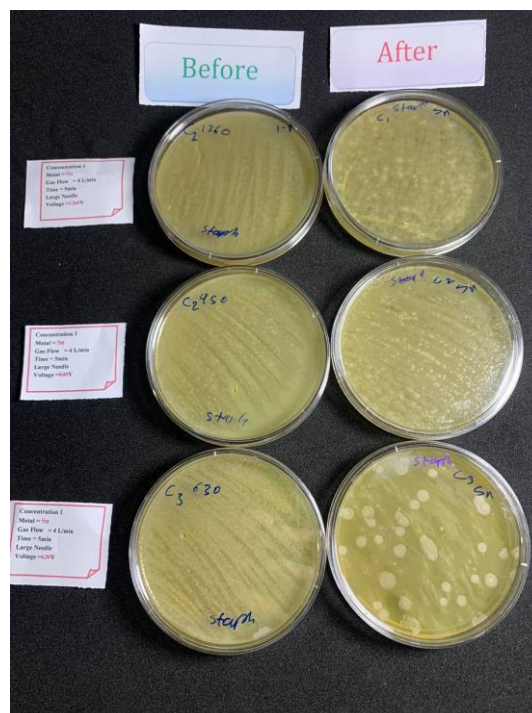


**Figure 4-b** image of E.coil before and after treatment plasma using Pb metal with different voltages

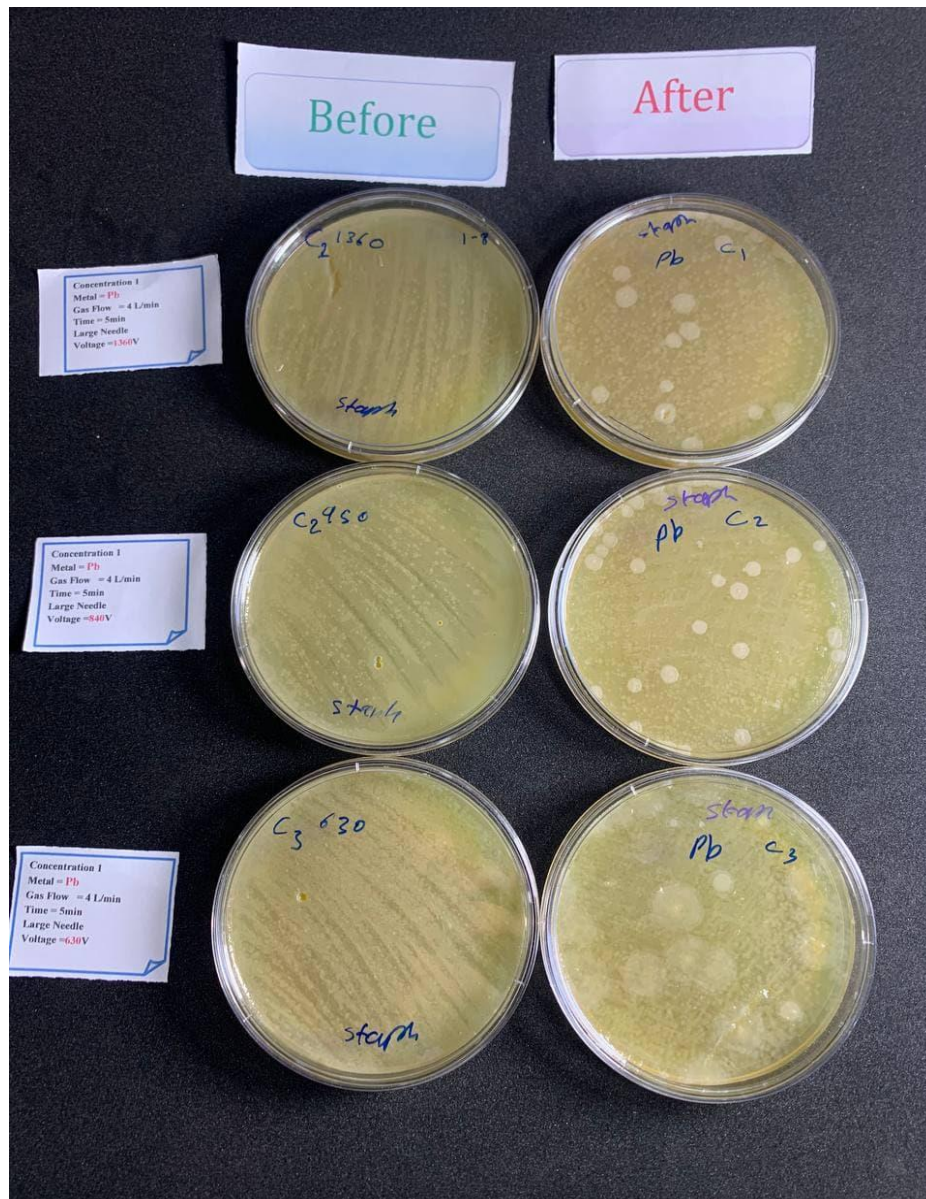




**Figure 4-c** image of E.coli before and after treatment plasma using Cu metal with different voltages

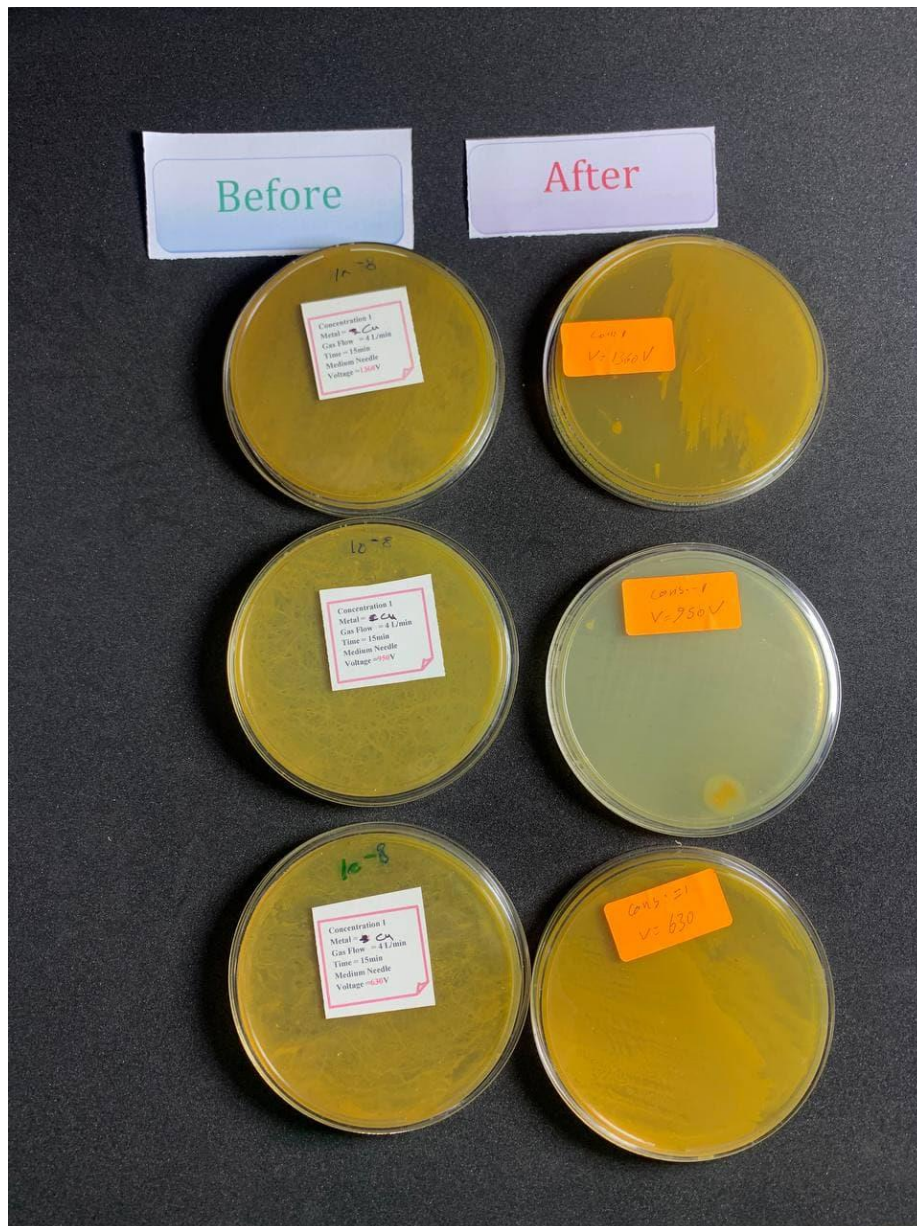


**Figure 5-a** image of S. aureus before and after treatment plasma using Sn metal with different voltages



**Figure 5-b** image of *S. aureus* before and after treatment plasma using Pb metal with different voltages





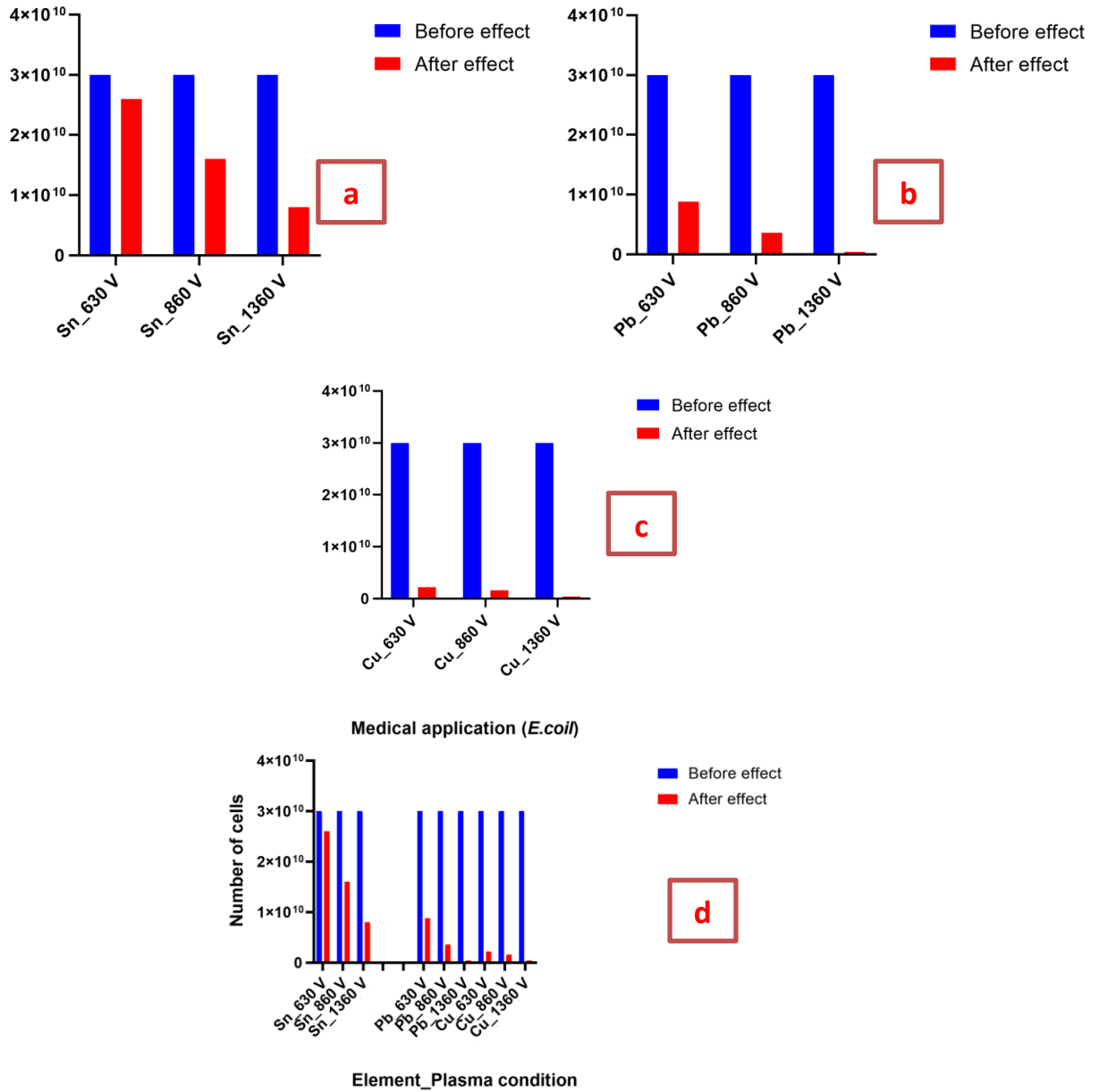
**Figure 5-c** image of *S. aureus* before and after treatment plasma using Cu metal with different voltages

## THE RESULTS AND DISCUSSION

### Inactivation rates of *E. coli*

The inactivation rate of the bacteria was defined as the percentage decrease in CFU counts following treatment. Fig shows the inactivation rates of *E. coli* at a treatment distance of 1.5 cm. Both inactivation rates reached 100% in 1 min in the treated area. Bacteria in other regions on the Petri dish were also inactivated. A 100% inactivation rate on the

whole Petri dish was achieved in 1360 Voltage with Cu –metal at 1 min, while the less active of the treatment using Sn metal and the effect will be shown in Fig 6a , 6b ,6c and 6d . This result was consistent of the inactivation of *E. coli* with different voltage by PMJ were studied [2]. The slightly different inactivation effect of plasma on *E. coli* was speculated that there were differences in the resistance to inactivation of the different metals and voltages.



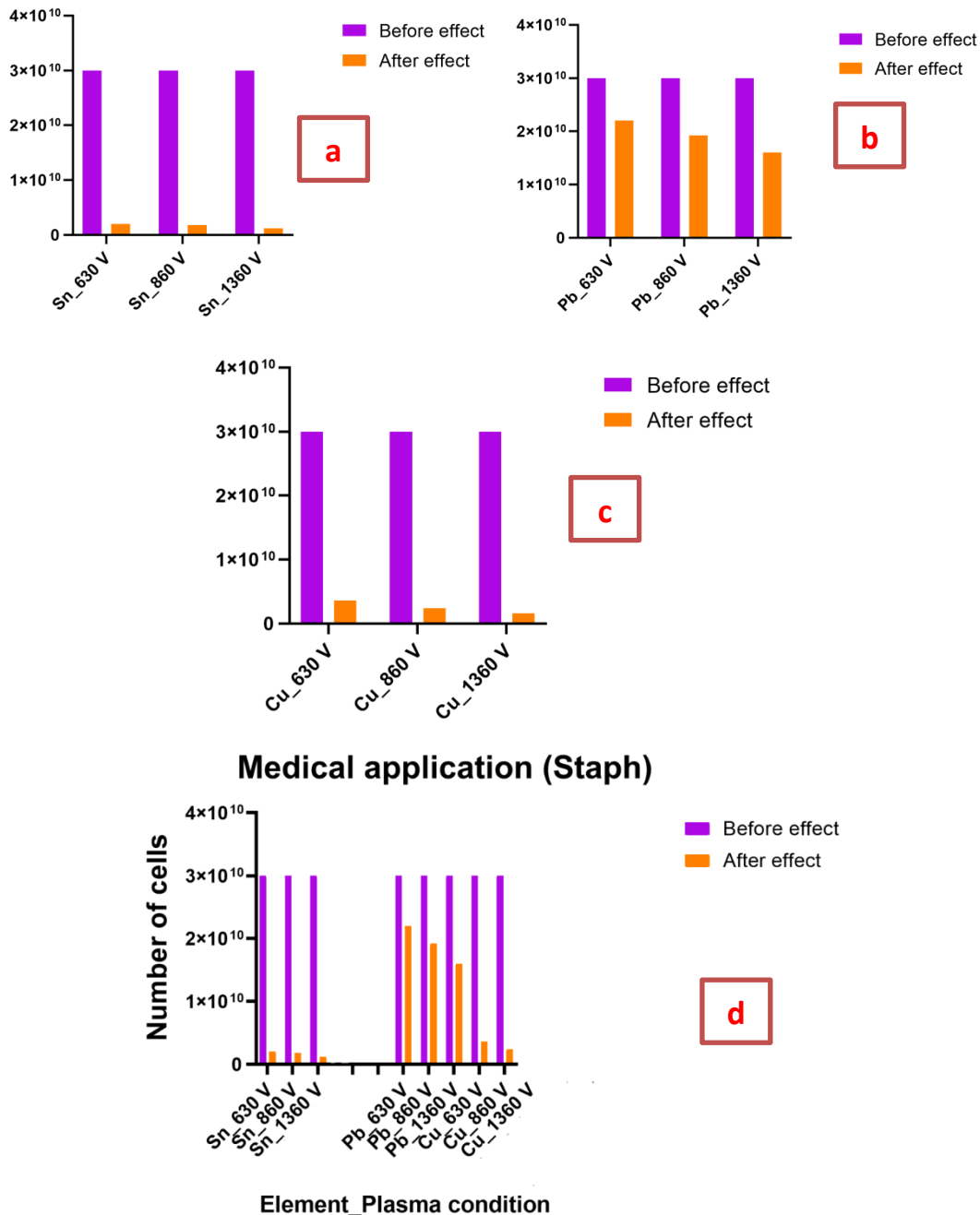
Figures (6) The inactivation rate of E.coil at different treating voltage (630 V , 860 V and 1360 V) and different metal where:- (a) using Sn metal (B) using Pb metal (C) using Cu metal (D) the compares effect of three different metals

### Inactivation rates of *S. aureus*

The inactivation rate of the bacteria was defined as the percentage decrease in CFU counts following treatment. Fig shows the inactivation rates of *S. aureus* at a treatment distance of 1.5 cm. Both inactivation rates reached 100% in 1 min in the treated area. Bacteria in other regions on the Petri dish were also inactivated. A 100% inactivation rate on the whole Petri dish was achieved in 1360 Voltage with Cu

–metal at 1 min, while the less active of the treatment using Sn metal and the effect will be shown in Fig 7a , 7b ,7c and 7d . This result was consistent of the inactivation of *S. aureus* with different voltage by PMJ were studied [6]. The slightly different inactivation effect of plasma on *S. aureus* was speculated that there were differences in the resistance to inactivation of the different metals and voltages.





Figures (7) The inactivation rate of *S. aureus* at different treating voltage (630 V , 860 V and 1360 V) and different metal where:- (a) using Sn metal (B) using Pb metal (C) using Cu metal (D) the compares effect of three different metals

*S. aureus* and *E. coli* follows different inactivation curves in the treated area and on the whole Petri dish, which may be attributed to the different distributions and diffusibilities of various germicidal agents, including charged particles, free radicals and excited neutrals. The treated area received more plasma jet as well as

more charged particle bombardment than in the periphery, which could partly account for the quick inactivation of bacteria in the treated area. However, some radicals have a longer lifetime and can thus travel laterally to the untreated area, leading to the gradual inactivation there. The inactivation of bacteria



at 630 v cm was slower than at 130 v. A longer treating distance means higher recombination rates of charged particles, leaving the main agent to be some long-lived radicals. The indirect and long-distance inactivation effect of plasma treatment implies its potential application in the disinfection of concealed slits, deep holes, and some other regions that are not readily accessible.

### CONCLUSIONS

We used a plasma needle that was developed using a DC power source. The plasma jet Produced by the plasma needle easily interacted with the simple of skin of human body without causing any sensation. A direct-current, atmospheric-pressure cold air PMJ was successfully employed to inactivate *S. aureus* and *E. coli*. Electronics images showed extreme cell morphology changes after plasma treatment, reflecting the lethal effect of plasma on bacteria. The inactivation rate increased with the applied voltages, reaching 100% within 1 min and Cu-metal treatment in the treated area. Although not directly exposed to PMJ, bacteria in the untreated area were also inactivated, with a longer time required to reach 100% inactivation. The effect of PMJ on bacteria at 1.5 cm high voltage was slightly better than that at 630 V.

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