

## Plasma Treated Liquid: Reactive Species and Influence on Saline Stress in Quinoa Seeds

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Atmospheric pressure plasma sources are increasingly used in life science, from biomedical fields to agricultural and food applications. The use of liquids as targets in plasma treatments leads to the absorption at the plasma-liquid interface of reactive species produced by the discharge. This study focuses on the optical diagnosis of a liquid-compatible plasma source (50 Hz, 15.5 kV) and correlating the physico-chemical changes in the treated liquids with the plasma operating parameters and the ability of liquids to store the reactive species long-term. Furthermore, the effects of the treated liquids on seed germination and response to saline stress were investigated.

topics: plasma physics, atmospheric pressure plasma, life sciences

### 1. Introduction

In the last decade, the interest towards understanding the processes that occur at the plasma-liquid interface has been increasing significantly [1–6]. This interest is mainly owed to the emergence of the plasma medicine field and the subsequent shift towards indirect treatments, i.e., replacing direct live target treatment with the administration of a plasma-treated medium [7–9], thus minimizing the risk of exposing living targets to high-voltage discharges. Naturally, these studies have shifted towards other life science applications, the most prominent of which in recent years is agriculture — from eliminating fungi and bacteria from seed surfaces to stimulating germination and plant growth [10–15].

In the plasma region ionization processes and electron collisions and recombinations lead to the formation of a multitude of reactive chemical species: atomic oxygen, nitric oxide, nitrous oxide, ozone, hydroxyl, hydrogen peroxide, nitrates, nitrites, and many more [12, 16–18]. All these reactive species have various lifespans, some of them persisting, others recombining with other radicals. At the plasma-liquid interface, some of these reactive species will diffuse in the target liquid, affecting its chemistry, thus making it more suitable for

various applications from surface decontamination and water purification to cell proliferation and seed germination [19, 20].

The work presented here proposes an electrode geometry that minimizes the risk of water contamination during treatment and allows for a relatively large volume of water to be treated, namely 30 ml. Furthermore, we analyze the effects of plasma on the treated liquid, both immediately after treatment and over time. This evolution of liquid properties is important for long-term storage and prospective uses outside laboratory conditions. Looking at agricultural applications, we evaluated the effects of using treated liquids on quinoa seed germination and response to saline stress.

### 2. Materials and methods

#### 2.1. Atmospheric pressure plasma source

When approaching the liquid treatment, our main focus was minimizing any possible contamination during plasma treatment. To that extent, we developed an experimental setup, presented in detail in Fig. 1, which ensures that the target liquid comes in contact only with the glass Petri dish (9 cm in diameter, 2 mm thick, 1.5 cm in height; acting as a dielectric layer). Stainless steel pin electrodes are

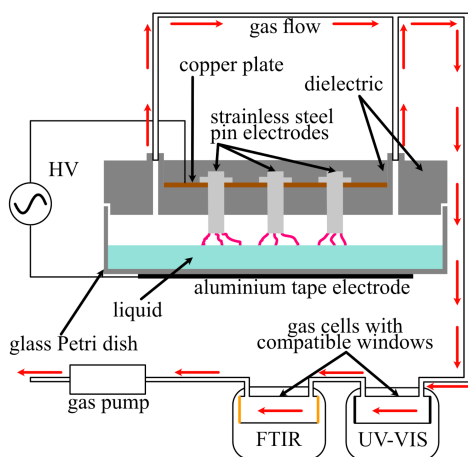


Fig. 1. Schematic representation of the experimental setup.

connected to a copper plate and enclosed in a dielectric lid with ventilation holes, connected to a gas circulating pump that allows us to extract and investigate the changes in gas chemistry throughout plasma treatment.

The entire ensemble is connected to an alternating current power supply (quasi-sinusoidal waveform, 50 Hz, 15.5 kV peak-to-peak voltage). The plasma ignited between the stainless steel pins and the target liquid has a multispark-like appearance.

The liquid was treated for 5 min in batches of 30 ml. For these specific experiments, the target liquid was distilled water (DW).

## 2.2. Plasma diagnosis methods

Current and voltage values were monitored using Pearson 6585 and Tektronix P6015 probes, respectively. During the rising (in the positive part) and on the falling (in negative part) of the quasi-sinusoidal voltage, around 300 current peaks are developed, with peak intensity ranging from 2 up to 60 mA and peak duration from 10 up to 60 ns.

Gas analysis was performed during treatment by extracting the gas from the discharge area and circulating it through a quartz gas cell, connected to an UV-Vis spectrometer (Thermo Scientific Evolution 300) and a glass gas cell with ZnSe windows connected to an infrared spectrometer (Jasco FT/IR-4700).

## 2.3. Liquid analysis methods

Reactive oxygen and nitrogen species with shorter life spans were assessed immediately after treatment using test strips (Quantofix) for nitrite, nitrate, and peroxide, and a colorimetric disk

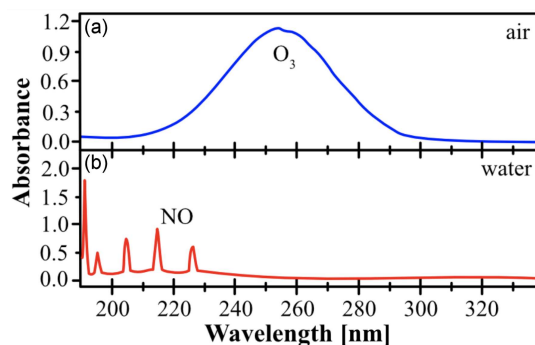


Fig. 2. Typical trace of gas phase ultraviolet-visible (UV-Vis) spectra acquired during plasma treatment. Panel (a) illustrates gas spectra obtained with discharge operating in air, panel (b) illustrates gas spectra obtained with DW in the discharge area.

checker for ozone using the diethyl-p-phenylene diamine method (HI38054). Conductivity and pH values were measured using the pH/conductivity meter SevenDirect SD23 (Mettler Toledo).

For reactive species, aging following treatment nitrite and nitrate concentrations were assessed using two photocolorimeters (Hanna Instruments). Nitrite concentration was evaluated using the ferrous sulfate method (HI97708) and nitrate using the cadmium reduction method (HI97728).

## 2.4. Saline stress and seed germination

For the saline stress, quinoa (*Chenopodium quinoa*) seeds from “Titicaca” cultivar were chosen; it is a high yielding, highly nutritional plant, with small disk shaped seeds. Saline stress was administered by using various concentrations of NaCl, however, in this paper we are focusing only on 300 mM NaCl solution, using both distilled water and treated distilled water (TW). The seeds were placed in Petri dishes, in batches of 50, on filter paper with 5 ml of liquid and monitored for 7 days.

## 3. Result and discussion

The infrared and UV spectra of the gas extracted from the discharge area are presented in Fig. 2 and Fig. 3. These measurements were performed both with and without liquid in the discharge area. While the absence of liquid leads to the presence of ozone characteristic peaks in both analyses, introducing liquid in the discharge space leads to the formation of nitrogen and oxygen radicals. These radicals are the ones diffusing at the plasma-liquid interface and lead to the presence of reactive species in liquid.

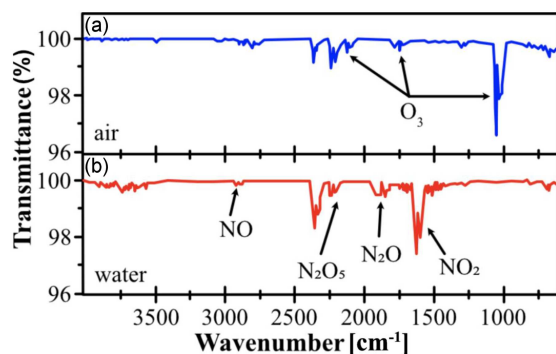


Fig. 3. Typical trace of gas phase Fourier-transform infrared spectroscopy (FTIR) spectra acquired during plasma treatment. Panel (a) illustrates gas spectra obtained with discharge operating in air, panel (b) illustrates gas spectra obtained with DW in the discharge area.

TABLE I

Analysis result comparison for DW and TW. Values presented below are an average of 5 distinct experiments. For DW, reactive species concentrations are below the *limit of detection* (LOD) or nonexistent.

| Measurement                              | DW   | TW  |
|--|------|-----|
| pH                                       | 6    | 3.5 |
| Conductivity [ $\mu\text{S}/\text{cm}$ ] | 1.8  | 155 |
| Nitrite [mg/L]                           | <LOD | 15  |
| Nitrate [mg/L]                           | <LOD | 100 |
| Peroxide [mg/L]                          | <LOD | 2   |
| Ozone [mg/L]                             | <LOD | 0.2 |

Analyzing the presence of reactive species and changes in pH and conductivity immediately following the treatment, yielded the results presented in Table I. Continuous monitoring for the first 30 min following treatment shows complete disappearance of ozone and peroxide, as well as significant changes in the nitrite and nitrate concentrations.

Due to the rapid changes that occur immediately after the treatment, monitoring of the reactive species aging followed a different protocol. Having a limited amount of liquid that can be treated (30 ml), a series of 15 consecutive treatments was performed, resulting in 450 ml of TW. All treatments were mixed in a sterile bottle and deposited at room temperature for further analysis. The first measurements were performed 30 min after the last 30 ml of TW was added and mixed thoroughly to eliminate sudden changes in concentration appearing immediately after treatment.

The evolution of nitrite and nitrite concentrations, as well as pH and conductivity values, presented in Fig. 4, shows the most significant changes occurring in the first 5 to 10 days, followed by the measured values. The second half of monitoring

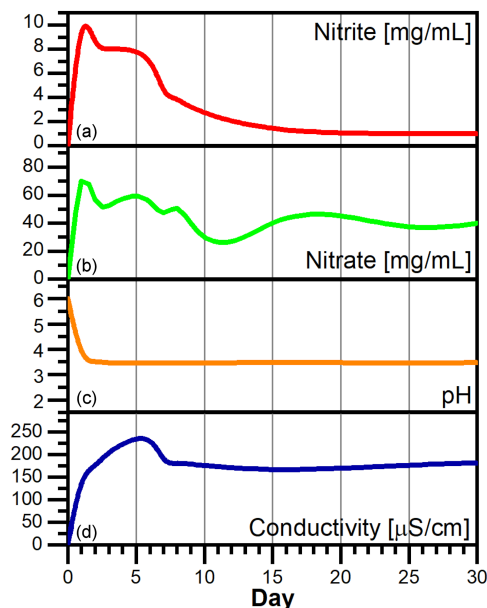


Fig. 4. Time evolution of (a) nitrite and (b) nitrate concentrations, (c) pH, and (d) conductivity. Day 0 represents the values for DW, while day 1 is the first measurement for TW, 30 after finishing all water treatments.

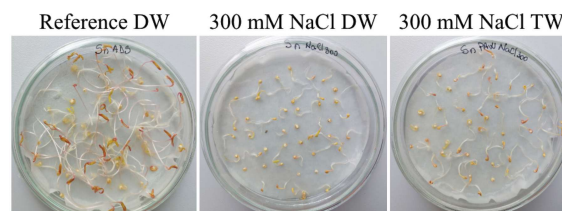


Fig. 5. Images of quinoa seeds after 7 days of saline stress.

TABLE II

Germination rates and biomass evaluation of the three experimental conditions. The germination rate was calculated after 7 days of incubation. Biomass values represent the average of 41 seeds.

| Experiment condition | Germination [%] | Biomass [g] |
|----------------------|-----------------|-------------|
| Ref. DW              | 87              | 0.66        |
| 300 mM NaCl + DW     | 83              | 0.39        |
| 300 mM NaCl + TW     | 90              | 0.46        |

period shows a much smaller variation in values, while, at the same time, being still vastly different from the initial values related to DW.

TW was used to assess the saline stress response and germination rates of quinoa seeds. The saline solution was obtained using both DW and TW, and the concentration presented below is of 300 mM

NaCl. All these results were compared with quinoa seeds placed in plain DW (from the same batch as the one used to obtain TW). As illustrated in Fig. 5, we observed an increase in germination rate when compared to both quinoa seeds with no saline stress and quinoa seeds that were watered with DW saline solution.

However, when evaluating fresh biomass of all three experimental conditions, both batches of seeds under saline stress exhibit a biomass of approximately half of the one calculated for the reference, as presented in Table II. This result does emphasize that, even though our experiments aid in increasing germination rates, under saline stress conditions the impact on biomass is minimal.

#### 4. Conclusions

The plasma source developed for liquid treatments presents one major advantage, namely by ensuring the target liquid comes in contact exclusively with the glass Petri dish, we managed to minimize the contamination risks. The liquid analysis results emphasize the ability of the treated water to preserve a lot of its properties for long-term use, without any costly storage solutions. Our findings demonstrate that the plasma-treated water has the potential to improve the germination of quinoa seeds under saline stress, but further studies should be done in order to improve its performance.

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