

Concentration-Dependent Effect of Hydrogen Peroxide Generated by Non-Thermal Plasma on the Viability of *Schistosoma mansoni* Cercariae

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Non-thermal (low-temperature) plasma is a modern physico-chemical instrument with significant biological effects based on the production of reactive forms of oxygen and nitrogen (RONS). In addition to direct plasma action, plasma-activated water (PAW) also plays an important role, and its effects may persist for an extended time due to the presence of stable reactive substances. The most important of these is the hydrogen peroxide H₂O₂, which is one of the main carriers of the biological effect of PAW. It is H₂O₂ that accumulates at measurable concentrations in plasma-activated solutions and contributes significantly to oxidative stress resulting in cellular damage and inactivation of micro-organisms [1].

Schistosomiasis remains widespread, particularly in tropical regions of Africa and South America, highlighting the need for effective and environmentally friendly water decontamination technologies. The life cycle of *Schistosoma mansoni* involves an intermediate host (freshwater snail) and a final mammalian host. Infectious cercariae are released from infected snails into the aquatic environment, where they actively seek a host and penetrate the skin. As the infective stage responsible for schistosomiasis transmission, their elimination from water is essential for disease control [2,3]. This project also serves as a preliminary study for future collaboration with Covmask s.r.o. and the Government of Ghana on the development of plasma-based water treatment methods.

While the effect of non-thermal plasma on bacteria, viruses and fungi is well documented [4,5], studies aimed at inactivating parasites, especially aquatic enteroparasites, are so far less numerous. However, the available works confirm the high potential of non-thermal plasma in this area as well. Pulse gas plasma discharge has been shown to effectively inactivate *Cryptosporidium parvum*, a major agent of intestinal infections in humans [6], and shows also high efficacy against *Acanthamoeba*, including its highly resistant developmental stages [7].

The study [2] represents a significant contribution in the field of parasite research demonstrating that low-temperature plasma generated by different types of corona

discharges, as well as plasma-activated water, effectively inactivate cercariae and miracidia *Schistosoma mansoni*. The efficiency of the inactivation varies significantly according to the type of discharge – the positive discharge led to complete inactivation within 2–3 minutes, while the negative discharge required 5–8 minutes and the direct action of the axial discharge was almost ineffective. Similar trends were observed when plasma-activated water was used, with PAW prepared using a positive discharge being the most effective, while PAW generated by an axial discharge showed a significantly weaker effect. In addition, preliminary microscopic observations indicate the existence of different mechanisms of inactivation for individual types of discharges [2].

Based on these facts, the effect of non-thermal plasma on the larval stages of *Schistosoma mansoni* is extremely significant, but the mechanisms of this action, particularly the role of the individual stable reactive components of plasma-activated water, remain under-quantified. In this context, hydrogen peroxide H_2O_2 , which is among the main carriers of PAW biological action and likely to be fundamentally involved in cercariae inactivation, deserves special attention.

The aim of this project is therefore to systematically investigate the concentration-dependent effect of hydrogen peroxide generated by non-thermal plasma on the viability of *Schistosoma mansoni* cercariae. The project is directed at the concentration of H_2O_2 for the inactivating of cercariae and at the same time at the follow-up of the temporal dependence of this effect, i.e. the exposition time needed on the induced lethal and sublethal biological changes.

The first step of the project began with the generation of Plasma Activated Water (PAW), optimizing parameters of positive transient spark discharge to achieve the highest possible concentration of H_2O_2 , at the same time as the lowest concentrations of NO_3^- and NO_2^- . In Figure 1 is seen the experimental apparatus used to generate PAW by positive transient spark discharge in point-to-liquid configuration and to subsequent exposure of water with cercariae.

The experiments looked at the influence of different resistance and capacitance values of ballast impedance on the resulting chemical composition of PAW. Testing was performed at $R = 5\text{ M}\Omega$, $10\text{ M}\Omega$ and $15\text{ M}\Omega$ with the capacity maintained constant at $C = 117,5\text{ pF}$. Subsequently, the impact of different capacity values, representing different numbers of capacitors involved, was also monitored in the context of constant resistance, namely $C = 117,5\text{ pF}$, $156,7\text{ pF}$, 235 pF and 470 pF .

After measuring the concentrations of H_2O_2 , NO_3^- and NO_2^- , respectively in the resulting PAW solutions, three solutions generated by a positive transient spark discharge were identified as having the most suitable properties. The optimal solutions were those produced at $R = 10 \text{ M}\Omega$ and $C = 117,5 \text{ pF}$ after 10 minutes of activation, containing approximately $20 \text{ mg/l H}_2\text{O}_2$, and solutions generated at $R = 5 \text{ M}\Omega$ with capacitances of $C = 235 \text{ pF}$ and $C = 470 \text{ pF}$, containing approximately $10 \text{ mg/l H}_2\text{O}_2$.

Based on the optimization results, two configurations of positive transient spark discharge with $R = 5 \text{ M}\Omega$, $C = 235 \text{ pF}$ and $R = 5 \text{ M}\Omega$, $C = 470 \text{ pF}$ were selected for subsequent biological experiments, representing the optimal compromise between the production of H_2O_2 and low concentrations of NO_3^- and NO_2^- . Biological experimental part of the project began with the collection of *Schistosoma mansoni* cercariae

released from infected *Biomphalaria glabrata* snails following light stimulation. For each chosen discharge configuration, the contaminated water with cercariae was subsequently directly treated by NTP for 5 and 10 minutes. After the exposure, the mortality of the cercariae was continuously evaluated by microscopic observation, depending on the time after treatment. The results obtained are shown in Figure 2.

Based on these findings, it can be assumed that direct NTP treatment and consequent action of PAW effectively inactivates *Schistosoma mansoni* cercariae through the formation of sufficient concentrations of hydrogen peroxide, which appears to be one of the key stable reactive species responsible for the observed biological effect. Cercarial mortality increased with exposure time, while comparable results were obtained for the selected discharge configurations, indicating similar inactivation efficiency under both optimized conditions.

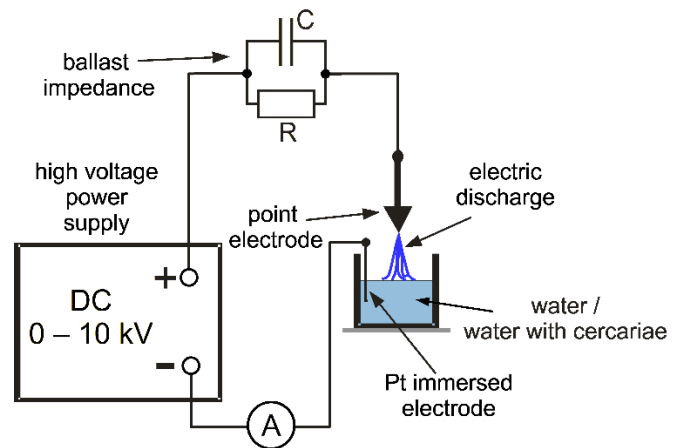


Fig. 1: Schematic representation of the experimental apparatus used for PAW generation and exposure of *Schistosoma mansoni* cercariae.

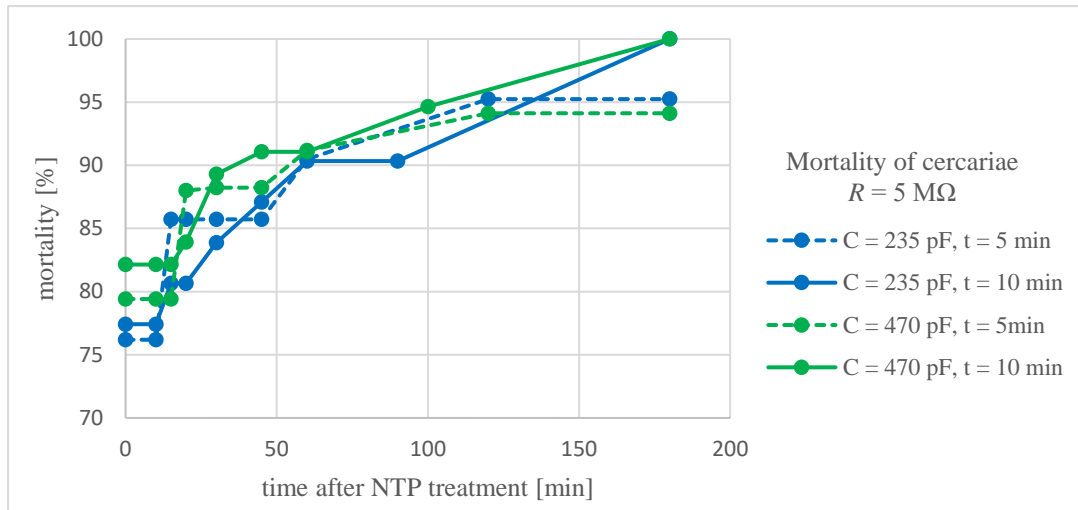


Fig. 2: Graph of time-dependent mortality of cercariae following direct NTP treatment at $R = 5 \text{ M}\Omega$, using capacitances of $C = 235 \text{ pF}$ and 470 pF with treatment times of 5 and 10 min.

Furthermore, the optimization of discharge parameters was shown to significantly influence the formation of reactive species and, consequently, the biological activity of the treatment. These findings demonstrate the potential of direct NTP treatment as an environmentally friendly approach for parasite control in water and provide a basis for the future development of sustainable water treatment technologies, particularly for improving drinking water safety in regions affected by schistosomiasis.

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