# 9th Central European Symposium on Plasma Chemistry (CESPC-9)

joint with

# COST Action CA19110 Plasma Applications for Smart and Sustainable Agriculture (PlAgri)

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Vysoké Tatry, Slovakia
September 4–9, 2022

# BOOK OF ABSTRACTS

including general information and program

**Edited by Karol HENSEL, Richard CIMERMAN, Aleksandra LAVRIKOVA, Mário JANDA, and Zdenko MACHALA**

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**Subtitle:** Book of Abstracts (inlcuding general information and program)
**Editors:** K. Hensel, R. Cimerman, A. Lavrikova, M. Janda, and Z. Machala
**Publisher:** FMFI UK, Bratislava
**Printing:** Neumahr s r.o,, Bratislava, 2022
**ISBN:** 978-80-8147-115-5
**EAN:** 9788081471155

# WELCOME

Dear colleagues,

On behalf of the local organizing committee of the Faculty of Mathematics, Physics and Informatics of the Comenius University in Bratislava and Astronomical-Geophysical Observatory (AGO) Modra, we are delighted to welcome you at the **9th Central European Symposium on Plasma Chemistry (CESPC-9)** joint with **COST Action CA19110 Plasma Applications for Smart and Sustainable Agriculture (PlAgri)** workshop, held in Vysoké Tatry, Slovakia, from September 4 to 9, 2022.

CESPC is a well-established small size symposium of <120 participants and friendly open atmosphere. It deals with fundamental problems of gaseous plasma physics and chemistry, modelling and diagnostics, new and nanostructured materials, energy technologies, environmental protection, surface processes, and plasma technologies in modern industry, medicine, food technology, and agriculture. It provides as an interactive platform enabling researchers of different backgrounds (chemistry, physics, engineering, material sciences, biology, medicine, agriculture, food technology) to meet and develop new ideas in the widely interdisciplinary field of plasma chemistry. The symposium promotes the transfer of scientific knowledge to industrial, agricultural, and clinical practices.

PlAgri workshop covers the working groups as follows: 2 (seeds), 3 (plants), 4 (agriculture wastewater and plasma activated water), and 5 (plasma in food industry). It aims to investigate the potential of low-temperature plasmas as a green alternative for treating seeds, plants, agricultural wastewater, plant growth media, manure, and the use of plasmas for treating food and packaging. It also studies undergoing processes of plasma treatment of this agricultural biological materials. The Action aims to break the classical field boundaries for a new dimension in sustainable agriculture with lower chemical and environmental impact, higher yield, and sustainability.

The following pages will guide you through the general information about both events including their history and the venue. This booklet also provide the detailed program of both CESPC-9 and PlAgri workshop as well as the abstracts of all oral and poster contributions that can be also found on the USB key. Event excursions are also presented.

We thank all LOC members for helping us in the organization of this event and, above all, all of you for your active participation. We also gratefully acnowledge the support for CESPC-9 provided by Central European Iniciative (CEI). You are all very welcome to Štrbské Pleso in Vysoké Tatry (the High Tatras), the smallest alpine mountain range in the world. Please enjoy the event with all the multidisciplinary-scientific, as well as social-collaborative aspects and have a good late-summer time in Vysoké Tatry!

Zdenko MACHALA, Karol HENSEL and Mário JANDA
Chairmen of **CESPC-9 symposium** and **PlAgri workshop**

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# GENERAL INFORMATION

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## Organizers


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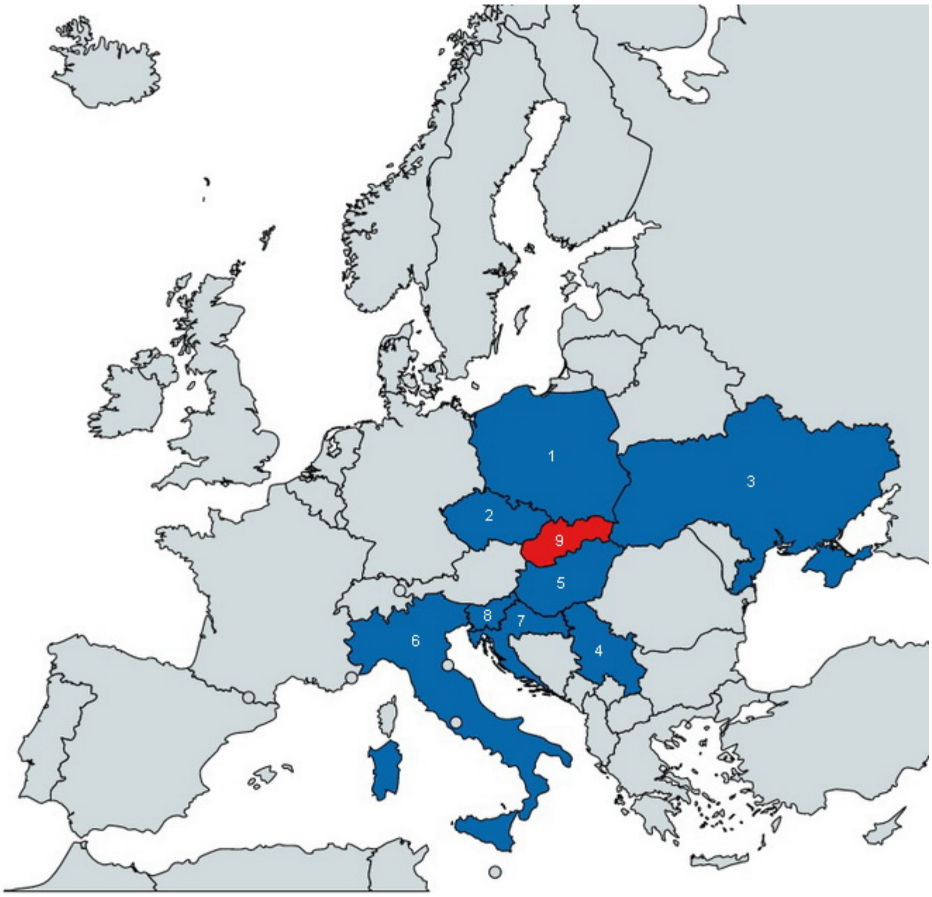
## Sponsors

# HISTORY

The first Central European Symposium on Plasma Chemistry (CESPC) was organized in 2006 in Gdansk, Poland. In the following years it was organized in various countries across Central and Eastern Europe. The upcoming ninth symposium (CESPC-9) is held in a very central part of Europe – in **Vysoké Tatry, Slovakia**. This time, CESPC-9 will be joint with COST Action CA19110 Plasma Applications for Smart and Sustainable Agriculture (PlAgri) workshop. Therefore, five-day format of the symposium along with workshop provides excellent opportunities for both formal presentations and informal discussions in between more than 100 participants.

| CESPC | Place | Dates | Organizers |
|---|---|---|---|
| 1 | Gdańsk (PL) | May 26-31, 2006 | J. Mizeraczyk and M. Dors |
| 2 | Brno (CZ) | Aug 31 - Sep 4,2008 | A. Brablec and P. Vašina |
| 3 | Kyiv (UA) | Aug 23-27, 2009 | V. Chernyak and V. Zhovtyanskij |
| 4 | Zlatibor (RS) | Aug 21-25, 2011 | M. M. Kuraica and B. M. Obradović |
| 5 | Balatonalmádi (HU) | Aug 25-29, 2013 | K. Kutasi and A. Tóth |
| 6 | Bressanone (IT) | Sep 6-10, 2015 | C. Paradisi and E. Marotta |
| 7 | Sveti Martin na Muri (HR) | Sep 3-7, 2017 | S. Milosević and N. Krstulović |
| 8 | Gozd Martuljek (SI) | May 26-30, 2019 | G. Primc and M. Mozetič |
| 9 | Vysoké Tatry (SK) | Sep 4-9, 2022 | Z. Machala, K. Hensel and M. Janda |

*General information of all previous CESPC events*



# VENUE

# Štrbské Pleso

The CESPC-9 symposium and PlAgri workshop are held in Hotel Sorea TRIGAN***, located directly in one of the best Slovak skiing resorts – Štrbské Pleso in Vysoké Tatry. Beautiful mountain lake Štrbské pleso is located just a few minutes from the hotel and the region provides great possibilities for hiking or nice walks after which you can relax in the hotel spa centre. The distance from Bratislava is ~350 km, from Vienna Airport ~450 km. International airport in Krakow, Poland is very close and provides wide range of connections.

# Vysoké Tatry

Vysoké Tatry (High Tatras) are mountains situated in the northern part of Slovakia, on the border with Poland. It is the smallest alpine mountain range in the world, although it is also considered a part of the larger Carpathian range. The High Tatras were created about 60 milions years ago heaving up the granite massif above the level of the surrounding land. It gained its current appearance by the glacier activity 2 mil. years ago. It was declared to be the first Slovak national park (TANAP) and in together with the polish part of the Tatras mountains declared by the UNESCO to be the "Biospheric reservation of the Tatras". The jewel of the High Tatras is Mount Kriváň (2,495 m) and its famous curved profile is an important symbol in Slovak art and culture. A visit to the High Tatras is very affordable when compared to other parts of Europe.



*Hotel Sorea Trigan, Štrbské Pleso, Vysoké Tatry*

# Slovakia

Slovakia is a country in Central Europe. Its territory spans about 49,000 square kilometers and its population comprising mostly ethnic Slovaks is over 5 million. The official language is Slovak, a member of the Slavic language family. The Slavs arrived in the territory of present-day Slovakia in the 5th and 6th centuries. In the 10th century, the territory was integrated into the Kingdom of Hungary, which later became the Austro-Hungarian Empire. After World War I and the dissolution of the Austro-Hungarian Empire, the Slovaks and Czechs established Czechoslovakia. Slovakia became an independent state on January 1, 1993 after the peaceful dissolution of Czechoslovakia. Slovakia is a high-income advanced economy. The country joined the European Union in 2004 is a member of the Schengen Area, NATO and the United Nations.

# EXCURSIONS

The excursions will be held on Wednesday, September 7, 2022 after the lunch. The participants can choose the more attractive option for them as follows:

**1. Kežmarok castle + The Wooden Articular Church**

**2. Predné Solisko** *(In case of bad weather conditions - **Belianska Cave**)*

# Kežmarok Castle + The Wooden Articular Church

**Kežmarok Castle**

Situated in the historical centre of the ancient Spiš city of Kežmarok stands a beautifully preserved castle, one of the most precious structures of cultural and historical importance. This castle became the starting point of the first popular hiking trip to the High Tatras. The initiator of this trip was princess Koscielecká, a wealthy widow of one Russian magnate, who married the local castle lord. Her new husband, however, did not approve of her hiking trip at all, seeing the trip was attended by a number of people from Kežmarok. When the princess returned, he accused her of amoral rambling in the wilderness with strange men and had her thrown into the castle prison, from where she could see the Tatras or Snowy Mountains as they used to be called back in their day. While the poor hiker sat in the prison and mulled over her wrongdoing for six long years, her husband merrily squandered all of her fortune to the last gold coin.

These days Kežmarok Castle is open to the public. It houses the local museum, where visitors can admire the attractive interiors of the castle - for example, the watchtower or the torture chamber - as well as interesting exhibitions focused on Kežmarok's history, medieval crafts, and guilds, Jewish culture or religious art.

**The Wooden Articular Church**

The Wooden Articular Church is on the UNESCO World Heritage list and it dates to the time of religious oppression. The first building of the Wooden Church was already standing in 1687. It was rebuilt into its current form in 1717 in incredibly only three months. An interesting fact is that, allegedly, during the construction there was not one metal nail used! The exterior walls are plastered with clay, which is a little bit misleading, the shingled roof being the only sign that it is indeed a wooden jewel.

# Predné Solisko

**Predné Solisko**

Predné Solisko (2,093 meters above sea level) is the last ridge of the long Solisko ridge that extends from the Furkotski peak to the southeast. Predné Solisko is a wonderful spot to enjoy the views of the surrounding country! A 4-seater Solisko Express cable car will take you from the lower station at Štrbské Pleso up to the altitude of 1,814 m. It takes only 7 minutes to surmount a vertical drop of 431 meters. From here you can take about 1h hike to the top of Predné Solisko with stunning views.

**Belianska Cave** *(instead of Predné Solisko in case of bad weather conditions)*

Belianska Cave is a stalactite cave in the Slovak part of the Tatra mountains, the largest and the only one open to the public in the High Tatras.

# PROGRAM

## CESPC-9 Topics

1. Fundamental problems
2. Modeling and diagnostics
3. New materials and nanomaterials
4. Energy technologies
5. Environmental protection
6. Bio and medical plasma technologies
7. Surface processes
8. Plasma in contact with liquids

## PlAgri Workgroups

1. Dissemination and communication
2. Low-temperature plasma treatment of seeds
3. Low-temperature plasma treatment of plants
4. Plasma treatment of agricultural wastewater, growth media, manure and production of PAW
5. Applications of plasma processes and technologies in food industry

## Presentation Guidelines

The CESPC-9 scientific program takes the first three days (September 5-7, 2022). The PlAgri workshop scientific program takes the following two days (September 8-9, 2022). Both CESPC-9 and PlAgri workshop will be composed of invited lectures, oral presentations and of poster sessions. In addition, PlAgri workshop will also include an introduction to each working group.

The lengths of the invited lectures and oral presentations are as follows:

**CESPC-9**
**Invited lecture:** 35 min (30 minutes for lecture + 5 minutes for questions and discussion)
**Oral presentation:** 20 min (17 minutes for presentation + 3 minutes for questions and discussion)

**PlAgri Workshop**
**Intro to working group:** 15 min
**Invited lecture:** 30 min (25 minutes for lecture + 5 minutes for questions and discussion)
**Oral presentation:** 15 min (12 minutes for presentation + 3 minutes for questions and discussion)

Presenters are kindly requested to respect the above length of presentations.

**Poster sessions**
Posters will be organized in two poster sessions corresponding to CESPC-9 and PlAgri workshop. The poster numbers listed in this booklet will be attached to the poster panels. The authors are requested to be present at their posters during the poster sessions. Material for poster mounting will be available on the poster board. The preferable size for preparing the poster is **A0 (VERTICAL portrait format: 84 cm (w) x 118 cm (h)).** Posters should be posted during the presentation day and are requested to be removed by the presenters right after each poster session. The organizers take no responsibility for leftover posters.

**Invited lecture / oral / poster presentation code (ID):** I/O/P-[presentation number]

# Program in Detail

# CESPC-9

## Monday, September 5

| | | | |
|---|---|---|---|
| $8^{15}$ | | Opening ceremony | |
| $8^{30}$ | I-1 | **Deanna Lacoste**<br>Non-equilibrium plasmas for combustion systems | Chair: *K. Hensel* |
| $9^{05}$ | O-1 | **Tim Nitsche**<br>Experimental insights in the development of an oxygen removal process for coke oven gas with ... | |
| $9^{25}$ | O-2 | **Callie Ndayirinde**<br>Plasma-catalytic ammonia synthesis: the effect of the metal composition on the performance of ... | |
| $9^{45}$ | O-3 | **Claudia Verheyen**<br>Microwave plasma for fertilizer and oxygen production in the Martian atmosphere | |
| $10^{05}$ | | Coffee break | |
| $10^{30}$ | I-2 | **Ester Marotta**<br>Plasma treatment of perfluoroalkyl substances in water | Chair: *K. Kutasi* |
| $11^{05}$ | O-4 | **Zdenko Machala**<br>Effects of cold plasma activated water on urinary tract infections: *in vitro* vs. *in vivo* | |
| $11^{25}$ | O-5 | **Joanna Pawłat**<br>Cold plasma treatment of selected types of foods | |
| $11^{45}$ | O-6 | **Thalita Nishime**<br>Influence of the reactor configuration on the treatment of rapeseed using a conical corona reactor | |
| $12^{05}$ | | Lunch | |
| $13^{30}$ | I-3 | **Cristina Canal**<br>Moving from 2D to 3D to evaluate plasma-conditioned liquids in cancer treatment | Chair: *A. Bogaerts* |
| $14^{05}$ | O-7 | **Kristian Wende**<br>Biomolecule oxidation by CAP derived species – a general concept in biomedical plasma applications | |
| $14^{25}$ | O-8 | **Francesco Tampieri**<br>How biopolymers in solution affect the generation and stability of plasma-generated reactive species | |
| $14^{45}$ | O-9 | **Stanislav Kyzek**<br>The effects of non-thermal plasma treatment on the structural and functional parameters of ... | |
| $15^{05}$ | | Coffee break | |
| $15^{30}$ | I-4 | **Štefan Matejčík**<br>Diagnostics of atmospheric plasma by ion mobility spectrometry (IMS) | Chair: *C. Canal* |
| $16^{05}$ | O-10 | **Kostyantyn Korytchenko**<br>Optical and electrical investigation of plasma generated by high-energy self-stabilized spark ignition system | |
| $18^{30}$ | | Dinner | |

# Tuesday, September 6

| | | | |
|---|---|---|---|
| 8 30 | I-5 | **Gregor Primc**<br>Inactivation of viruses in irrigation water by combining advanced oxidation techniques | Chair: *P. Lukeš* |
| 9 05 | O-11 | **Zlata Kelar Tučeková**<br>Optimization of plasma-activated media generation for decontamination of thermally sensitive materials | |
| 9 25 | O-12 | **Robin Menthéour**<br>Synergic antibacterial effect of pulsed electric field and plasma activated water | |
| 9 45 | O-13 | **Josef Khun**<br>Comparison of non-thermal plasma produced by cometary and point-to-ring discharges for ... | |
| 10 05 | | Coffee break | |
| 10 30 | I-6 | **Annemie Bogaerts**<br>Plasma-based $CO_2$ conversion: Improving the performance by a post-plasma carbon bed | Chair: *D. Lacoste* |
| 11 05 | O-14 | **Marley Becerra**<br>Investigation of $CO_2$ decomposition in a pulsated warm arc plasma by optical emission spectroscopy | |
| 11 25 | O-15 | **Richard Cimerman**<br>Nonthermal plasma regeneration of deactivated catalysts after plasma-catalytic removal of ... | |
| 11 45 | O-16 | **Thomas Vazquez**<br>Indoor air decontamination by cold atmospheric plasma and photocatalysis | |
| 12 05 | | Lunch | |
| 13 30 | I-7 | **Tomáš Homola**<br>Recent developments in applications of plasma to the manufacture of flexible solar cells | Chair: *H. Kersten* |
| 14 05 | O-17 | **Miran Mozetič**<br>Hydrophilization of fluorinated polymers | |
| 14 25 | O-18 | **Matteo Gherardi**<br>Control strategies for aerosol-assisted atmospheric pressure plasma deposition of fluorinated silane thin films | |
| 14 45 | O-19 | **Slavomír Sihelník**<br>Dry cleaning and activation of flexible glass using nonthermal plasma before PEDOT:PSS coating | |
| 15 05 | | Coffee break | |
| 15 30 | I-8 | **Holger Kersten**<br>On the combination of conventional and non-conventional probe diagnostics for process plasmas | Chair: *B. Mitu* |
| 16 05 | O-20 | **Senne Van Alphen**<br>Modelling study of $CO_2$ conversion enhancement in microwave plasmas using a quenching nozzle | |
| 16 25 | O-21 | **Eduardo Morais**<br>$CH_4$ coupling in nanosecond pulsed plasma discharges: 0D modelling to unravel the effect of ... | |
| 18 30 | | Dinner | |
| 20 00 | | Poster session | |

# Wednesday, September 7

| Time | | |
|---|---|---|
| $8^{30}$ | **I-9** | **Petr Lukeš**<br>Chemistry induced by atmospheric plasma in aqueous liquids<br><u>Chair:</u> *E. Marotta* |
| $9^{05}$ | **O-22** | **Nikola Škoro**<br>Correlation between properties of plasma treated liquids with characteristics of atmospheric pressure ... |
| $9^{25}$ | **O-23** | **Michael Schmidt**<br>Non-thermal plasma for generation of antimicrobial aerosol |
| $9^{45}$ | **O-24** | **Ana Sainz García**<br>Forest material treatment by PAW |
| $10^{05}$ | | Coffee break |
| $10^{30}$ | **I-10** | **Bogdana Mitu**<br>Plasma driven synthesis of NPs and their immobilization<br><u>Chair:</u> *M. Mozetič* |
| $11^{05}$ | **O-25** | **Ionut Topala**<br>Revision of 3.4 um band destruction rates under ion beam irradiation of hydrogenated amorphous ... |
| $11^{25}$ | **O-26** | **Kerstin Sgonina**<br>Selective study of ion-substrate interactions using the VUV-photoionization chamber |
| $11^{45}$ | | Lunch |
| $13^{00}$ | | Excursion |
| $19^{30}$ | | Gala Dinner |

# PlAgri Workshop

## Thursday, September 8

| | | | |
|---|---|---|---|
| $8^{20}$ | | Opening ceremony | |
| $8^{30}$ | | **Intro to WG 5 Plasma for Foods** - *Matteo Gherardi* | |
| $8^{45}$ | I-11 | **Tomislava Vukušić-Pavičić**<br>The effects of direct plasma treatment and indirect (PAW) treatment on physicochemical and functional ... | Chair: *M. Gherardi* |
| $9^{15}$ | I-12 | **Fernando Alba-Elías**<br>Applications of atmospheric plasma in the food and medical industry | |
| $9^{45}$ | O-27 | **Filippo Capelli**<br>Plasma decontamination of food packaging material | |
| $10^{00}$ | O-28 | **Klaas De Baerdemaeker**<br>Cold plasma for bacterial decontamination: impact of food matrix composition and relative humidity of ... | |
| $10^{15}$ | | Coffee break | |
| $10^{35}$ | | **Intro to WG4 Plasma Activated Water** - *Zdenko Machala / Wolfgang Gernjak* | |
| $10^{50}$ | I-13 | **Chedly Tizaoui**<br>Non-thermal plasma and advanced oxidation processes for micropollutants removal in water | Chair: *Z. Machala* |
| $11^{20}$ | O-29 | **Ludmila Čechová**<br>Effect of plasma and plasma activated water on growth media used in hydroponics | |
| $11^{35}$ | O-30 | **Vladimir Scholtz**<br>Comparison of the effect of plasma activated water and artificially prepared activated water | |
| $11^{50}$ | O-31 | **Jan Čech**<br>CaviPlasma: A plasma source capable of application-scale generation of plasma treated water for ... | |
| $12^{05}$ | | Lunch | |
| $13^{30}$ | I-14 | **Rune Ingels**<br>Do we need the Haber Bosch? | Chair: *W. Gernjak* |
| $14^{00}$ | O-32 | **Elise Vervloessem**<br>Experimental and computational study of nitrogen fixation mechanisms from (humid) air and nitrogen in ... | |
| $14^{15}$ | O-33 | **Kinga Kutasi**<br>Role of metals on fixation of $NO_2^-$ in plasma-activated liquids | |
| $14^{30}$ | O-34 | **Arijana Filipić**<br>Inactivation of viruses in irrigation waters | |
| $14^{45}$ | O-35 | **Raluca Alina Bisag**<br>On the use of plasma activated water (PAW) for agricultural purposes | |
| $15^{00}$ | | Coffee break | |

# Thursday, September 8

| Time | | |
|---|---|---|
| 15 20 | | **Intro to WG2 Plasma for Seeds** - *Nevena Puač* |
| 15 35 | **I-15** | **Božena Šerá** (Chair: *N. Puač*)<br>Seed treatment with non-thermal plasma from the point of view of seed germination and ... |
| 16 05 | **I-16** | **Pankaj Attri**<br>Impact of plasma/electric field treated seeds on germination, morphology, gene expression, and ... |
| 16 35 | **O-36** | **Jonas August**<br>From anhydrobiosis to germination: effect of an air atmospheric cold plasma treatment on ... |
| 16 50 | **O-37** | **Plamena Marinova**<br>Cold plasma treatment effect on the germination and seedlings growth of durum wheat genotypes |
| 17 05 | **O-38** | **Vida Mildažienė**<br>The persistance of effects of seed treatment with cold plasma, vacuum and electromagnetic field: ... |
| 18 30 | | Dinner |
| 20 00 | | Poster session |

# Friday, September 9

| Time | | |
|---|---|---|
| 8 30 | | **Intro to WG3 Plasma for Plants** - *Joanna Pawłat / Vida Mildažienė* |
| 8 45 | I-17 | **Sara Di Lonardo** (Chair: *V. Mildažienė*)<br>Cold plasma effects on plants: challenges and future in its use |
| 9 15 | O-39 | **František Krčma**<br>Cold plasmas application on onion bulbs |
| 9 30 | O-40 | **Rasa Žūkienė**<br>The evaluation of cold plasma effect on morphometric and biochemical parameters in ... |
| 9 45 | O-41 | **Karol Hensel**<br>Effect of plasma activated water, its chemically equivalent solutions and arsenic stress on ... |
| 10 00 | O-42 | **Sabrinne Bousselmi**<br>Effects of plasma treated water on seed germination and growth of blue lupine (*Lupinus angustifolius L.* ) ... |
| 10 15 | | Coffee break |
| 10 35 | I-18 | **Milica Milutinović** (Chair: *J. Pawłat*)<br>Molecular response to PAW in model plant species |
| 11 05 | O-43 | **Kazunori Koga**<br>Development of experimental system for plasma irradiation effects on plants using marchantia polymorpha |
| 11 20 | O-44 | **Liutauras Marcinauskas**<br>Application of plasma and pulsed electric field for the treatment of microalgae |
| 11 35 | O-45 | **Eugen Hnatiuc**<br>Preparing for large scale use of cold plasma discharges: Pitfalls and challenges |
| 11 50 | | Closing ceremony |
| 12 05 | | Lunch |

# ABSTRACTS

## CESPC-9

## Invited Lectures



## Oral Presentations

# Poster Presentations

# Non-equilibrium plasmas for combustion systems

**D. A. Lacoste**
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In view of the urgency of environmental problems such as global warming, energy demand, and pollutant emissions, there is a need for new combustion strategies. Plasma-assisted combustion (PAC) is one of them and it has attracted a strong interest from the scientific community for the last 15 years [1, 2]. The primary concept of PAC is to enhance combustion by mean of an electric field, using a minimum amount of energy and causing fewest pollutant penalties. The electrical energy input should be negligible compared to the thermal energy released by combustion or, alternatively, the electrical energy input should be less than the thermal energy saved. In this context, non-equilibrium plasma discharges are very interesting because they can create reactive chemical species and heat the gas in a controlled manner. Depending on the targeted effect, non-equilibrium plasmas are tuned to promote the production of different chemical species coupled or not with gas heating. Recently, nanosecond repetitively pulsed (NRP) discharges have demonstrated a good potential for combustion enhancement at pressures up to 5 bar. At this pressure, corresponding to the pressure in micro-gas turbine engines, for the combustion of methane, the glow regime of the NRP discharges is more efficient than the spark regime. Compared to what was obtained by many groups at atmospheric conditions, this result is unexpected. Possible explanations are proposed.

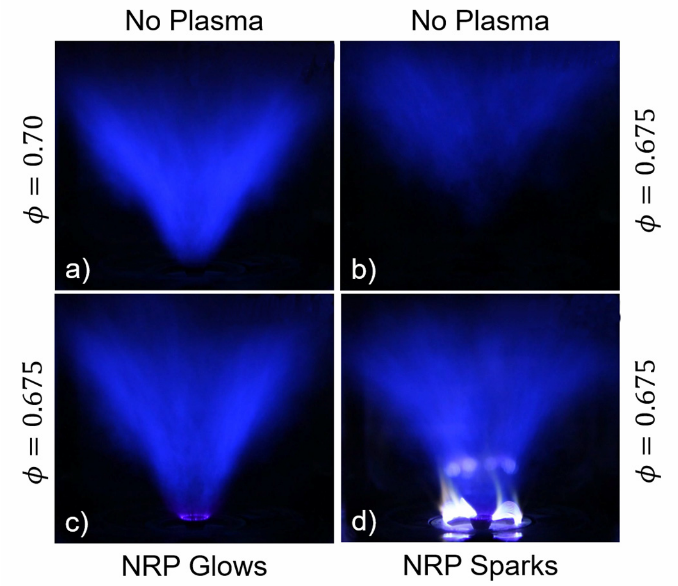


Fig. 1. Direct visualization of methane-air swirl flames at 3 bar. a) Stable flame with no discharges and an equivalence ratio of 0.7. b) Unstable flame with no discharges and an equivalence ratio of 0.675. c) Flame with an equivalence ratio of 0.675 stabilized by NRP glow discharges. d) Flame with an equivalence ratio of 0.675 stabilized by NRP spark discharges.

*This work was supported by the King Abdullah University of Science and Engineering through the baseline fund BAS/1/1396-01-01.*

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[1] Y. Ju, W. Sun, *Prog. Energy Combust. Sci.* **48**, 21-83 (2015).
[2] S. Starikovskaia, D. A. Lacoste, G. Colonna, *Eur. Phys. J. D* **75**, 231 (2021).

# Plasma treatment of perfluoroalkyl substances in water

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Plasma has recently demonstrated to be able to degrade even the most recalcitrant organic contaminants in water, among which there are perfluoroalkyl substances. These belong to the class of PFAS (poly- and perfluorinated substances), man-made chemicals of high thermal and chemical stability, soluble in water, acid resistant and oil repellent, which are routinely used in many industrial and consumer applications, such as electroplating, surface coatings and as a major ingredient in the manufacture of aqueous film forming foams to extinguish petroleum-based fires. PFAS persistence in the environment is thus due to their extraordinary stability and has made them ubiquitously distributed in the environment causing contamination of ground, surface and drinking water. A particularly high concern is associated to these compounds due to their ability to bioaccumulate in animal and human organisms and cause adverse health effects [1]. Failure of conventional water treatment technologies in degrading PFAS has promoted active search for novel efficient approaches based on advanced oxidation/reduction processes, including plasma [2]. Most studies on plasma activated degradation of PFAS in water consider perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), which have been for a long period the most used PFAS in industrial applications and are thereby the most diffused in the environment. Indeed both can be efficiently and quantitatively degraded by plasma treatment [3-10]. However, the degradation of these long chain precursors generates many poly- and perfluorinated shorter chain carboxylic acids, whose production and fate are not always easy to be determined. Research in our laboratory is focused on two targets, one concerning the development and optimization of plasma reactors dedicated to PFAS degradation, the other dealing with the identification of the products of the plasma activated process, on the mechanisms of their formation and on their evolution in time. In the talk an overview of the most recent advancements on the characterization of plasma induced PFAS degradation mechanisms will be given, discussing and comparing our findings with the state of the art.

**References**

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# Moving from 2D to 3D to evaluate plasma-conditioned liquids in cancer treatment

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The chemistry of Cold Atmospheric Plasma (CAP) leads to the generation of a variety of reactive oxygen and nitrogen species (RONS) which play a key role in selective cancer cell death without damaging surrounding healthy tissues. This has led to advancing research on plasmas in a variety of cancer types. Taking advantage of the reactivity of plasmas in liquids, plasma-conditioned liquids (PCL) can be generated and represent a very interesting alternative to direct CAP treatment because they open the door to minimally invasive therapies [1] [2].

With a focus on bone, we have investigated a rare cancer, osteosarcoma (OS), but also metastatic bone cancers, that have a wide prevalence and affect a high number of population. OS is a malignant type of bone cancer that arises in periods of increased bone formation (in pediatric patients and young adolescents). Curative strategies for this type of tumors has remained essentially unchanged for decades and overall survival for most advanced cases is still dismally low.

The use of PCL can be a useful tool in the treatment of this malignancy [3], and we have investigated the generation of RONS in different biocompatible liquids [4], ranging from saline solutions to more complex biopolymer-containing solutions [5]. In this talk we will discuss some of our research on the nature and extent of the modifications induced by CAP treatment on biopolymer solutions. The results allowed observing that the concentration of biopolymers affects the concentration of RONS detected in the liquid phase, pointing out direct interactions between RONS and biopolymer chains. The RONS generated in different solutions and hydrogels will be discussed in relation to their biological effects, as investigated in 2D monolayer cultures [4] and in a new 3D-OS tumor model specifically developed to study PCL as anti-tumoral agent. Employing these 3D tumor models allows considering the effect of microenvironment and of different tumor cell subpopulations, which we have found to be critical for the success of the therapy.

*Authors acknowledge the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Grant agreement Nº 714793) and Cost Action 20114 PlasTHER. MINECO is acknowledged for PID2019-103892RB-I00/AEI/10.13039/ 501100011033 project. Authors acknowledge Generalitat de Catalunya for SGR2017 1165 and for the Icrea Academia award of CC.*

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# Diagnostics of atmospheric plasma by ion mobility spectrometry (IMS)

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We present application of IMS and IMS-MS (IMS combined with mass spectrometry) techniques for diagnostics and monitoring atmospheric discharges such as plasma jet and corona discharge. These techniques are suitable to monitor neutral species formed in discharges and the decomposition of volatile organic compounds (VOC's) by the atmospheric discharges. IMS offers high detection sensitivity combined with fast response time and sufficient spectral resolution and therefore is suitable for online monitoring of these compounds.

IMS is a suitable technique to detect trace gases, such as volatile organic compounds (VOCs) and electronegative gases. Several laboratories have developed IMS or (IMS-MS) techniques for the analysis of complex chemical compounds. In the present work we have used IMS spectrometer and IMS-MS spectrometer of own design [1,2]. The ionisation method was based on Atmospheric Pressure Chemical Ionisation (APCI) technique based on Corona Discharge (CD) ion source. For atmospheric pressure discharge monitoring we have used IMS in positive and negative polarities. The decomposition of VOC's in a corona discharge reactor was carried out in positive polarity. We have monitored decomposition of different phthalates [3]. The IMS allowed detection of the Phthalates at sub ppm level. In the negative polarity we have studied formation of $NO_2$ in the DBD plasma jet in Ar and in two different corona discharge reactors (point to plane and wire – cylinder geometries). The absolute concentration of $NO_2$ have been determined based on calibration using $NO_2$ standards. The method allows detection od $NO_2$ at ppm levels.

Using this technique we were able to detect neutral species formed in atmospheric pressure discharges in ambient air and to monitor decomposition of VOC's by the atmospheric pressure discharges.

*This research was supported by the Slovak Research and Development Agency under contract No. APVV-19-0318 and Slovak Grant Agency VEGA project Nr. 1/0489/21.*

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# Inactivation of viruses in irrigation water by combining advanced oxidation techniques

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Water scarcity is among the most pressing problems of the modern world. Water usage estimates vary, but about 70% of worldwide water is used for irrigation for growing crops [1]. With the latest advancements in climate change and shortage of food, pressing issues with water scarcity will only escalate. Stated facts point to minimizing agricultural water usage, which can be achieved by expanding the network of closed-loop irrigation systems and innovative water management. Thanks to already improving irrigation management and practices, FAO estimates that the amount of water used by agriculture in developing countries will increase by only 14%. [1]. Using closed systems for water irrigation exposes several problems, like agrochemical residues, the balance of ions in the nutrient solution, and pathogens' spread. The latter is particularly pressing in closed-loop irrigation systems, as one single plant can release pathogens (bacteria, moulds, viruses) through the root system into the circulating water, infecting other plants. Some closed-loop irrigation systems use UV disinfection, which is economical but quite susceptible to water turbidity and requires several filters, while others, to a smaller extent, use membrane filtration, which is prone to constant filter cleaning. To address the spread of pathogens in closed-loop and semi-closed-loop irrigation systems, we have researched the possibility of joining two advanced oxidation methods: hydrodynamic cavitation and gaseous plasma. In this sense, we have designed a device that exploits the effect of hydrodynamic cavitation called the "supercavitation regime" that enables a formation of a single stable water bubble filled with water vapour [2]. In this way, we created conditions to ignite a gaseous plasma in water vapour and hence generate oxidizing species and, to a smaller extent, UV radiation. We built and tested two systems, small and medium scale, each with different characteristics for the inactivation of plant viruses. Systematic measurements were performed with MS2 bacteriophage, a surrogate for human enteric viruses, with both systems' best-achieved inactivation rate of 5-log. The method proved to be non-cytotoxic and efficient in inactivating (or even destroying) MS2 bacteriophage and is thus considered an environmentally friendly disinfection method as it uses no chemicals.

*The authors acknowledge the financial support from the Slovenian Research Agency core funding "Thin-film structures and plasma surface engineering" (P2-0082) and project "Waterborne virus inactivation efficiency of a prototype device combining non-equilibrium plasma and hydrodynamic cavitation" (L7-3184).*

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# Plasma-based $CO_2$ conversion: Improving the performance by a post-plasma carbon bed

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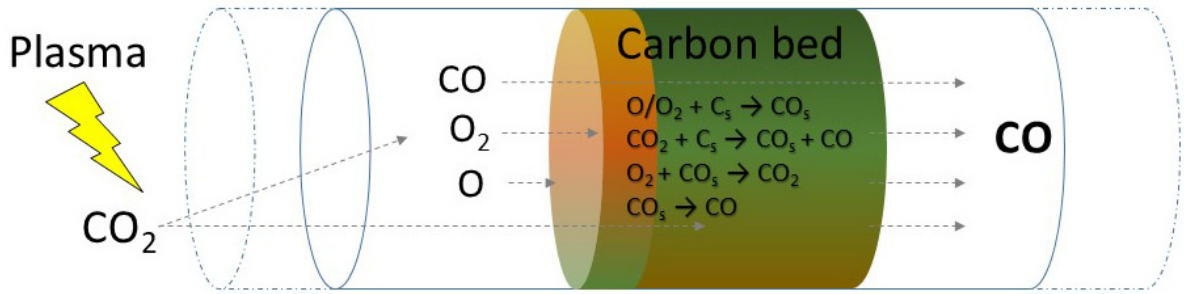
Plasmas are gaining increasing interest for $CO_2$ conversion into value-added chemicals and renewable fuels [1]. After a general introduction and overview of the state-of-the-art on plasma-based $CO_2$ conversion by various types of plasma reactors, I will discuss what are the current limitations and how they can be overcome, e.g., by smart reactor design and quenching after the plasma [2]. A well-known limitation is the recombination of CO with $O/O_2$ after the plasma. A solution for this problem is to place a carbon bed after the plasma [3]. Hence, in this talk, I will discuss in more detail how placing a carbon bed after a gliding arc plasma reactor can help to increase the $CO_2$ conversion and CO yield, by trapping the $O/O_2$ on the carbon bed (to avoid these recombination reactions), and (if the temperature is high enough) by the reverse Boudouard reaction ($CO_2(g) + C(s) \leftrightarrow 2CO(g)$). In addition, the carbon bed allows to remove $O_2$ and obtain a (more or less) pure $CO_2$/CO stream.

Our experimental results show that the $CO_2$ conversion is enhanced by almost a factor two (from 7.6 % to 12.6 %), while the energy efficiency rises from 28 % to 45 %, corresponding to a drop in energy cost from 42 $kJ.L^{-1}$ (without) to 25 $kJ.L^{-1}$ (with carbon bed). In addition, the CO concentration even increases by a factor three, and $O_2$ is completely removed from the exhaust mixture.

We also measured the temperature as a function of distance from the reactor outlet, as well as the $CO_2$, CO and $O_2$ concentrations and the temperature in the carbon bed as a function of time, which is important for understanding the underlying mechanisms. Indeed, these time-resolved measurements reveal that the initial enhancements in $CO_2$ conversion and in CO concentration are not maintained in our current setup. Therefore, we present a model to study the gasification of carbon with different feed gases (i.e., $O_2$, CO and $CO_2$ separately), from which we can conclude that the oxygen coverage at the surface plays a key role in determining the product composition and the rate of carbon consumption. Indeed, our model insights indicate that the drop in $CO_2$ conversion and in CO concentration after a few minutes is attributed to deactivation of the carbon bed, due to rapid formation of oxygen complexes at the surface

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# Recent developments in applications of plasma to the manufacture of flexible solar cells

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The current manufacture of modern energy-harvesting systems and other electronics based on silicon does not meet the requirements of the steps involved in low-cost fabrication. Rapid and low-cost roll-to-roll manufacture – the future of commercialization for flexible and printed electronics – requires flexible and low-cost substrates such as PET, PEN and, more recently, green materials such as nano-paper as well. The temperature at every single fabrication step is crucial with such materials and cannot exceed a certain threshold, generally 150 °C or less. Low-temperature plasma can, therefore, provide an excellent way forward for future manufacturing methods.

We present a proprietary, large-area plasma of extremely high-volume power density, up to 100 $W.cm^{-3}$, capable of generating diffuse, homogeneous and cold plasma (<70 °C) in the open air, as well as in other technical-grade gases including nitrogen, argon, methane, hydrogen, carbon dioxide and pure water vapour. Although the temperature of the plasma is very low, the population of energetic states is sufficient to induce physical/chemical changes on the surfaces of a range of nanostructured materials and semiconductors, such as graphene oxide, titanium dioxide, perovskites, and others, resulting in various crystallinity, optoelectronic and wettability changes, depending on the gas employed for plasma generation. The low temperature of the plasma and rapid treatment times, in the order of 1–10s, enables the integration of plasma processing into roll-to-roll manufacture, a significant step forward in the further commercial success within the emerging field of flexible and printed electronics.

In this contribution, we present two examples of fast (< 1 min) low-temperature plasma processing of thin films in perovskite solar cells: *i)* plasma treatment of indium-tin-oxide electrodes as a replacement of time-consuming chemical treatment before deposition of conductive films, and *ii)* plasma treatment of mesoporous titanium dioxide electron transporting layers (Fig. 1), as a replacement of time-consuming and high-temperature sintering.

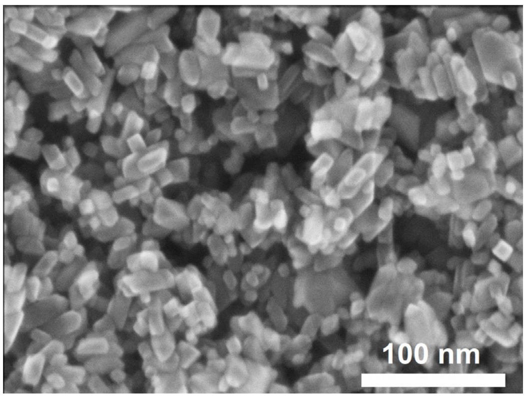


Fig. 1. Scanning electron microscopy image of $TiO_2$ mesoporous layer sintered in atmospheric nitrogen plasma for 5 min and further used in perovskite solar cell.

*This research was supported by the Czech Science Foundation project 19-14770Y.*

# On the combination of conventional and non-conventional probe diagnostics for process plasmas

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Diagnostics of electrons and ions in plasmas and fluxes of charged and neutral species toward plasma-facing surfaces by non-optical methods will be discussed. The focus is laid on the fundamentals of conventional methods as Langmuir probes (LPs), Faraday cups (FCs) and retarding field analyzers (RFA), but as well as on the principles of non-conventional diagnostics as calorimetric and force probes (CPs, FPs) [1]. These rather simple methods are useful tools for the measurement of overall, not species resolved, ions and neutral species fluxes toward surfaces. For example, RFAs provide overall ion energy distribution functions, whereas CPs and FPs can even deliver information about fluxes of fast neutrals.

Although many of these diagnostics have their roots in the beginnings of plasma research, they were gradually refined to match the requirements of plasma environments in industry, such as rf-discharges, reactive plasmas, dusty plasmas, and atmospheric pressure plasmas. Examples for "non-conventional" diagnostics, which are also applicable in plasma processes, are the determination of the total energy fluxes from plasma to substrate by calorimetric probes [2,3] and the measurement of momentum transfer due to sputtered particles or changes of plasma pressure by force probes [4,5].

Of particular interest is the combination of different types of probes, e.g. retarding field analyser (RFA) and passive thermal probe (PTP). The PTP serves as collector, in front of which three centrally aligned grids are operated as the retarding field system [6]. In this setup the collector does not only measure the incoming ion current depending on the voltage applied to the grids of the RFA, but also the incoming energy flux density of the impinging ions or neutrals, respectively. The ion energy distribution (IED) is determined regarding the energy exchange of the neutral background gas with the ions extracted from the plasma source.

The current trend in the miniaturization of sensors, adopted from the manufacturing of MEMS, will allow measurements with high spatial resolution in miniaturized plasma sources, like plasma jets or micro discharges [7], respectively.

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# Chemistry induced by atmospheric plasma in aqueous liquids

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The knowledge of plasma chemical processes at gas/liquid interface is of great importance for the understanding of fundamental mechanisms of interaction of non-equilibrium plasmas with a liquid state. Depending on the type of discharge, its energy, and the chemical composition of the surrounding environment various types of plasma-chemical reactions can be initiated and a number of primary and secondary species can be formed by plasma in the liquid either directly, or transferred from the gas phase discharge plasma being in contact with the liquid [1]. Among these processes, the oxidative properties of reactive oxygen species (OH radical, atomic oxygen, ozone, hydrogen peroxide) and nitrogen species (nitric oxide, nitrogen dioxide radical) are generally accepted to play central role in the chemical and biological effects of plasma produced in gas-liquid environments. In addition, secondary chemical and biological effects can be induced in the plasma-treated liquid through the post-discharge reactions of chemical species produced by plasma in the liquid either directly, or transferred from the gas phase discharge plasma via gas-liquid interface (e.g., $H_2O_2$, ozone, nitrite, peroxynitrite). Many of these chemical species are not stable in the liquid and subsequent reactions can take place giving rise to new transient species as OH•, $O_2^{•-}$, NO• and $NO_2$• radicals, which have highly cytotoxic properties and cause prolonged activity of plasma-treated solutions after the exposure to the discharge. This extended chemical and biological effect phenomenon was called by different names (e.g., plasma activated water) and is subject of study in different plasma medicine and agriculture motivated applications.

Nevertheless, the properties of plasma activated liquids and duration of their activity are affected by many factors which determine a type, quantity and also lifetime of the reactive species being formed in plasma treated liquid. For aqueous solutions that were treated by air-liquid-phase plasmas, great attention is paid to the chemistry and biocidal effects of peroxynitrite and acidified nitrites [2, 3]. Formation of reactive oxychlorine species and subsequent Cl-related chemistry and biocidal effects might be initiated by plasma in saline solutions [4, 5]. Chemical composition of cell culture media gives additional complexity to the aqueous chemistry in plasma activated liquids because presence of organic compounds [6]. Therefore, chemical and biological effects in plasma activated liquids are result of complex interactions of plasma at gas-liquid interface and chemical reactions of its products in the liquid. In this talk the main chemical processes initiated in plasma-treated liquids will be discussed.

*This work was supported by the Czech Science Foundation (project No. 19-25026S).*

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# Plasma driven synthesis of NPs and their immobilization

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Materials in their nanoparticle forms exhibit unique properties in comparison to their bulk counterparts [1], such as high catalytic activity, cohesive energy, peculiar electrical, magnetic, optical, and biological properties. New opportunities were opened in several fields among which sensors or biomedical applications, while at the same time such systems were raising concerns regarding their toxicity. While the chemical synthesis routes for obtaining materials in form of nanoparticles were intensively investigated, physical synthesis methods were also developed, among which some implying plasmas, like sputtering, arc discharge, in liquid laser ablation, and more recently cold atmospheric pressure plasma sources [2].

In the present work we show some of the advancements regarding the usage of low and atmospheric pressure plasma systems for NPs synthesis and regarding their incorporation in nanocomposite materials. As such, we will present results on the utilization of magnetron sputtering in classic mode or with the gas aggregation component and evidence the obtaining of various types and morphologies of nanoparticles, depending on the plasma conditions. Afterwards, we will continue with the results on the NPs synthesis starting from silver, cobalt and tungsten salts interacting with different types of atmospheric pressure plasma jets and show the role of the plasma species in the salt reduction and nano/microparticle formation.

*This work was supported by the Romanian Ministry of Research, Innovation and Digitization under the Nucleus Program LAPLAS VI – contract no. 16N/2019, projects PCCDI 46/2018 and PCCDI 63/2018.*

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# Experimental insights in the development of an oxygen removal process for coke oven gas with non-thermal plasma

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Non-thermal plasma (NTP) is applied to gas mixtures with high $O_2$ contents as air to oxidize the contained trace gases with a wide variety of chemical properties (e.g. volatile organic compounds). Is a high $O_2$ content necessary for the oxidation reaction or would NTP also preferentially activate $O_2$, if it is a minor component?

This research question is adressed by investigations on the removal of $O_2$ traces in complex gas mixtures such as coke oven gas (COG). This application is of particular importance, because the lower the $O_2$ content in the COG, the better the $H_2$ present in the COG can be recovered by pressure swing adsorption (Fig. 1). $H_2$ is a key component within the Carbon2Chem® project, because it allows the conversion with carbon containing components in steel mill gases to industrial chemicals such as methanol or urea [1].

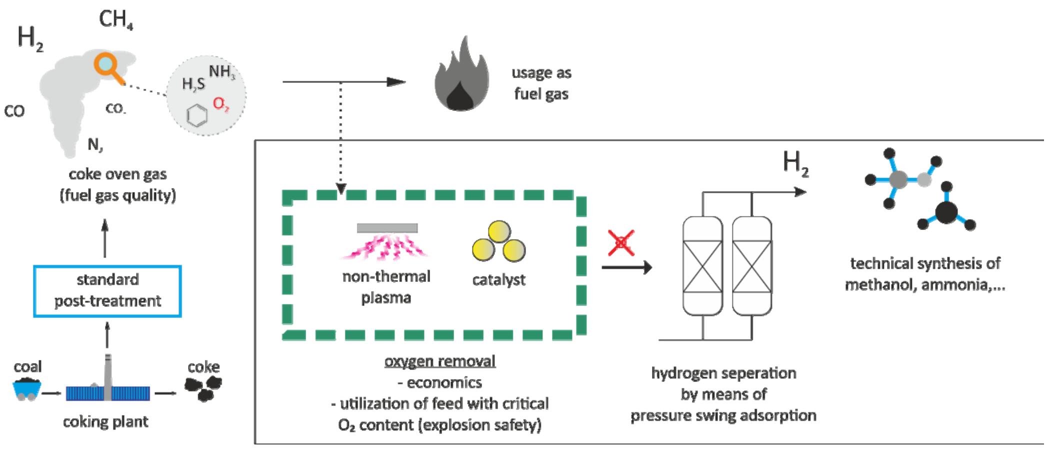


Fig. 1. Utilization of non-thermal plasma for $O_2$ removal in coke oven gas in the Carbon2Chem® project

For the laboratory research, model COG compositions (major components: $H_2$, $CH_4$, $CO_2$, CO, $N_2$; minor component: $O_2$) are composed by dosed mixing of bottled gases. The used atmospheric NTP reactor is a coaxial (packed-bed) reactor with dielectric barrier discharge (DBD). With this reactor, a gas feed of 0.05–0.2 $Nm^3/h$ is treated with NTP generated at a power consumption of 20–40 W. The reaction results are monitored with IR spectrometers, a thermal conductivity detector, and an electrochemical sensor.

First successful conversions of 1,000 ppmV $O_2$ in model COG mixtures could be observed with the NTP reactor without significant changes of the COG composition. The conversion is further enhanced by combining the plasma process with a catalyst (plasmacatalysis). The final investigations of the electrical parameters and the reactor setup lead to promising conversion results. Detailed information on these investigations and the results will be presented at the symposium.

*The work is performed in collaboration with our partners in the research project Carbon2Chem®, which is funded by the German Federal Ministry of Education and Research.*

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# Plasma-catalytic ammonia synthesis: The effect of the metal composition on the performance of Co-based $Al_2O_3$-supported catalysts

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Ammonia is an essential chemical not only due to its use in fertilizers and other applications, but also because it could serve as a hydrogen carrier [1]. Currently, ammonia is mainly produced via the highly polluting and energy-intensive Haber-Bosch (HB) process [2]. Consequently, a lot of research has been conducted towards finding carbon-free, sustainable routes to $NH_3$. Recently, plasma-catalytic nitrogen fixation has gained a lot of attention as a potential green alternative for the HB process as it combines the advantages of both catalysis and plasma [1].

Even though quite some advances have been made in the field of plasma-catalytic synthesis of $NH_3$, it is not yet clear how the knowledge from thermal catalysis translates into plasma catalysis [3,4]. Among other open questions, the role of the active metals on the $NH_3$ production rate and the interaction or synergy between the catalysts and plasma remain unclear. Previously reported findings suggested the slightly enhanced performance of Co-based catalysts in plasma-catalytic $NH_3$ synthesis over other metals data [4]. Furthermore, adding various metals (e.g. Ce, basic Ca or Ba) evidently improved the performance of Co catalysts in thermo-catalytic $NH_3$ synthesis [5]. Herein, we studied the effect of six different Co-based catalytic packing materials supported on $Al_2O_3$, including the added Ce and basic Mg and La: Co, CoCe, CoMg, CoLa, CoCeLa and CoCeMg, on the formation of $NH_3$ from $H_2$ and $N_2$ in a dielectric barrier discharge plasma reactor. Various characterization techniques, including Raman spectroscopy, X-ray powder diffraction, scanning electron microscopy energy dispersive X-ray analysis and $N_2$ sorption, were used to determine the structural and physicochemical properties of the catalysts.

The best performing catalyst at a 1:1 $H_2$:$N_2$ ratio and a total gas flow rate of 100 mL/min was found to be CoLa/$Al_2O_3$ which yielded an $NH_3$ concentration of 9000 ppm, an energy consumption of 83 MJ/mol and a production rate of 38 mg/h. Yet, the differences in $NH_3$ concentration, energy consumption and production rate between the different catalysts were limited, which agreed with the previous findings that various metals perform similarly in plasma catalysis [4]. At the same time, plasma properties such as deposited power and the current and voltage profiles varied significantly, depending on the catalyst. These data suggest that the catalysts act as plasma modifiers (i.e., changing the discharge properties and hence the gas phase plasma chemistry). This effect dominates over the direct catalytic effect defined by the chemistry on the catalyst surface.

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# Microwave plasma for fertilizer and oxygen production in the Martian atmosphere

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Mars exploration is of great scientific interest for many private as well as public organizations, of whom many are exploring the possibility of crewed missions. Due to enormous mission costs, there is a great need for in-situ resource utilisation (ISRU) technology to harvest Martian resources for fuel, life support and materials over the coming decades. In 2021 NASA's Martian rover *Perseverance* performed the 'Mars $O_2$ in-situ resource utilisation experiment' (MOXIE) [1], producing for the first time, extra-terrestrial $O_2$ using solar-harvested electricity-producing ~ 6g of $O_2$ from compressed Martian ambient using a full sol energy allocation of 1 kWh. To compare human consumption of $O_2$ is about 1 kg/day. MOXIE uses a solid oxide electrolysis cell (SOXE) for the conversion of $CO_2$ to $O_2$. However, due to the long start-up time (~2 hours), the technique is largely inflexible. Furthermore, longer stays on Mars will require on-site food production for which nitrogen fixed fertilizers [2] are needed. For this, the Nitrogen available in the Martian atmosphere (i.e., ~2 %) might provide a solution. In our setup [2], inspired by MOXIE conditions, a vacuum system is employed to lower the pressure inside the reactor to ~0.34 bar, while using a typical swirling mass flow rate of 10 L/min required to operate the reactor. In Fig. 1 production rates for $O_2$, CO and $NO_x$ of 47.0, 76.1 and 1.25 g/h, respectively, are shown with corresponding energy costs of 0.021, 0.013 and 0.79 kWh/g. Notably, the $O_2$ production rates are considerably higher than those currently demonstrated by MOXIE (~30 times), while the $NO_x$ production rate represents a ~7% fixation of the $N_2$ fraction present in the Martian atmosphere. The proposal for $N_2$ fixation (NF) with the Martian atmosphere using plasma, has, to the best of our knowledge, not been previously interrogated. Along with our experimental results, chemical kinetics models used to study the key reaction pathways will be discussed. In summary, MW plasma-based conversion shows great potential as an ISRU technology on Mars, for $N_2$ fixation and $O_2$ production using the local atmosphere. The technology also has the key benefit of a rapid start-up time and flexibility to use intermittent Martian solar electricity.

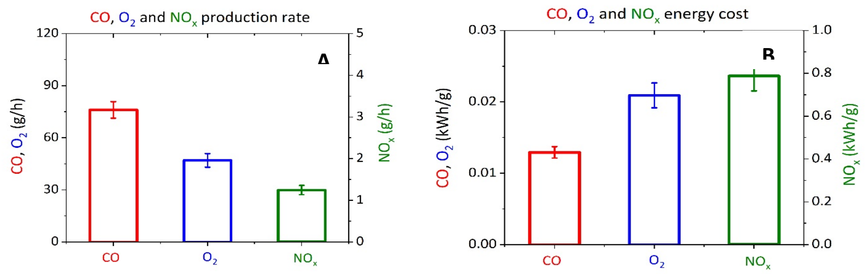


Fig. 1. A: Absolute production rates (g/h) of CO, $O_2$ and $NO_x$; B: equivalent energy cost (kWh/g), in a MW plasma using a Martian simulant mixture of $CO_2/N_2/Ar$ (96/2/2 %) at 10 L/min flow rate, 0.34 bar pressure and 1 kW absorbed power. Note the $NO_x$ data is indicated on the right-hand y-axes.

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# Effects of cold plasma activated liquids on urinary tract infections: *in vitro* vs. *in vivo*

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Urinary tract infections (UTI) are often caused by resistant uropathogenic bacteria and can lead to sepsis or chronic renal failure [1]. Cold plasma activated water liquids (PAL) are rich and tunable mixtures of reactive oxygen and nitrogen species (RONS) and have shown antimicrobial properties [2] with applications e.g. in wound disinfection. The aim of our study is to evaluate PAL as a potential treatment of UTI *in vitro* vs. *in vivo* in an animal model [3].

We tested four types of PAL prepared by different atmospheric air discharge settings: Streamer Corona (SC), Transient Spark with Electrospray (TS-ES), Transient Spark Batch (TS-B) and Glow Discharge (GD). Different types of activated liquids we examined: saline, PBS (Phosphate Buffered Saline) and deionised water. Concentration of $H_2O_2$, $NO_2$-, $NO_3$- were analyzed in each of these PALs, at different storage temperatures (-80°C, -18°C, 4°C, 23°C). The chemical analysis was carried out immediately after plasma activation and after 24-hour storage at each tested temperature. The alarming result is that freezing of PAPBS resulted in the strong decrease of the $NO_2$- concentration, which resulted in a decreased antibacterial effect.

Based on *in vitro* tests using a uropathogenic *E. coli* bacteria, PAL generated in the GD had the strongest antimicrobial effect in comparison to other types of PAL and was further tested *in vivo*. Single transurethral PAL application had no effect on bacterial load in the 24h mouse model of UTI. Upon investigating the treatment failure, we found that urine completely prevented any antimicrobial effects of PAL and PAL treatment of immunocompetent cells - neutrophils resulted in their reduced viability and loss of mitochondrial membrane potential.

These results do not support the hypothesis that the *in vitro* antimicrobial effects of PAL can be translated to the *in vivo* model of UTI. This could be explained by the attenuating effect of urine on the antimicrobial activity of PAL and its toxicity on immune cells. The detailed mechanism of the effects of urine on PAL requires further investigation.

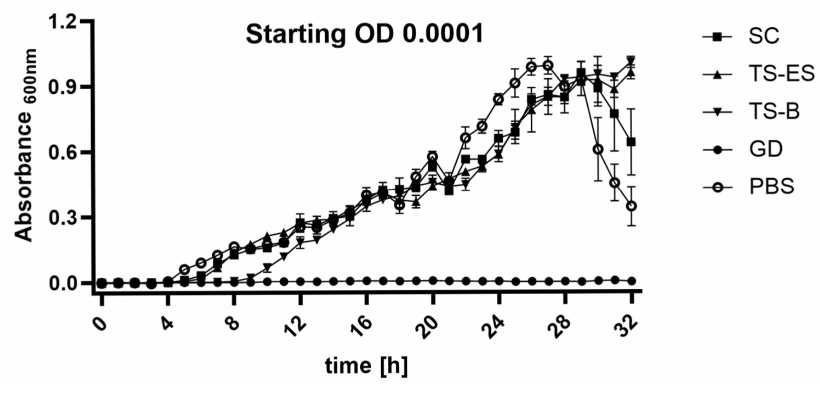


Fig. 1. Antimicrobial effect of four different types of PAL on uropathogenic *E. coli*: OD 0.0001 = 8×10$^4$ CFU/ml. GD PAL showed the highest antimicrobial effect, as no growth was detected throughout 32 hours.

*This work was supported by Slovak Research and Development Agency, grant APVV-17-0382 and APVV-18-0287.*

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# Cold plasma treatment of selected types of foods

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Non thermal plasma (NTP) is used as alternative technology for conventional thermal and pure chemistry-based food preservation. Glide arc reactor (GAD) was applied for conditioning of bakery products and fresh juice. The aim of the work was to investigate the effect of the use of NTP on the amount of microbes produced during bread and fresh juice storage.

In the case of GAD treated gluten-free and mixed wheat-rye bread after 600 s no mesophilic bacteria or fungi were found. 120 s of treatment resulted in complete lack of growth of yeast and mould in gluten-free and wheat-rye bread. A decrease in the moisture content of the bread was observed. In the case of gluten-free breads, this may be beneficial to a certain level because this bread is significantly higher in humidity than traditional bread. In addition, the breads we tested were natural without any improving additives.

The same reactor was used for processing of tomato (*Solanum lycopersicum* L.) juice in air. Tomato juice properties such as pH, total carotenoid and lycopene content (spectrophotometric method) and content of vitamin C (Tillmans dye method) were tested.

Plasma influence on background microflora, including the total aerobic mesophilic viable count and the total yeast and mold count was investigated.

On the first day after the preparation, the control (plasma untreated) juice samples exhibited a relatively high number of mesophilic aerobic microorganisms of 3.1 $\log_{10}$CFU/g. On the consecutive days of storage, this number increased significantly due to the microbial multiplication to the level of 5.0 $\log_{10}$ CFU/g after 7 days and 5.8 $\log_{10}$ CFU/g after 10 days of storage. The control juice samples exhibited the presence of lactic acid bacteria at a level of 1.9 $\log_{10}$CFU/g. Their number substantially increased on the subsequent storage days to reach 2.8 $\log_{10}$CFU/g, 3.7 $\log_{10}$ CFU/g, and 5.6 $\log_{10}$ CFU/g after storage days 4, 7, and 10, respectively. Coliform bacteria were present at a very low level of 0.6 $\log_{10}$ CFU/g on the first day, but they multiplied intensively throughout the storage time and reached 1.2 $\log_{10}$ CFU/g, 2.7 $\log_{10}$ CFU/g, and 2.8 $\log_{10}$CFU/g after 4, 7, and 10 days, respectively. Similarly, the small number of yeast detected on the first day, i.e. 0.6 $\log_{10}$CFU/g, increased to 2.6 $\log_{10}$CFU/g after 4 and 7 days of storage and 3.6 $\log_{10}$CFU/g after 10 days.

The 300-s CAP treatment resulted in significant improvement of the microbiological quality of the tomato juice and extended its shelf life to 10 days (in accordance with the allowable total number of aerobic microorganisms and the number of yeasts specified for pasteurized juices by the Regulation of the Polish Minister of Health of January 13, 2003). The total count of aerobic microorganisms was reduced by 2.4 $\log_{10}$ CFU/g after 7 days of storage and by 3.3 $\log_{10}$ CFU/g after 10 days in comparison to the control. The number of lactic acid bacteria was reduced by 1.4 $\log_{10}$ CFU/g after 7 days of storage and by 5.0 $\log_{10}$ CFU/g after 10 days. Regardless of the storage time, the counts of coliform bacteria and yeasts were reduced to < 10 CFU/g (< 1 $\log_{10}$ CFU/g), i.e. below the limit of quantification.

# Influence of the reactor configuration on the treatment of rapeseed using a conical corona reactor

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Plasma agriculture is a novel application that has been drawing more and more attention in the past years. Its importance derived from the urgent search of new alternative solutions for the agricultural sector as a result of the continuous increase in food demand. Plasma had emerged as a green technique adequate for use in pre- and post-harvest applications [1]. It has been demonstrated that cold plasmas are capable of promoting seed germination and seed viability, decontaminating plant material and enhancing plant growth [2]. Countless plasma sources have been reported for the treatment of several different plant species. Where new and unique operation parameters have to be adjusted in order to acquire good results. Thus, it is crucial to investigate which important plasma parameters play a role during seed treatment.

In the present work, a conical corona reactor was employed for the treatment of seeds. The corona reactor was already shown to improve rapeseed germination [3]. However, it was also reported that the amount of seeds influences the generated discharge (direct treatment mode). Here, the effects of different reactor configurations for seed treatment were studied. For instance, the difference between direct and indirect treatment of rapeseed was performed by altering the electrode's configuration. Rapeseed germination was shown to be accelerated by both treatment conditions, as shown in Figure 1. Even though the indirect treatment condition allows the treatment of more seeds simultaneously, it led to a less pronounced rapeseed germination acceleration.

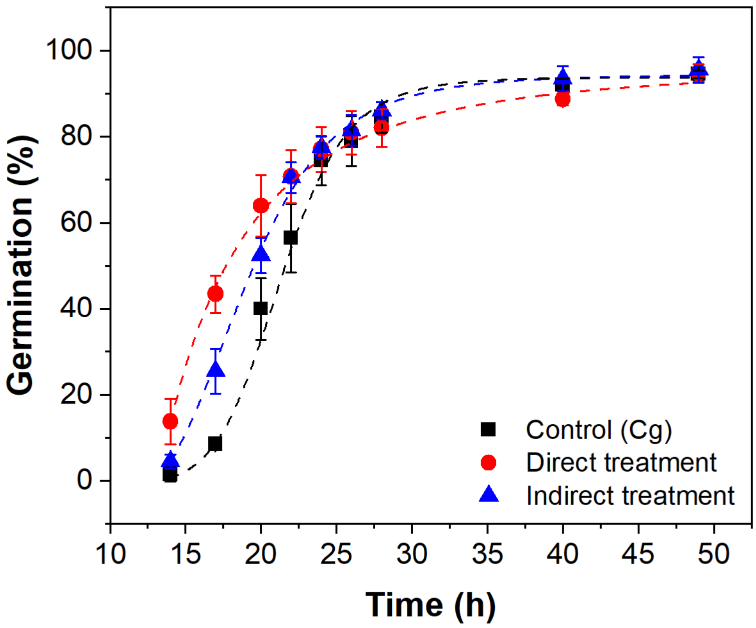


Fig. 1. Germination acceleration of rapeseed treated with direct and indirect mode using a conical corona reactor (treatment time of 5 min).

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# Biomolecule oxidation by CAP derived species: A general concept in biomedical plasma applications

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In plasma medicine, the application of plasmas for curative or preventive measures has been established successfully. Among the generated reactive entities, reactive oxygen and nitrogen species were attributed to play a significant role. While it is accepted that long-lived species such as hydrogen peroxide can penetrate into (model) tissues, the mode of action of short-lived species like atomic and singlet oxygen or peroxynitrite remains to be clarified. We hypothesize that the oxidation of biomolecules such as amino acids, lipids, proteins, and carbohydrates modulate or control downstream physiologic processes. Starting from amino acids (tyrosine, cysteine) and phospholipids (POPC), we found distinctive oxidation products and could show the incorporation of gas-and liquid phase derived atoms indicative for gas-liquid interphase reactions. Via model lipids and peptides, proteins and carbohydrates, a site and sub-structure specificity of plasma-derived reactive species could be shown (1-3). In catalase, phospholipase, or filamentary proteins, the newly introduced chemical modifications modulated protein activity and recognition and subsequently changed cell physiology. Accordingly, it can be stated that the oxidative modification of biomolecules is a regular event in plasma medicine potentially relevant for the outcome or diagnostic of CAP treatment.

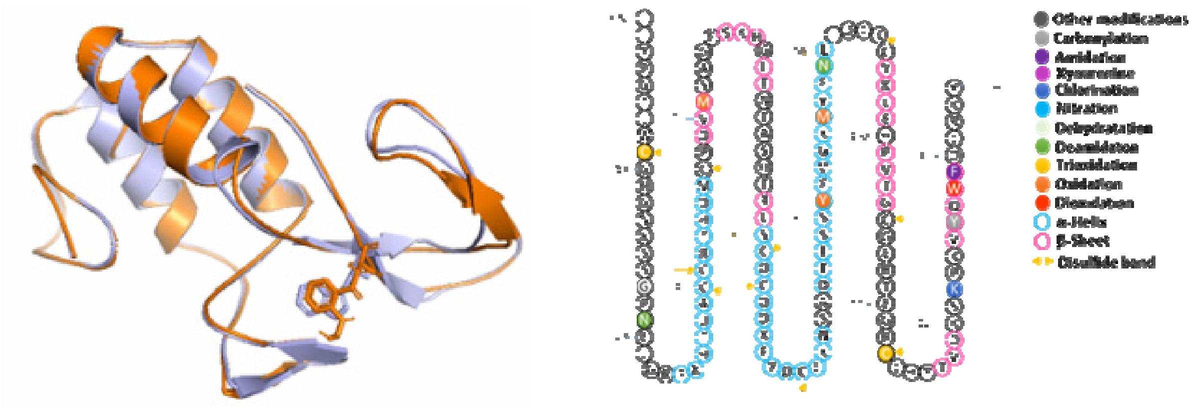

Fig. 1. Enzyme Phospholipase A2: the oxidation of the Tryptophan 128 by singlet oxygen leads to structural changes inhibiting lipid membrane approach and ultimately enzymatic activity

*This work was supported by the German Ministry of Research and Education (BMBF), grants no. 03Z22DN12 (to K. W.), 03Z22DN11 (To S.B.), and 03Z22Di1 (to K.-D. W.).*

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# How biopolymers in solution affect the generation and stability of plasma-generated reactive species

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Recently, non-thermal plasma (NTP) has been proposed and extensively investigated as a relevant tool for various biomedical applications, ranging from tissue decontamination to regeneration and from skin treatment to tumor therapies [1]. This versatility is due to the different kinds and amount of reactive oxygen and nitrogen species (RONS) that can be generated by the NTP treatment and put in contact with the biological target.

In our previous studies we have shown that solutions of biopolymers, when treated with NTP, can enhance the generation of RONS and influence their stability, resulting thus in the ideal media for direct and indirect treatments of biological targets [2-4]. The direct effects of the plasma treatment on the structure of biopolymers, as well as the chemical mechanisms responsible for the enhanced generation of RONS, are not yet fully understood. This knowledge would be extremely useful in tuning the parameters of the treatment to achieve target-specific outcomes.

In this study, we aim at filling this gap by investigating, on the one hand, the nature and extent of the modifications induced by NTP treatment in alginate solution, and, on the other hand, at using this information to explain the mechanisms responsible for the enhanced generation of RONS as a consequence of the NTP treatment.

The approach we use in this research is twofold: *i*) direct study of NTP-treated alginate solution, by techniques conventionally used in polymer science (size exclusion chromatography, rheology, scanning electron microscopy) and *ii*) study of a molecular model sharing its chemical structure, by chromatography coupled with mass spectrometry and by molecular dynamics simulations.

Our results point out the active role of alginate chemistry during direct NTP treatment. Short-lived RONS, such as ·OH radicals and O atoms, can modify the polymer structure, affecting its chemical groups and causing partial fragmentation. Some of these chemical modifications, like the generation of organic peroxide from the biopolymer, are likely responsible for the secondary generation of long-lived RONS such as hydrogen peroxide and nitrite ions.

*This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Grant agreement Nº 714793). Authors belong to the SGR2017 1165.*

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# The effects of non-thermal plasma treatment on the structural and functional parameters of human spermatozoa

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Nowadays, the number of couples that benefit from assisted reproduction (AR) is increasing due to various environmental and lifestyle factors. As high-quality reproductive cells contribute to the success of AR, it is essential to collect and process structurally and functionally competent gametes [1]. As pointed out by many studies, bacterial contamination of ejaculates has become an essential contributor to excessive sperm degradation in AR clinics [2]. However, the advantages of supplementing antibiotics to semen extenders have been questioned for a couple of years now. Despite the ability of antibiotics to prevent bacteriospermia, an increased number of reports have emerged emphasizing on the potentially toxic effects of traditional antibiotics on the sperm and embryo vitality [3]. These findings, coupled with a dramatically increasing bacterial resistance to antibiotics, have contributed to the need for finding appropriate alternatives to reduce their widespread use during semen handling.

Significant antibacterial properties of non-thermal plasma (NTP) have converted this technology into a promising solution for this problem. As substantial data available on the specific *in vitro* effects of NTP on male reproductive cells are currently missing, our study was designed to investigate selected quality parameters of human spermatozoa exposed to NTP generated by RPS40 plasma generator for 15 to 90 s. This plasma source is based on the technology of diffuse coplanar surface barrier discharge that was previously used for treatment of various cell types. Sperm motility characteristics, membrane integrity, mitochondrial activity, production of reactive oxygen species, DNA fragmentation and lipid peroxidation were investigated in our study. Sperm cells were analyzed immediately and 2 hours after the NTP treatment. Exposure to NTP for 15 s or 30 s had no negative effects on the structure and function of sperm. However, longer NTP treatment impaired all the sperm quality markers in a dose-dependent manner. Our findings indicate that appropriate plasma exposure conditions need to be carefully selected in order to preserve the sperm vitality.

Assuming lower NTP doses used in our experiments are effective enough to exhibit substantial antibacterial effects, the RPS40 plasma generator could become an interesting strategy to decrease the bacterial contamination of semen samples without harmful effects on the sperm cells.

*This work was supported by the Slovak Research and Development Agency grants APVV-16-0216, APVV-15-0544 and by the project VEGA 1/0460/21.*

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# Optical and electrical investigation of plasma generated by high-energy self-stabilized spark ignition system

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The environmental performance of internal combustion engines with forced ignition is improved when operating under lean mixture conditions. High-energy ignition systems are therefore being developed to ensure reliable ignition of lean mixtures. Here we present optical and electrical investigation of plasma generated in ambient air by a novel high-energy self-stabilized spark ignition system (Fig. 1). Aluminum and copper electrodes were used in point-to-point configuration, with the spark gap size from 0.5 to 13 mm. The discharge voltage was measured by TEKTRONIX P6015A and HVP-39pro PINTEK voltage dividers, while the current was measured by Ion Physics CM-500-L current transformer and by Honeywell CSNM191 current sensor.

We observed two current pulses. The first current pulse appears in the time interval 0 to 3 µs as a consequence of a high-voltage pulse (about 20 kV) supplied to the spark gap. The discharge current after the first current pulse gradually increases up to 3 ± 1 A, when the second current pulse appears. The delay between two current pulses increases with growing spark gap size from 60 to 125 µs, corresponding to gap size 0.5 mm and 13 mm, resp. The duration of the second current pulse is about 150-300 µs with a current amplitude of 140-180 A, depending on the spark gap size. The experimental-computational method was used to estimate the energy input to plasma. It was found that the main part of the energy, 231-541 mJ, is deposited during the second current pulse.
The electrical signals were correlated to temporally resolved optical signals measured by photodetector and high speed Andor iStar iCCD camera coupled to high-resolution spectrometer. Thanks to time resolved emission spectra of $N_2$ SPS (0-0 transition) we observed that the gas temperature from ~4000 K to ~6000 K during the time delay between two current pulses. Time resolved emission spectra of Al, Cu, H, N, O, $N^+$, and $Al^+$ atomic and ionic lines enabled us to estimate evolution of electron excitation temperature ($T_{exc}$) and electron density in generated plasma. It was found that $T_{exc}$ of N, $N^+$ and Cu species is about 10-20 kK, and the highest electron density, $3\text{-}4\times10^{17}$ cm$^{-3}$, correlates to the maximum of the second pulse current (Fig. 2).

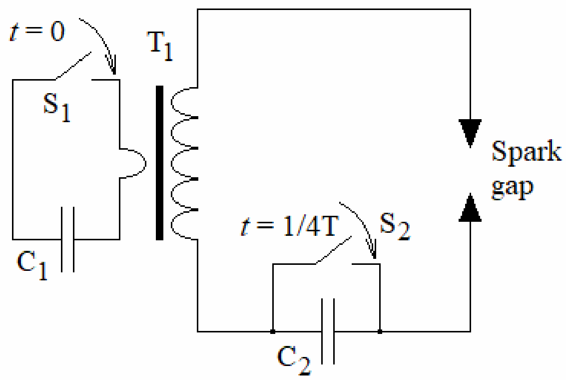


Fig. 1. Schematic diagram of the developed spark ignition system, $T_1$ - transformer, $C_{1,2}$ - capacitors, $S_{1,2}$ - switches.

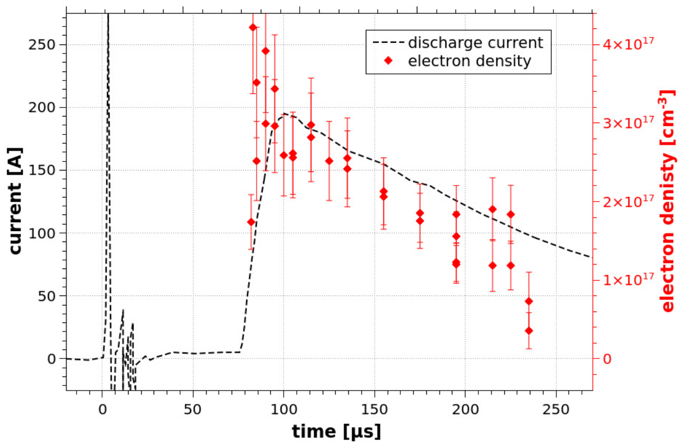


Fig. 2. Discharge current and electron density calculated from Stark broadening of Hα line.

*This work was supported by Slovak National Grant Agency VEGA, project No. 1/0596/22 and Slovak Research and Development Agency APVV-20-0566.*

# Optimization of plasma-activated media generation for decontamination of thermally sensitive materials

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The plasma-activated gaseous media can decontaminate surfaces of different materials at remote distances. Thus, this work's main aim was to determine parameters for efficient decontamination and/or sterilization of thermally sensitive materials at atmospheric pressure. For this purpose, the multi-hollow surface dielectric barrier discharge was used for plasma activation of gaseous media to produce an atmosphere with a high ratio of hydrogen peroxide and/or ozone. These active species were generated in pure water vapor [1] and oxygen with an admixture of water vapor.

The thermal and electrical properties of the used plasma source were measured. Optical emission spectroscopy was used to analyze the characteristics of generated plasma. The reactive species in plasma-activated gas and condensed activated vapor were detected and compared for different plasma parameters, such as water vapor concentration, gas flow and source power input.

The generated media was then applied to microorganisms in the form of planktonic bacteria, bacterial biofilm, and biological indicators for standard sterilizers. The germicidal efficiency of short and long-time exposure to plasma-activated media was evaluated by standard microbiological cultivation and fluorescence analysis using a fluorescence multi-well plate reader. The test was repeated at different distances from the surface of the plasma source.

The decontamination efficiency of plasma-activated water vapor increased with the exposure time and the plasma source power input. Similar results were obtained for the decontamination by plasma-activated oxygen and oxygen with the admixture of water vapor. The main results of the optimization process are a prototype of the decontamination chamber and verified technology for the construction of scalable commercial plasma devices for utilization in medicine and bioresearch.

*This work was supported by project no. TJ04000329 funded by the Technology Agency of the Czech Republic. This research was also supported by project no. LM2018097 funded by the Ministry of Education Youth and Sports of the Czech Republic.*

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# Synergic antibacterial effect of pulsed electric field and plasma activated water

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Bacterial contamination is an economic and human problem, particularly in food or medical applications. Nosocomial infections became a global scourge with 700,000 deaths each year and could cause up to 10 million deaths by 2050, a figure that is increasing due to the appearance of resistant bacterial strains. Over the past decades, pulsed electric fields (PEF) and cold plasma-activated water (PAW) have demonstrated their antibacterial efficacy and can be used as an alternative antibacterial solution. We investigated the antibacterial treatment of the strain *Escherichia coli* ATCC 25922 by PAW and coupled with an application of PEF.

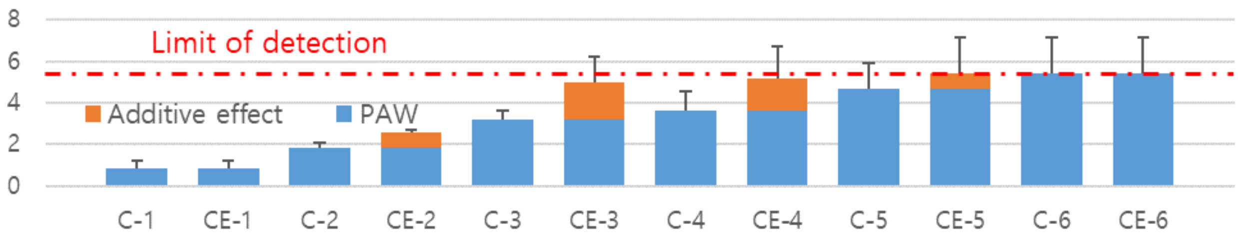


Fig. 1. Antibacterial effect on E. coli in water: 10 min incubation in PAW TS-BC closed reactor in blue and the additive effect from PAW + PEF. C-1 no added H2O2, C-2 to C-6 is, respectively, added 100 µM, 200 µM, 1 mM, 2 mM, 10 mM H2O2. The letter E is for an additional PEF treatment.

The PAW is generated by cold air plasma of transient spark discharge (TS) operating at 1 kHz in a closed or open atmosphere of air in batch water system. It is generated by a DC generator delivering a voltage of 14-16 kV to a needle electrode placed at 10 mm from the surface of 5 ml of deionized water with an immersed ground electrode. The open-air chemistry favored formation of $H_2O_2$ over $NO_2$- in PAW, while the closed air produced a strong dominance of $NO_2$-.

The PEF is obtained by applying 10 000 electrical pulses of 12.5 kV at a frequency of 100 Hz with a duration of 200 ns delivered between two electrodes 2mm apart. The effect on the viability of the bacteria was measured by measuring CFU by incubating the bacteria grown on the agar. The reactive oxygen and nitrogen species (RONS), hydrogen peroxide $H_2O_2$, and nitrite $NO_2$- in the PAW were measured by UV-visible absorption spectrometry, as well as the pH measurements.

The PEF+PAW coupling makes it possible to significantly increase the antibacterial effects of the different treatments. In addition, understanding the mechanisms involved in PEF+PAW treatments could open other possibilities for using these two technologies in the medical field: improving wound healing, stimulation of the immune response, cancer treatment, and in the food processing.

*This work was supported by Slovak Research and Development Agency APVV-17-0382.*

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# Comparison of non-thermal plasma produced by cometary and point-to-ring discharges for portable devices usable in biomedical applications

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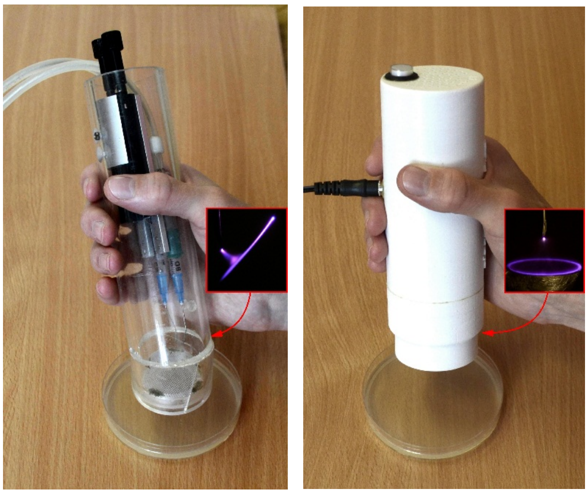
The capability of some medically important pathogens to develop defense mechanisms against traditional drug therapy has led to increased interest in the use of non-thermal plasma (NTP). NTP is an effective tool for inhibiting bacteria, viruses, fungi, etc. due to its special mechanism of action, which is based on damage to microbial membranes. We developed two types of NTP sources for biomedical applications that use a DC cometary discharge in the point-to-point electrode system and a DC corona discharge in the point-to-ring electrode system (Fig. 1).



(a) (b)

Fig. 1. The developed plasma devices based on the cometary (a) and corona (b) discharges.

The physical and microbicidal properties of these plasma sources were compared. Despite some differences in the electrical parameters, optical emission spectroscopy showed that both these sources generate NTP plasma of similar composition. To explore the microbicidal properties, we tested the effect on 12 strains of different microorganisms (bacteria, yeast, and micromycetes). In general, the cometary discharge forms smaller but more rapidly emerging inhibition zones, while the corona discharge affects a larger area but with a less pronounced microbicidal effect. However, over long-term exposure, both plasma sources have approximately the same microbicidal efficiency in terms of their use to inactivate all microorganisms tested.

Later, we improved the plasma flow channel of the plasma source based on point-to-ring corona discharge and achieved a higher microbicidal effect by orders of magnitude.

*This work was supported by the Czech Science Foundation Project GAČR GF21-39019L and Charles University COOPERATIO Institutional grant.*

# Investigation of $CO_2$ decomposition in pulsated warm arc plasma by optical emission spectroscopy

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Gliding arc plasmas can most efficiently decompose carbon dioxide $CO_2$ at atmospheric pressure [1]. However, the space-resolved physical properties of gliding arcs have not yet been well studied due to the intrinsic limitations when performing plasma diagnostics on a moving thin plasma channel. In order to overcome these limitations, a non-gliding warm arc plasma source has been recently developed in our laboratory. The arc plasma is generated between two metallic electrodes within a gap of 22 mm within a tubular glass envelope as shown in Fig. 1. Furthermore, the developed warm arc plasma source can be fed by square wave voltage pulses of different width, in contrast to standard gliding arc reactors working under direct or alternating voltages. The resulting quasi-square current wave flowing through the plasma have an initial overshoot of 4 A and a flat current below 1 A, lasting less than 1 ms as shown in Fig. 2. Optical emission spectroscopy is used as plasma diagnostic tool to investigate the spatial and transient changes of the plasma composition. As it can be seen in Fig. 3, the spectrum is dominated mainly by the gas emission in the C2 Swan band and by the 777 nm O I triplet line. No metallic lines are observed, in contrast to previous spectroscopic measurements in a similar geometry at low currents [2]. Interestingly, the optical emission spectra of microwave plasmas, which are also efficient to dissociate $CO_2$ but at low pressures, are also dominated by the $C_2$ Swan Band [3]. The space-averaged, time variation of the spectral peaks measured by two photomultipliers coupled to their corresponding monochromators will be also discussed.

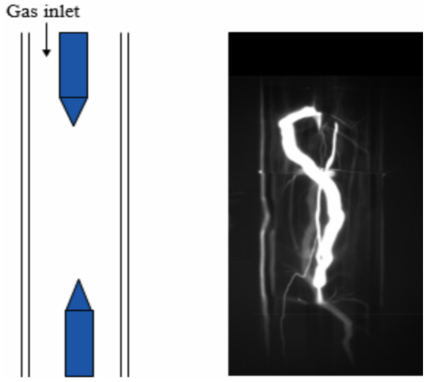


Fig. 1. Schematic of the electrode system and still photograph of the produced warm arc.

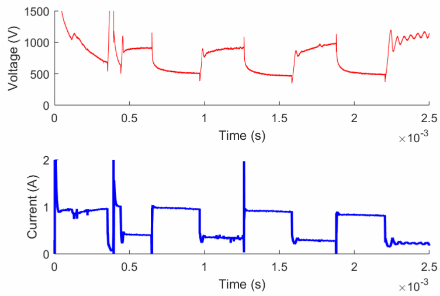


Fig. 2. Example of a train of current pulses applied to the warm arc plasma. The current scale is truncated at 2 A.

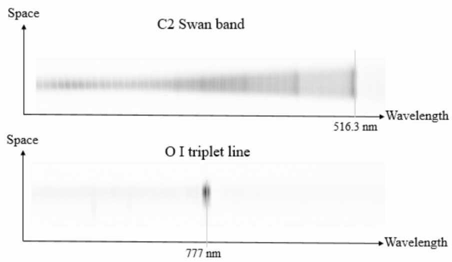


Fig. 3. Measured side-on spectral image for a CO2 arc plasma.

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# Nonthermal plasma regeneration of deactivated catalysts after plasma-catalytic removal of toluene and naphthalene

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Nonthermal plasma (NTP) is well known for its ability to create a highly reactive chemical environment. However, chemical reactions induced by NTP significantly suffer from a low reaction selectivity [1]. This fact must be particularly considered for environmental applications of NTP (e.g., air pollution control) since low reaction selectivity may lead to formation of undesired by-products. In order to enhance the reaction selectivity and, thus, to decrease a yield of undesired by-products, NTP is commonly combined with a catalyst (i.e., plasma catalysis). The selectivity of the plasma catalysis is much higher than NTP alone, however, in real conditions undesired by-products can be still present [2]. This is often a case when plasma-catalytic removal of complex volatile organic compounds (VOCs) or polycyclic aromatic hydrocarbons (PAHs) is employed. The undesired by-products usually create solid carbon deposits on the surface of the catalysts leading to their deactivation (i.e., loss of their catalytic activity and/or selectivity over time) [3]. After a catalyst deactivation, catalyst can be regenerated (restored), recycled, or discarded. From economic and environmental point of view, first option should be preferred to the other two.

In this work, the utilization of NTP generated by atmospheric pressure dielectric barrier discharge (DBD) for regeneration of deactivated catalysts was investigated. The experiment consisted of two consecutive steps. Firstly, the cylindrical DBD reactors packed with various catalytic materials ($Pt/\gamma Al_2O_3$, $TiO_2$, $BaTiO_3$) were employed for plasma-catalytic removal of toluene or naphthalene with synthetic air as a carrier gas. Toluene and naphthalene were chosen as model VOC and PAH compounds, respectively. Their removal resulted in formation of several gaseous and solid by-products, some of them were found as solid carbon containing deposits on the surface of catalysts. Secondly, deactivated catalysts were regenerated by DBD reactors of the same geometry for several hours with oxygen as a carrier gas at ambient or elevated temperature ($100^oC$).

Regeneration of the catalysts led to formation of gaseous products ($CO_2$, CO and HCOOH) as a result of oxidation of solid deposits and their concentration as a function of time was evaluated. The results showed that concentration of gaseous products during regeneration decreased over time and rate of the decrease depended on the specific product as well as catalytic material. In addition, an efficiency of the catalyst regeneration strictly depended on the catalytic material, what is probably linked with different discharge modes governing the various DBD reactors. The analysis of gaseous products was performed by FTIR. Surface of the catalysts was analysed by SEM and optical microscopy. Finally, regenerated catalysts were reused for toluene removal in several repetitive cycles. The results obtained with regenerated and non-regenerated were compared.

*This work was supported by grants of Slovak Research and Development Agency APVV-20-0566 and Slovak Grant Agency VEGA 1/0822/21.*

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# Indoor air decontamination by cold atmospheric plasma and photocatalysis

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With millions of deaths and many restrictions, the recent Covid-19 pandemic has strongly impacted the world, and the risk of a new deadly variant is real. In addition to viruses, the air pollution (chemical pollutants, bacteria, other pathogenic aerosols, tobacco smoke, etc.) is responsible for respiratory, cardiovascular, and oncological diseases and millions of deaths every year. Outdoor as well as indoor spaces can contain harmful pollutants, and nosocomial infections in the hospitals can also be spread through air contaminants. Therefore, achieving a high indoor air quality would be a major advance for preventing other COVID-related restrictions and for health issues in general. However, older technologies for air purification (using non-destructive methods such as HEPA filters) suffer from many drawbacks (pressure losses in heating, ventilation, and air conditioning, filter replacement, maintenance costs, possible pollution source, low efficiency for small particles, etc).

In this study, we propose using indoor air decontamination based on cold atmospheric plasma (CAP) combined with photocatalysis. Both technologies have shown to be efficient for the decomposition of a wide range of pollutants, and they have also recently proved that they were effective for inactivation of viruses such as SARS-CoV-2 [1, 2]. Combining these two techniques may offer a very effective hybrid air decontamination device, as studies suggest a synergetic effect [3]. Our decontamination device combines cold atmospheric plasmas generated by two Dielectric Barrier Discharges (DBD), and a $TiO_2$ coating wrapping the whole device interior which is activated by four UV C lamps placed inside the reactor. We studied the effect of each component of the device taken separately (i.e. DBD only, UV only, UV with photocatalytic coating, and their combinations).

To assess the efficacy of the air purification device, we followed a norm (AFNOR XP B44-200) that describes the testing methods and the pollutants to use. It is a single-pass method, where we measured the concentration of the pollutant before and after the single pass into the air decontamination device. We also performed the tests in a closed environment, especially for the study of the influence of humidity in the decontamination process. The tested pollutants were formaldehyde and acetaldehyde, as representative indoor pollutants.

The results show that in dry condition ($RH < 30\%$), the photocatalysis is more effective than the DBDs only. The overall efficacy of the device appeared to be promising, given the very short residence time of the pollutant in the reactor. With higher levels of humidity, we observed an increase in the DBD efficacy for the removal of pollutants. We also monitored the concentration of ozone generated by the DBDs, which is well decomposed by the photocatalytic process.

*This work was supported by Slovak Research and Development Agency APVV-17-0382 and APVV-20-0566. The air decontamination device was developed together with IQ Capital, s.r.o. Slovakia.*

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# Hydrophilization of fluorinated polymers

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A standard method for hydrophilization of polymer materials is a brief treatment with non-equilibrium gaseous plasma. Reactive plasma species interact chemically with the solid material in the surface film and cause the formation of various new functional groups. Many groups are highly polar, so their presence causes an increase in the polar component of the surface energy and thus hydrophilization of the polymer. Standard polar functional groups contain oxygen, so a natural solution for polymer hydrophilization is treatment with oxygen plasma. The large concentration of polar surface functional groups will assure the hydrophilic surface finish. However, the plasma treatment does cause not only functionalization but also etching of the modified surface film. The hydrophilization will be successful when the etching is less pronounced than the functionalization. The etching and functionalization rates depend on the type of polymer and the polymer temperature. Fluorinated polymers are etched much more extensively than functionalized upon treatment with oxygen plasma, so the wettability is hardly improved upon the plasma treatment [1]. A method useful for hydrophilization of any polymer, including fluorinated ones, is a subsequent treatment with plasmas rich in vacuum ultraviolet radiation and oxygen radicals [2]. The radiation from the first plasma causes bond scission in the surface film of the polymer and this depletion of fluorine. The second plasma treatment causes functionalization with the polar, oxygen-containing functional groups. One of the best sources of photons capable of scising C-F bonds is a low-pressure hydrogen plasma sustained with an inductively coupled radiofrequency discharge in the H-mode. The radiation in the range of wavelengths from about 100 to 150 nm, and simultaneous treatment with H atoms, will cause the substitution of the fluorine in the surface film with hydrogen in a time span of about one second [3]. The functionalization with polar, oxygen-containing functional groups depends on the fluence of O-atoms on the polymer surface. Best results are obtained in the range of fluencies between $10^{20}$ and $10^{21}$ $m^{-2}$. Any plasma capable of supplying these fluences of O atoms would do.

*This work was supported by Slovenian Research Agency, Grant no. L2-2616 (Selected area functionalization of polymeric components by gaseous plasma) and Core Fund P2-0082 (Thin-film structures and plasma surface engineering).*

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# Control strategies for aerosol-assisted atmospheric pressure plasma deposition of fluorinated silane thin films

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Plasma enhanced chemical vapor deposition (PECVD) is a unique technique to produce coatings with characteristics suitable for a wide range of applications, including electronics, aerospace, and medicine [1]. Despite the technology is well-established at low pressure (LP), atmospheric pressure (AP) PECVD is receiving a lot of attention due to the absence of expensive vacuum equipment and the possibility of industrial on-line processing [2]. Whatever the pressure, researchers always had a strong interest in controlling the PECVD process by correlating the deposition conditions to the resulting coating properties [3]. To achieve this goal, the so-called Yasuda Parameter W/FM (where W is the discharge power and FM the precursor flow rate) was proposed for LP-PECVD processes [4]. Nonetheless, its implementation at AP has been questioned since the precursor is typically mixed with an inert gas, thus requiring a careful evaluation for each experimental setup [5]. An additional layer of complexity is added when the precursor is introduced in aerosol form, since particle size distribution, precursor evaporation and particle charging might influence plasma characteristics [6]; nonetheless, the introduction of aerosolized precursors is an extremely interesting technique to deposit coating retaining most of the chemical moieties of the precursor, by limiting their fragmentation, and to encapsulate nano/micro-metric functional additives within the coating [7]. In this work, the use of W/FM as control parameter for a PECVD process from an AP plasma jet and an aerosolised fluorinated silane precursor is investigated. ATR – FTIR and XPS results show that a tailored fluorine content in the coatings can be tuned according to W/FM: the lower is the degree of fragmentation of the precursor in the plasma discharge, the higher is the fluorine retention in the deposited coatings. These findings are further supported by water contact angle measurements which reveal a higher coating hydrophobicity for low W/FM values. Scanning electron microscopy images indicate that W/FM affects the deposition rate while not inducing substantial differences from a morphological point of view. Moreover, it is shown that coatings deposited under the same W/FM obtained with different combinations of discharge power and precursor flow rate exhibit same chemistry and wettability, suggesting the validity of W/FM as control parameter. Since the interest towards the AP-PECVD of fluorinated silane coatings arises from the need to develop new antimicrobial biomaterials for orthopedic implant applications, results of the stability and antimicrobial activity of the deposited coatings are presented.

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# Dry cleaning and activation of flexible glass using nonthermal plasma before PEDOT:PSS coating

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Substrate conditioning is an important part of coating processes, and it varies with the materials involved. Coating of ultra-thin flexible glass (UTFG) is specific due to its low weight and high fragility, while its advantageous chemical and optical properties predestine UTFG as a substrate, barrier and packaging material for various optoelectronic applications. Coating techniques with conductive inks are often applied in flexible electronics manufacturing. UTFG has lower surface energy than a common float glass, which normally hinders the coating process, and to improve the coating quality, it is advisable to increase the substrate's surface energy. The present study compares standard wet cleaning method and dry atmospheric-pressure plasma treatment applied prior to deposition of conductive polymer PEDOT:PSS layers. Glass substrates for PEDOT:PSS layers are usually pre-cleaned in ultrasonic bath using three liquids. Plasma generated with dielectric barrier discharges (DBDs) in ambient air was tested as a potential alternative viable for UTFG cleaning. A 30 µm thick UTFG was used as the substrate for two different deposition techniques of various PEDOT:PSS dispersions—spin and spray coating. Two different DBD geometries (volume and coplanar) were compared to study the additional effect of substrate activation induced by plasma. DBDs are promising plasma sources for direct exposure of UTFG due to their nonthermal character. Volume DBD (VDBD) plasma source is the most industrially known configuration for modifying flexible materials. The comparison with coplanar DBD geometry was realized by applying Diffuse Coplanar Surface Barrier Discharge (DCSBD). Both plasma sources were tested in curved implementation, which can be employed in roll-to-roll systems. Plasma was generated in ambient air at atmospheric pressure to meet the ecologic and economic aspects of modern industrial production. Before coatings, surface analyses of UTFG targeted its hydrophilicity, chemical composition, and morphological changes, and were carried out with WCA measurement, XPS analysis and AFM, respectively. The quality of PEDOT:PSS layers was studied with a focus on their uniformity, thickness and electrical conductivity. SEM imaging, profilometry and four-point probe measurements were carried out to explore these properties. Both applied coating techniques showed similarly significant improvement of PEDOT:PSS layers deposited on pre-conditioned UTFG. Plasma treatment exhibited the same cleaning efficiency as ultrasonic cleaning but in a substantially shorter time—in just one second—and therefore proved efficient for PEDOT:PSS coating without any additional requirements for cleaning. Moreover, rapid exposure of UTFG to DBD plasma did not cause any damage or deterioration of its extraordinarily smooth surface. Simultaneously, the gentle cleaning effect of plasma exposure induced the activation of the UTFG surface due to an increase in the present oxygen-based functional groups. The electric properties of PEDOT:PSS layers were in concordance with the increased surface energy of plasma-activated UTFG before coating. Compared with common wet cleaning, the use of VDBD plasma-modified UTFG resulted in a significantly lower sheet resistance of consequently deposited PEDOT:PSS layers.

*This work was supported by the Czech Ministry of Education, Youth and Sports grant no. LM2018097, the Technology Agency of the Czech Republic no. FW03010109 and the Slovak Grant Agency no. VEGA 1/0488/19.*

# Modelling study of $CO_2$ conversion enhancement in microwave plasmas using a quenching nozzle

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Due to ecological problems associated to fossil fuels, the future energy landscape is expected to move further towards renewable energy sources (solar, wind, etc.). In this context, plasma technology is gaining increasing interest to use renewable electricity for converting greenhouse gases like $CO_2$ into value-added chemicals and fuels, thereby closing the so-called "carbon loop".

Microwave plasmas have proven to be successful for $CO_2$ dissociation, achieving very high conversion and energy efficiency. [1] At atmospheric pressure, these plasmas are characterized by high temperatures up to 6000 K, in which thermal conversion of $CO_2$ into CO and O is the dominant dissociation pathway. However, the conversion via this pathway is limited by the recombination of CO back into $CO_2$:

$$CO + O + M \rightarrow CO_2 + M$$

$$CO + O_2 \rightarrow CO_2 + O$$

These recombination reactions typically occur after the plasma, where the gas temperature is still high (i.e. around 3000 K), losing a significant part of the CO that was formed in the plasma. It is suggested that fast quenching of the gas after the plasma could slow down recombination reactions and increase the CO retention. [2] In this work, computational fluid dynamics simulations are performed to investigate the effect of a cooling nozzle right after the plasma to induce such a fast gas quenching and maximize the CO retention. Experiments performed by Hecimovic et al. [3] demonstrated a significant enhancement of the $CO_2$ conversion and energy efficiency using this nozzle, particularly at pressures close to atmospheric pressure and for lower $CO_2$ flow rates. Our simulations show that the nozzle increases mixing with the cool gas surrounding the plasma, leading to fast quenching. This effect indeed delivers the greatest enhancement at lower flow rates, as recombination is more prevalent in these regimes due to the longer residence time in the afterglow. This way the CO retention is drastically enhanced, increasing the $CO_2$ conversion from 5% to 35% for a nozzle with 2.5 mm diameter at a flow rate of 5 slm at atmospheric pressure.

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# $CH_4$ coupling in nanosecond pulsed plasma discharges: 0D modelling to unravel the effect of pressure and temperature on product selectivity

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$CH_4$ conversion into olefins (especially ethylene) is a desirable strategy as it holds the promise to reduce emissions of greenhouse gases and generate key value-added raw materials for the chemical and polymer industries. The use of plasma technology in $CH_4$ conversion is of particular interest because it allows for the thermodynamically unfavourable process of $CH_4$ splitting to occur via energy-efficient avenues [1]. This contribution presents a 0D kinetic model to study the gas phase chemistry involved in the non-oxidative $CH_4$ coupling into $C_2$ products under nanosecond pulsed plasma discharges (NPD). The work aims to uncover the main routes for $CH_4$ dissociation (within 1–5 bar) and identify underlying limitations, offering insights to experiments in this research area.

The model comprises 60 species (stable and vibrationally excited molecules, radicals, ions and electrons) and 2088 reactions. It describes a co-axial reactor geometry and the NPD power inputs were integrated via time-resolved pulsed power profiles. Pulses were emulated by asymmetric triangles (Fig 1a) with differing intensity and width depending on applied pressure. The model was numerically solved by ZDPlasKin (in tandem with BOLSIG+) whereby, under varying physical conditions (*e.g.* plasma power, electric field and electron and gas temperature), the continuity equation is evaluated for each species, computing the probability of all reactions as a function of time. As shown by previous studies [2], gains in $CH_4$ conversion and selectivity towards ethylene can be correlated with increased pressures, hence the model was tested in the 1–5 bar range, with notably good agreement between experimental and modelled results (Fig 1b).

Reaction pathways and heating and cooling mechanisms were studied. This was decisive to determine major avenues of $CH_4$ dissociation into radicals and ions and pinpoint critical back-reactions slackening $CH_4$ coupling into higher products. We also delineate the effect of applied pressure on average gas temperatures, in turn drawing correlations with conversion and selectivity trends. This analysis revealed rapid and sluggish pathways and their dependency on pressure and temperature, readily rendering approaches to tune selectivity towards $C_2H_2$ or $C_2H_4$ and to improve $CH_4$ conversion rate and energy efficiency in NPD experiments.

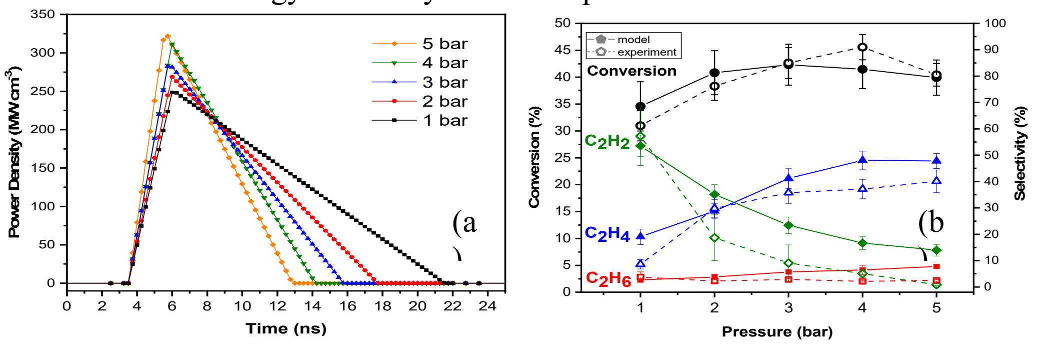


Fig. 1. (a) Pulses in the range of 1 to 5 bar emulating experimental power discharges and (b) $CH_4$ conversion and selectivity profiles of $C_2H_6$, $C_2H_4$ and $C_2H_2$ as a function of applied pressure.

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# Correlation between properties of plasma treated liquids with characteristics of atmospheric pressure plasma devices

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Non-equilibrium plasma at atmospheric pressure that operate in contact with liquids has proven to induce various chemical reactions in liquids. The most important chemically reactive species formed in the gas discharge above the liquid target are reactive oxygen and nitrogen species (RONS), electrons, UV radiation etc [1]. In complex interactions these species penetrate and/or react with liquid-phase molecules producing short and long-lived species in the target. Thus, adjusting of the plasma chemistry in the gas phase results in different outcomes of the liquid treatment which supports employment of the plasma treatments for different applications [2,3]. With respect to the purpose of the plasma treatment, it can be used for creation of new species, i.e. for liquid activation and creation of plasma activated water (PAW) and plasma activated medium (PAM), or for destruction of dissolved pollutant molecules – for decontamination processes. In this work we will present both kind of plasma treatments featuring comparison of properties of the treated liquids depending on different plasma setups, operating parameters etc. Treatments of pure and contaminated water (with organic dye AB25) were performed by using pin-type of plasma sources operating with He and Ar. These jets were operated with continuous sine signal at the frequency around 350 kHz. The setups were designed to enable precise determination of power delivered to the plasma and the sample. We investigated the influence of the working gas as well as sample volume to the RONS concentration in the activated water. For PAW treatments, we observed much higher production of $H_2O_2$ in case of Ar plasma than in He. We tested variation in the efficiency of the plasma decontamination by adding multi-pin source and for conditions of recirculation of the contaminated sample. The effective treatment surface parameter proved to be significant for regulation of the decontamination efficiency. With respect to the treatments of the cell media, we used a dielectric barrier discharge (DBD) jet configuration that operated with continuous signal at around 80 kHz. We studied the influence of the input power on production of RONS in the PAW and PAM. It was shown also that important operating parameter for this kind of plasma jet was gas feed gas humidity. Understanding and optimizing the production of RONS in the plasma system is important due to their roles in major biological processes, thus fine tuning of the produced species is of great importance.

*This work was supported by NOWELTIES project - EU H2020 MSCA ITN No. 812880 and by Ministry of Education, Science and Technological Development of Republic of Serbia grant number 451-03-68/2022-14/200024.*

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# Non-thermal plasma for generation of antimicrobial aerosol

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To overcome the ongoing Covid-19 pandemic new therapeutical approaches are needed. A local antiviral treatment of the upper respiratory tract may be a promising approach to reduce SARS-CoV-2 virus load. Applications utilizing non-thermal plasma technology already showed antimicrobial activity treating bacteria containing liquids[1] as well as airborne microorganisms[2]. Recently, also human coronaviruses were treated successfully by using plasma activated water[3]. However, a plasma-assisted in situ treatment of viral loads in the respiratory tract is not available so far.

One approach of local antiviral treatment is the application of plasma-treated air or aerosol, respectively. Thus, a coaxial dielectric barrier discharge (DBD) was combined with an airflow or with an aerosol generator for producing plasma activated aerosols. The aerosol was generated out of distilled water or saline solution. To avoid accumulation of condensed aerosol inside the reactor, it was operated vertically and the gas passage zone is made smooth and free of edges. The size distribution of the aerosols was monitored optically with and without discharge operation to avoid the aerosol entering the human respiratory tract further than the trachea. Discharges were investigated electrically, i.e. voltage amplitude and current, and the ozone concentration of the treated gas was measured.

In order to investigate the feasibility of this approach, the discharge configuration was characterized by using dry and moist synthetic air as process gas. Due to the toxicity of ozone, which is commonly produced in DBDs operated in oxygen containing atmospheres, the plasma source was especially designed for production of low ozone concentrations. At a high voltage amplitude of 4 kV and a frequency of 28 kHz the device generated less than 50 ppm of ozone in dry synthetic air.

As a benchmark test, *Escherichia coli* cultured on tryptic soy agar served as a model microorganism. The agar plates were positioned in a gas tight glass vessel containing the plasma treated gas or aerosol. Multiple treatments were carried out for different time periods.
In this presentation, the inactivation of *E.coli* by non-thermal plasma treated gas and aerosol will be discussed. Additionally, research on the influence of gas composition and plasma on the plasma chemistry will be reported.

*This work was supported by the German Federal Ministry of Education and Research, grant no. 03COV06A.*

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# Forest material treatment by PAW

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This work studies the effect of Plasma Activated Water (PAW) to inactivate *Brettanomyces bruxellensis* in oak barrels and to eliminate TCA (2,4,6-tricloroanisol) from corks of wine. Oak barrels play a key role since they provide aromas and taste sensation to wine. On the other hand, barrels need to be disinfected, otherwise food safety could be negatively affected. Barrel sulphuring is the most used method; however, this technique will be forbidden as a European prohibition (Directive 98/8/CE2) mentions. TCA is known as "the cork disease" and at least 4% of worldwide wines are affected by it. TCA is the most relevant issue within wine industry because once the wine gets TCA organoleptic properties, which are impossible to remove, it is not suitable for sale. In order to cover both topics, PAW was used which was generated as Fig. 1a shows. PAW treatment was applied by immersion of contaminated samples (fragments of oak staves and corks) in a container with PAW during 3 hours. Fig. 1bc illustrates results achieved by PAW treatments. All PAW showed pH below 4. Nitrates, nitrites and reactive species were analyzed. Among these last ones, OH*, NO* y $NO_2$* (products after phenol reaction) were identified by chromatography [1] and $HNO_2$ by UV-vis. It is thought that those short-life species come from the instability of nitrites in an acidic medium. Moreover, the longer the generation time of PAW, the higher the amount of reactive species. The biocidal capacity could be affected by a synergetic effect of the reactive species identified and the TCA reduction could be cause of OH* [2]. Finally, the chosen PAW (PAW-5 min) achieved a reduction of 3.49 log of *Brettanomyces* and 75.2% decrease of TCA in artificial contaminated corks.

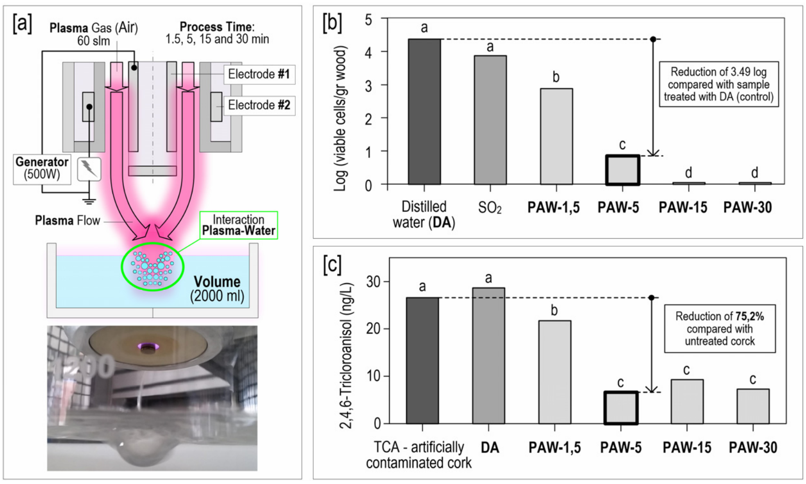


Fig 1. [a] PAW generation scheme; [b] Logarithmic growth of *Brettanomyces* after destilled water (AD) and PAW treatment; [c] TCA mean concentration (ng/l) of artificial contaminated corks after destilled water (AD) and PAW treatment. Different letters mean statistically significant differences (p < 0,05).

*This work was supported by National Grant PID2019-105367RB-C21 and PID2020-113658RB-C2.*

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# Revision of 3.4 um band destruction rates under ion beam irradiation of hydrogenated amorphous carbon as interstellar dust analogues

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The 3.4 um IR absorption band, assigned to CH stretching vibrations of $CH_2$ and $CH_3$ aliphatic groups, widely observed in the diffuse interstellar medium and also been observed for extragalactic sources, is associated with the presence of carbonaceous interstellar (IS) dust. Since the first reports in early 1980's on 3.4 um band observations in space, a debate was present on whether this band can be used as an indication for the presence of complex organic molecules in space. As result, various plasma devices are employed for carbon dust deposition of hydrogenated amorphous carbon (HAC or a-C:H) samples in order to match the astrophysical observations and related processes: capacitive coupled or inductive coupled radio frequency discharges, laser ablation plumes, pulsed discharge nozzle, spark and arc discharges, magnetron discharges, dielectric barrier discharges (last plasma technique to enter the scene [1,2]).

The present work will review our results on Dielectric Barrier Discharge (DBD) studies, as a new method for low temperature deposition of HACs for interstellar dust analogues. Comprehensive characterization of HACs is performed by microscopy and spectroscopic techniques, allowing to subtract relevant quantities such as $CH_2/CH_3$ ratio, H/C ratio or sp2/sp3 ratio. The talk will close by introducing our recent results on HACs energetic processing, using 3 MeV $H^+$ and 1 MeV $H^+$, $C^+$ and $Si^+$ aiming to revise the effects of analogues' morphology and density on the 3.4 um band intensity drop [3]. The characteristic times for CH bond destruction as compared with the dynamical times of diffuse regions and dense clouds allow to group the literature results into two families: data that support the scenario of unlikely aliphatic CH bonds destruction by cosmic rays and data that support a possible aliphatic CH bonds destruction by cosmic rays.

*This work received partial support from National Grant no. PN 19 06 02 01 and PN 19 06 02 02, Romanian Government Programme through the Programme for Installations of National Interest (IOSIN), from Spanish National Research Council (CSIC) through the i-Link project number LINKA20353 and from French Laboratory of Excellence CaPPA (Chemical and Physical Properties of the Atmosphere).*

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# Selective study of ion-substrate interactions using the VUV-photoionization chamber

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Cold atmospheric pressure plasmas (CAPs) are a powerful tool to study the effects of plasma treatment on biological substrates or other surfaces. By changing the design of the plasma source or the experimental setup it is possible to gain insight to the isolated effect of different reaction partners and mechanisms. Previous studies showed that for example the high reactivity of CAPs with biological substrates is in addition to the reactive neutral oxygen and nitrogen species, also based on additive or synergistic effects of these reactive species with charged species, photons, and electric fields [1].

In contrast to photons or electric fields, the study of the isolated effect of ions with biological substrates or on thin film growth is more challenging as their isolated production under atmospheric pressure conditions is not trivial. However, several authors have proposed the important role of ions next to the electric field for the treatment of biological substrates [2,3]. To prove the expected enhanced effect of ions due to their charge and internal energy, knowledge about the ion composition and absolute ion fluxes to the substrate is needed.

To investigate the isolated effect of ions on substrates and ion-based thin film deposition, an experimental setup has been developed in which photoionized ions are directed towards the substrate. The ions are generated in a helium atmosphere with a small admixture of $O_2$, $C_2H_2$ or other gaseous species by vacuum ultraviolet (VUV) radiation of the helium excimer emission (60−100 nm) generated by a helium driven atmospheric pressure plasma. Mass spectra and relative ion fluxes along the substrate position are measured by ion mass spectrometry. Combined with spatial resolved current measurements the absolute ion flux of $4 \cdot 10^{13}$ $s^{-1}cm^{-2}$ can be determined. A good agreement between mass spectrometry and current measurements can be observed. The deposition of polymerlike thin films with ions generated from $C_2H_2$ and a deposition rate around 0.005 nm/s will be discussed.

*This work was supported German Research Foundation (DFG) - 356768018.*

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# Biocidal effect of i-PAW

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The effect of three different Iced Plasma Activated Water (i-PAW) on the microbiota of cuttlefish (*Sepia officinalis*) along its storage was evaluated in this work. Three different generation process (Direct, Recirculated and Bubbles) were used in order to generate PAW which were characterized in terms of nitrates, nitrites, hydrogen peroxide, hydroxyl radical (OH*), nitrogen monoxide (NO*) and nitrogen dioxide ($NO_2$*). Concentration of those species (some of them by fenol reaction [1]) before and after freezing were analyzed. Then, those PAW were frozen in flakes (i-PAW) and cuttlefish was stored with that i-PAW at 2°C for 12 days. Moreover, the microbial load of the cuttlefish (mesophilic and psychrophilic bacteria) was evaluated at different times of storage. Regarding reactive species concentrations, the highest ones of OH*, NO* and $NO_2$* were detected in the "bubbles" PAW before freezing. These chemical species seem to be generated by secondary reactions due to the instability of nitrites at acidic pH (pH < 3.5). It is worth mentioning that $H_2O_2$ was not detected in any PAW studied. Furthermore, just after thawing (≈6ºC) PAW (pH > 3.5) displayed a significant reduction of OH*, NO* and $NO_2$* (reactive specie + fenol) concentration. It is believed that secondary reactions are affected by that pH (> 3.5). The microbial loads of cuttlefish fillets stored with i-PAW made of recirculation-PAW and bubbling-PAW were significantly lower than those of the control (stored in ice prepared from distilled water) during all the storage time, increasing shelf life of cuttlefish at least, two days, microbiologically speaking. Considering the above, the results of this study suggest that i-PAW is a very interesting alternative for extending the shelf life of cuttlefish and that bubbling would be the most promising PAW generation process for this purpose.

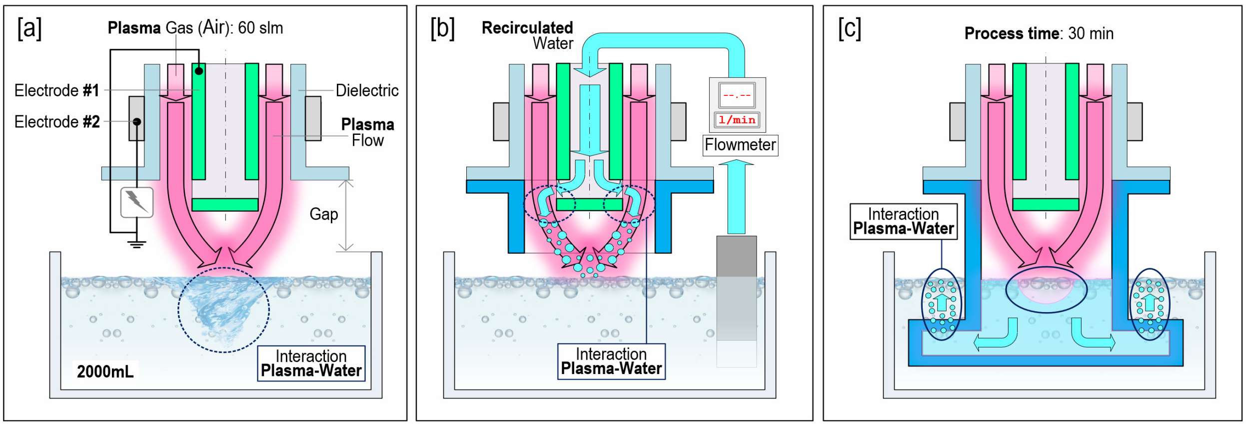


Fig. 1. PAW generation methods: [a] Direct; [b] Recirculated; [c] Bubbles.

*This work was supported by National Grant PID2019-105367RB-C21 and PID2020-113658RB-C2.*

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# CaviPlasma: A large-throughput technology for plasma treatment of contaminated water using peroxide chemistry

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Plasma treatment of contaminated water in industrial scale amounts and flow rates represents an ultimate goal, however the performance of reported plasma technologies hinders testing on samples larger that tens of milliliters, typically [1]. Technological and plasma physical key challenges lie in large volume treatment at reasonable timeframe [2,3]. We introduce a recently developed plasma source that tackles these challenges using a synergistic effect of hydrodynamical cavitation and electrical discharge in cavitating environment, called “CaviPlasma” [4]. The CaviPlasma performance potential is obvious from three simple numbers: 1 $m^3/h$ – flow rate of table-top, lab-scale unit; 1 $kWh/m^3$ power supply consumption; >10 mg/l hydrogen peroxide formation efficiency per passage. This meets or exceeds the limit of applicability >5 mg/l $H_2O_2$ at flow rate > 5 gal/min stated in [5]. We have achieved the peroxide yield of $G(H_2O_2)$ = 9.5 g/kWh, which ranks CaviPlasma to high efficiency plasma sources.

CaviPlasma combines hydro-mechanical effects, i.e., mechanical stress and efficient mixing of agents generated using an electric discharge in the reduced-pressure environment of void of cavitation cloud, with electro-mechanical stress and UV radiation. This results in very promising biocidal and decontamination effects on plasma-treated contaminated water. Representative examples of the results are focused on the removal of toxic cyanobacteria from water achieving a complete inactivation even after a single passage [6], or the remediation of wastewater sampled at municipal treatment plant from the residual contamination of pharmaceuticals, hormones and their metabolites (> 90% reduction).

*The Czech Science Foundation is gratefully acknowledged for support of this research under project No. GA22-11456S "Complex phenomena of hydrodynamic cavitation plasma jet and their impact on advanced oxidation processes in liquids". The research is supported also by the Technological Agency of Czech Republic under project No. SS01020006 and Slovak Research and Development Agency APVV-17-0382. This work results within the collaboration of the COST Action CA19110.*

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# Evaluation of the effect of plasma activated water on plants contaminated with heavy metals using laser-induced breakdown spectroscopy

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Heavy metal contamination of the environment is a significant problem that we face nowadays. The accumulation of heavy metals in soil is a big concern in agriculture because of the effects of heavy metals on food safety, crop growth and health of soil organisms. Plants grown in heavy metal-contaminated soil can exhibit altered metabolism, growth reduction, lower biomass production and metal accumulation [1].

Determination of exact distribution of heavy metal pollutants in plants is paramount for an assessment of toxicity and possible health risks. Laser-Induced Breakdown Spectroscopy (LIBS) is based on the principle of laser ablation, which occurs when a high-energy laser beam strikes the surface of a sample. During laser ablation, a microplasma is generated. The laser pulse incident on the sample serves as a source for subsequent vaporization, atomization and excitation of the ablated mass. This method is capable of the detection of broad range of elements. Additionally, it gives us information about spatial distribution of studied elements in the sample which enables a retrospective study of pollutant migration within the plant. It is possible to map the whole plants or only chosen parts of plant samples [2].

Plasma activated water (PAW), a new application of non-thermal plasma, has been used in several studies to increase seed germination and plant growth. In this study, we evaluated whether the growth rate of plants contaminated by cadmium can be increased with the application of plasma-activated water, as well as the accumulation of heavy metals can be reduces in industrial hemp *Cannabis sativa*. In short toxicity test, seedlings of *Cannabis sativa* were grown in mixture of PAW and $CdCl_2$ solutions with concentrations of 50 $\mu mol \cdot dm^{-3}$, 250 $\mu mol \cdot dm^{-3}$ and 500 $\mu mol \cdot dm^{-3}$. Control samples were simultaneously grown in PAW and in solution of $CdCl_2$ without PAW. Toxicity of tested solutions was assessed on the basis of macroscopic toxicological endpoints. LIBS was used to demonstrate the spatial distribution of cadmium in plant samples.

*This work was carried out within the frame of COST Action CA19110. PP and JK gratefully acknowledge the support by Brno University of Technology under grant no. FSI-S-20-6353.*

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# Multi-hollow surface dielectric barrier discharge: Variations of gaseous products under conditions of various air flow rates and relative humidities

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Evaluation of gaseous products (reactive species) generated by the discharge is crucial for its employment in practical applications especially under ambient air conditions.

In this work, variations of the gaseous products of the multi-hollow surface dielectric barrier discharge (SDBD) in dry air were studied with respect to various discharge powers (1–5 W), air flow rates (0.25–2.4 L/min) and air relative humidities (0–80%). The discharge was generated by a perforated ceramic substrate in a configuration with the air-exposed electrode [1]. A unique geometry allowed the air to pass through holes (hollows) of the substrate inside which the discharge was formed enabling efficient production of several gaseous products. Out of them, production of ozone $O_3$, nitrous oxide $N_2O$, nitric oxide NO, nitrogen dioxide $NO_2$, dinitrogen pentoxide $N_2O_5$ and nitric acid $HNO_3$ were evaluated by means of FTIR spectroscopy and UV absorption in terms of concentration (ppm) and production yield (g/kWh).

The work demonstrated a critical impact of both air flow rate and air relative humidity on prevailing discharge mode ("$O_3$ mode" vs. "NOx mode") and, thus, on production and composition of gaseous products. Whereas the air flow rate particularly determines the air residence time in a zone of generated discharge as well as gas heating, the air relative humidity influences the discharge from physical (processes of discharge formation, propagation, and its characteristics) and chemical aspects (production of H, OH, $HO_2$ radicals). When the discharge operated in the "$O_3$ mode", $O_3$, $N_2O$, $N_2O_5$ and $HNO_3$ were observed among the gaseous products, while with the discharge transitioned to the "NOx mode", $N_2O$ and $HNO_3$ along with NO and $NO_2$ were present. In dry air, the threshold of specific input energy (energy density) for a transition of the discharge from "$O_3$ mode" to "NOx mode" was approx. 1100 J/L, while with an increase of air relative humidity from 20 to 80%, it gradually decreased from approx. 600 to 450 J/L, respectively.

The work demonstrated that when the multi-hollow SDBD is generated under ambient air conditions, it generates various gaseous products in a wide range of concentrations that can be easily regulated by proper working conditions [2]. Therefore, the discharge may eventually find many possible applications, particularly in plasma pollution control and biomedicine.

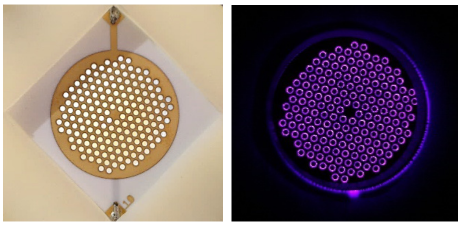

Fig. 1. The photographs of perforated ceramic substrate (left) and multi-hollow SDBD (right).

*This work was supported by grants of Slovak Research and Development Agency APVV-20-0566 and Slovak Grant Agency VEGA 1/0822/21.*

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# Preparation of protective hydrophobic layers on aluminum using plasma polymerization at atmospheric pressure

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Preparation of hydrophobic layers, and polymerization in general, is conventionally realized by the means of chemical processing. Besides monomers, these methods also require the use of solvents and catalysts, which produce additional toxic waste. Plasma polymerization (PP) is a method by which polymer-like layers are deposited using a plasma source. Highly reactive particles produced by the plasma activate a gaseous or liquid monomer present in the working gas in order to start the PP process [1]. The prepared functional layers are mechanically and chemically resistant, insoluble, thermally stable and homogeneous due to their high degree of monomer cross-linking, and they adhere well to different types of surfaces [2]. Further advantages of PP include generally lower costs than conventional polymerization methods and the ability to control multiple working conditions (input power, exposure time, electrode system configuration, etc.) to create layers of a precisely defined thickness and chemical functionality. Therefore, plasma polymerization can be considered a promising and environmentally friendly method of hydrophobic-layer preparation.

Many works on the topic of pp(plasma polymerized)-hydrophobic layers deal with polymerization of hexamethyldisiloxane (HMDSO). The presented work builds on our previous promising research in this field related to PP of HMDSO on aluminum [3] and glass substrates [4]. The main objective of this work is to carry out, study and optimize PP of HMDSO using plasma generated by a Diffuse Coplanar Surface Barrier Discharge (DCSBD) on aluminum substrate. Nitrogen was used as the carrier gas with admixtures of HMDSO in different concentrations. Water contact angle measurements were used to evaluate wettability of the sample surfaces and confirmed the hydrophobic effect of the pp-HMDSO layer. ATR-FTIR and XPS analyses were conducted to show the presence of the characteristic chemical groups corresponding to the pp-HMDSO layer as well as to determine its chemical composition.

We observed the PP process at various working conditions and showed how they are related to the resulting hydrophobicity of the prepared layers. The effect of plasma post-treatment on already polymerized layers was also studied. Importance of securing the optimal distance between the sample and the DCSBD ceramic was demonstrated.

*This work was financially supported by the Slovak Grant Agency for Science VEGA (No. 1/0811/21).*

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# Adhesion improvement of large area flexible PTFE foils by atmospheric pressure plasma

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Polytetrafluoroethylene (Teflon, PTFE) as an indispensable polymer has been widely used in modern society because of its excellent physical and chemical bulk properties. However, due to its low surface and weak boundary layer, the surface wettability and adhesion properties of PTFE are poor. This greatly limits the application of PTFE in the field with higher demand for the adhesion and wettability, especially in the biomedicine area. Therefore, it is very important to improve the adhesion and wettability of the PTFE surface. Nowadays, environmental protection and high efficiency are urgent needs in the industry. Plasma is the preferred option to satisfy those requirements [1,2]. The main objective of this work is to rapidly modify the surface of large area flexible PTFE foils by Diffuse Coplanar Surface Barrier Discharge (DCSBD) in order to improve the adhesion and wettability. The flexible PTFE foils were treated by the low-temperature non-equilibrium DCSBD plasma generated in ambient air, Argon and the mixture of $H_2/N_2$ (5% volume content of $H_2$). The plasma modification of PTFE foils was done in a special closed roll-to-roll device that enables in-line plasma treatment of flexible substrates up to the width of ~ 200 mm. The adhesion test of the flexible PTFE foils was performed by 90 degree peel adhesion test. The wettability and aging behavior were assessed by water contact measurement of sessile droplets. The surface chemistry and roughness were investigated by X-ray Photoelectron Spectroscopy (XPS) and Atomic Force Microscopy (AFM). It can be found that the adhesion and wettability of the flexible PTFE foils can be effectively improved by DCSBD plasma, however a significant effect of used working gas was observed. The mixture of $H_2/N_2$ seems to be as the most effective gas for modification of PTFE. The oxygen-containing functional group are introduced to the surface of the flexible PTFE foils after plasma treatment and the surface roughness of plasma-treated flexible PTFE foils also increases. The results of the aging behavior show the plasma-treated flexible PTFE foils exhibited only a partial recovery of the original wettability after two weeks.

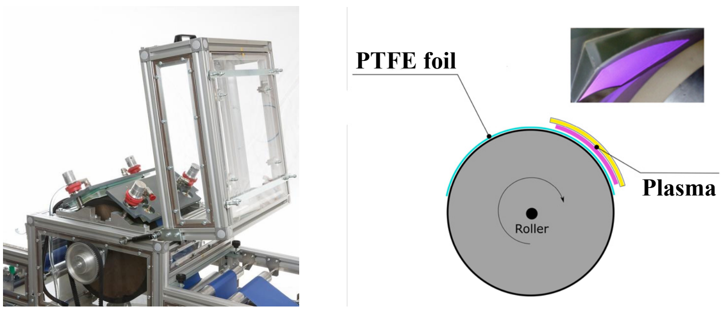


Fig. 1. Experimental setup for continuous modification of flexible Teflon foils by DCSBD plasma.

*This work was supported by the Czech Ministry of Education, Youth and Sports grant LM2018097.*

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# Applications of surface dielectric barrier discharge generated from liquid electrode

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This contribution presents the special type of Surface Dielectric Barrier Discharge (SDBD). An atmospheric pressure plasma technique was developed for technically simple treatment of inner and/or outer surfaces of hollow dielectric bodies, e.g., plastic tubes. The research results aimed at the application of the discharge will be presented.

Using water solution as a discharge electrode makes it possible to combine basic features of both water discharges and surface dielectric barrier discharges [1]. The SDBD is generated from the contact line between the liquid electrode, air and dielectric material (tube).

To illustrate the application potential of such SDBD and its basic physical properties, authors present a simple example of hydrophilization of the inner and outer surfaces of polytetrafluoroethylene (PTFE) and polyvinyl chloride (PVC) tubes which play a crucial role in the plastics industry and continues to be a polymer of significant importance. The effect of plasma treatment on PTFE and PVC tubes was verified by the contact angle, XPS and SEM measurements.

The next step in the technique development was the construction of several reactors enabling continuous plasma treatment on the laboratory and industrial scale and their successful testing. The technical details of the experimental setup and the results of polyethylene tube treatment are discussed in [2,3].

One of the experiments carried out by the authors was the deposition of liquid precursor (hexamethyldisiloxane) on the PTFE tube's external and internal wall surface with the help of this discharge type. Samples prepared in the dynamic mode were treated by the continual movement of the plasma ring across the tube surface. The motion of the plasma ring was achieved by a mechanism on the principle of two connected vessels. A better description of the apparatus can be found in [4].

Another possible application of such a plasma source was the plasma treatment of PTFE tubes to investigate the possibility of altering the properties of *Pseudomonas aeruginosa* cell biofilms formed on the inner surface of tubes. Experiments revealed the ability of plasma treatment to partially remove biofilms and inactivate the biofilm-forming cells. Thus, if it was not possible to remove all of the biofilm-forming cells, they were inactivated.

*This research has been supported by the projects LO1411 (NPU I) and LM2018097, funded by the Ministry of Education Youth and Sports of the Czech Republic.*

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# Image analysis evaluation of the non-thermal plasma inactivation of dermatophytes

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Following our previous study [1] on the treatment of onychomycosis with non-thermal plasma (NTP) and nail hygiene, we investigated the dynamics of the inactivation by NTP plasma of *Trichophyton iterdigitale*, *Trichophyton benhamiae*, *Trichophyton rubrum,* and *Microsporum canis* isolated strains. We also attempted to obtain data on the susceptibility of these dermatophytes. Three strains of each species were exposed on agar plates at different time intervals to plasma generated by DC corona discharge in a point-circle arrangement. Although all strains were sufficiently sensitive to plasma exposure, significant differences were observed in their sensitivity and inactivation dynamics [2].

Using Image Analysis software, the studied strains were classified into four types according to their growth trends observed after plasma exposure. Figure 1 shows the characteristics of these types. The different types are defined as follows: The "Strong effect" type showed almost complete growth suppression after exposure. In contrast, the "No effect" type included strains whose growth was not suppressed at all. The intermediate "Soft effect" type included strains whose growth was only slightly inhibited. In the 'Kick off effect' type, an initial growth retardation followed by rapid growth was observed.

These differences did not correlate with the species classification of individual strains, but could be assigned to four arbitrarily constructed types of strain response to NTP based on their sensitivity. These results suggest that plasma sensitivity is not an inherent property of fungal species, but varies from strain to strain.

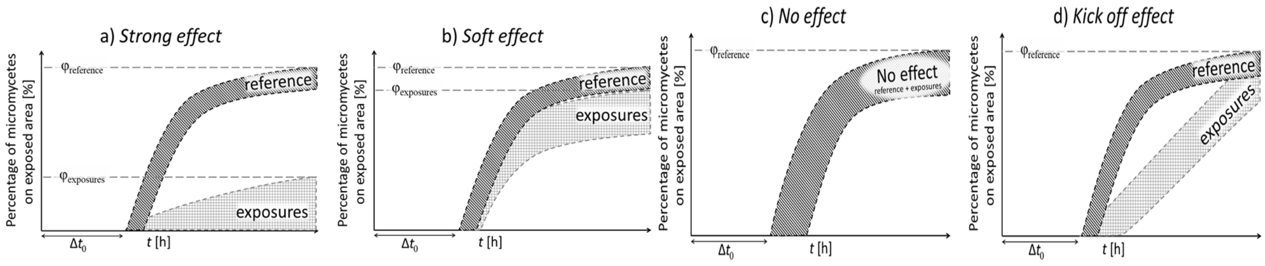


Fig. 1. The characteristic growth types of exposed micromycete strains after NTP exposure [2].

*This work was supported in part by a COOPERATIO Institutional grant from the 1st Faculty of Medicine.*

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# Plasma-assisted processing of PET waste flakes for use in wood-based composites

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Polyethylene terephthalate (PET) represents a significant fraction of the total amount of plastic waste [1], chiefly due to its popularity in the bottling industry. At the same time,bwell-functioning recollection systems for post-consumer PET bottles operating in many EU countries makes possible to acquire and reuse relatively well-defined PET recycle material. Besides direct reuse of waste PET for new bottles or PET fibers production, some of the recently tested possibilities for recycling PET bottles was to use them as an additive or filler in diverse set of composite materials. In [2] authors list a number of published works dedicated to adding PET particles into concrete, e.g., to reduce final concrete density. In [3], the properties of wood particleboards involving PET flakes fractions were investigated. The addition of PET flakes improved boards' water resistance (i.e., reduced thickness swelling and water absorption). However, it considerably reduced their mechanical properties due to the poor bonding of the used adhesive with PET fraction. In the past we have demonstrated a possibility to address this issue by plasma pre-treatment (activation) mediated by coplanar DBD - DCSBD [4]. Our further elaboration of the effective method of plasma activation led to a new design of plasma treatment reactor - a shaftless screw DBD conveyor [5,6]. The tests showed better energy efficiency (10 × ) and higher feedthrough of treated material (20 g/min vs. 5 g/min) than in [4]. Most importantly, mechanical properties of manufactured particleboards were equal to that of aggregated wood without any PET add-on [6]. Experimental optimization shows that the effect of plasma treatment is critically determined by the actual chemical composition of adhesive used in the wood-based composite. Different brands of used urea-formaldehyde adhesive exhibit distinct sensitivity to the effect of plasma activation. In the presented contribution, data addressing this application-wise important observation will be presented. Furthermore, experiments unraveling the chemiluminescence response for different types (colors) of PET flakes will be shown. These data are important for our development of rapid analytical tool for assessing the quality of plasma treatment.

*This research work was supported by the Project for the Development of the Organization "DZRO Military autonomous and robotic systems".*

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# Comparative study of the different cold atmospheric-pressure plasma sources' effects on the surface modification of thermally sensitive BOPP film

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Polymers are distinguished by their low density, flexibility, ease of manufacture, and cost-effectiveness. One of the most used polymers in a wide variety of applications is polypropylene. Biaxially oriented polypropylene (BOPP) has many significant properties, including chemical resistance, excellent optical properties, high strength, lightweight, and exceptional moisture barrier properties. For these reasons, BOPP films are using commonly in the electrical industry to produce capacitors and food packaging. However, there are some limitations to using the BOPP film in composite and laminated materials and printing, dyeing, and coating industrial areas, in which, from an environmentally friendly point of view, water-soluble inks and solutions are used. This fact is a consequence due to the innately hydrophobic and low-surface energy character of polypropylene. For these reasons, it is necessary to increase BOPP film's wettability and adhesion properties without changing the bulk properties [1,2], which can be achieved by its surface treatment.

Among the many surface treatment technologies, the cold atmospheric-pressure plasma represents an efficient and modern method for surface modification mainly due to the advantages of short exposure times, fast speed, simple operation, and easy control [3]. In our comparative study, the thermally sensitive BOPP film was treated by different atmospheric-pressure plasma sources for 1, 5, and 10-second exposure times and in different working gases. The plasma sources included nitrogen MSDBD [4], a linear nitrogen DCSBD-based plasma system, an argon cylindrical plasma jet array [5], and a DCSBD generated in ambient air and nitrogen gas [6]. The water contact angle (WCA), SEM, Profilometer, and XPS analyses were used to determine the wettability, morphology, and surface chemical composition of the BOPP plasma treated film. The adhesion properties were evaluated by the peel force measurement. The most significant change in the WCA reaching the value of 37.9 ° compared to 100.8 ° for the reference sample was obtained immediately after the linear nitrogen DCSBD-based plasma system treatment. Moreover, the adhesion increased by approximately 4 times after plasma treatment, mainly using DCSBD generated in air.

*This work was supported by the project LM2018097 funded by the Ministry of Education, Youth and Sports of the Czech Republic.*

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# The role of $HNO_2$ in the generation of plasma activated water

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Transient spark (TS), a DC-driven self-pulsing discharge generating highly reactive atmospheric pressure air plasma, was employed as a rich source of $NO_x$. In dry air, TS generates high concentrations of NO and $NO_2$, that increased approximately linearly with increasing input energy density ($E_d$) and reached 1200 and 180 ppm, at $E_d$ = 400 J/L, respectively. In humid air, the concentration of $NO_2$ decreased down to 120 ppm in favor of $HNO_2$ that reached approximately 100 ppm at $E_d$ = 400 J/L. The advantage of TS is its capability of simultaneous generation of the plasma and the formation of microdroplets by the electrospray (ES) of water directly inside the discharge zone. The TS discharge can thus efficiently generate plasma activated water with high concentration of $H_2O_2$(aq), $NO_2^-$(aq) and $NO_3^-$(aq), because water microdroplets significantly increase the plasma-liquid interaction interface.

We compare TS with water ES in a one stage system and TS operated in dry or humid air then followed by water ES in a two-stage system (Fig. 1). We show that gaseous $HNO_2$, rather than NO or $NO_2$, plays a major role in the formation of $NO_2^-$(aq) in PAW. With deionized water at the inlet, $NO_2^-$(aq) reached concentration up to 2.7 mM [1]. In buffered solution, without a decrease of pH, $NO_2^-$(aq) reached concentration up to ~8 mM due to its suppressed conversion of $NO_3^-$(aq). This is approximately the upper limit predicted by Henry's low solubility coefficient at ~100 ppm $HNO_2$ concentration in the humid air treated by transient spark discharge.

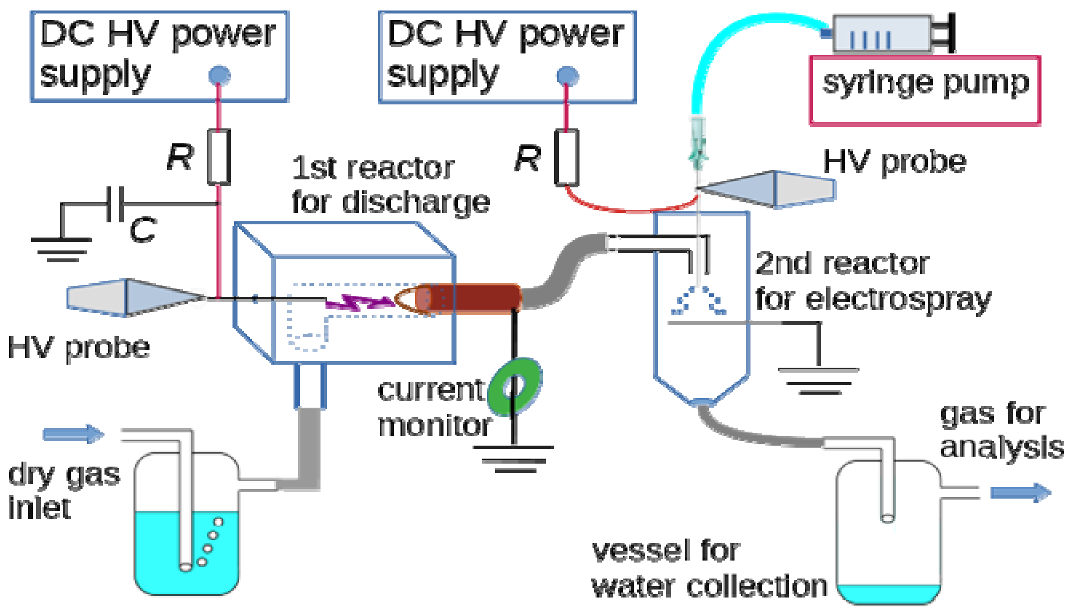


Fig. 1. Schematic of the experimental setup of two-stage system, R – resistor, C – capacitor.

*This work was supported by Slovak Research and Development Agency project under the contracts no. APVV-17-0382 and APVV-20-0566.*

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# *In vivo* effect of cold plasma activated water on wound healing

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Cold plasma has been applied with promising results in the field of biomedicine, specifically in oncology or as a decontamination tool in different environments. It has been also published that cold plasma improves wound healing *in vivo* in mice [1]. The aim of our study was to use cold plasma activated water (PAW) for wound healing after surgery *in vivo* in mice. Glow discharge PAW was produced. Totally 7 male mice were used – 4 in PAW group and 3 in control (CTRL) group. PAW or distilled water were applied 5 times in three days starting 3 hours after the surgery. Photos of wounds were taken daily as well as scoring of wound healing according to early wound healing score system [2]. Results show no difference between PAW and CTRL group in any of the scoring days (Fig. 1). There exist many reasons why application of PAW did not ameliorate the healing. Wounds after the surgery might not be the optimal model for such an experiment. Model of wound infection with bacteria might show different results. More in vivo studies are needed to reveal the direct effect of PAW on wound healing.

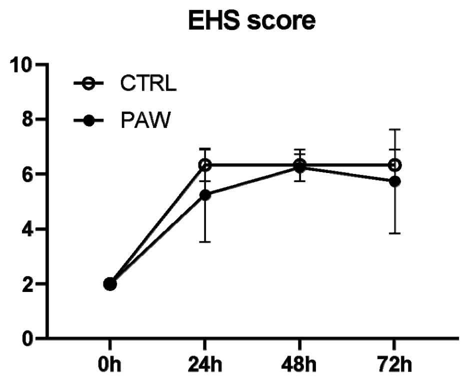


Fig. 1: Early healing score (EHS) system used to evaluate wounds of mice after the surgery.

*This work was supported by the Slovak Research and Development Agency (Grant Number APVV-17-0382) and by the Grant Agency of Ministry of Education, Science, Research and Sport of the Slovak Republic (VEGA 1/0674/21).*

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# In-line plasma pre- and post-treatment as parts of technology for manufacturing of nanofiber-based filters with the improved performance properties

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Fibrous filter devices represent a simple and economical method for efficient removing particles at a submicrometric-size scale from air and water streams. Usually, a small fiber diameter increases filtration efficiency. Therefore, many techniques are emerging for the synthesis and processing of fibrous materials at the submicrometric/nanometric level. Among them, electrospinning is one of the most efficient techniques used for the fabrication of nanostructured membranes for water and air filtration from synthetic polymers. Such media can be applied to produce filters and protective aids with FFP3 filtration efficiency, including protection against viruses with increasing importance precisely in connection with the COVID-19 pandemic.

The key point for creating a functional nanofiber membrane for air and water filters is to meet basic properties such as filtration efficiency, improved water flux, mechanical resistance, and resistance to fouling and chemicals. The advanced nanofiber-based filters' design and manufacturing urgently require new environmental-friendly and cost-effective surface treatment without using organic solvents and caustic solutions. Moreover, self-supporting electrospun nanofiber layers usually have poor mechanical properties. Therefore, the nanofibers are deposited on a polymer textile substrate while being requesting strong adhesion between the nanofiber layer and the substrate. To address this need, as an alternative, the atmospheric-pressure plasma offers to be used for both surface activation (plasma pre-treatment) of the substrate to achieve its higher adhesion to the electrospun nanofiber layer and post-treatment of this layer for the improvement of the performance properties of water nanofiber filters.

In this contribution, we present the applied research results focused on the transfer and adjustment of the diffuse coplanar surface barrier discharge (DCSBD) technology for the specific high-throughput nanofiber filter production needs of NAFIGATE Corporation a.s. within the joint project. The tape peel force measurement results confirmed that a few seconds lasting plasma pre-treatment of polymer textile substrates carried by DCSBD promotes its adhesion force significantly. Plasma post-treatment of the nanofiber layer resulted in higher wettability and water flux confirmed by strike-through time and gravimetric measurements. The SEM analysis of morphology revealed that the treatment did not destroy the surface of nanofibers due to the gentle character of the plasma. At the final stage of the project, the DCSBD technology was successfully tested for in-line pre-treatment of the PP nonwoven substrate prior to electrospinning and post-treatment of PVDF nanofiber layer for better wettability and utilization for the water filters in the production premises of NAFIGATE Park s.r.o.

*This work was supported by project No. E01-14 funded by the ELIIT initiative and project "R&D centre for plasma and nanotechnology surface modifications (CEPLANT)", LM2018097 funded by the Ministry of Education, Youth and Sports.*

# Photocatalysis of laser synthesized colloidal Ag-doped ZnO nanoparticles

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In this work pulsed laser ablation of the Ag coated ZnO target in water is performed in order to synthesize the colloidal solution of Ag-doped ZnO nanoparticles. The ratio of Ag dopant in ZnO nanoparticles strongly depends on the thickness of the Ag layer at the ZnO target. Synthesized nanoparticles were characterized by experimental methods such as XRD, XPS, SEM, EDS, ICP-OES and UV-VIS spectrophotometry in order to determine and analyze their crystal and stoichiometric structure, size distribution, shape, optical properties (photoabsorbance, bandgap) and mass concentration. The photocatalytic performance in the degradation of Methylene Blue (MB) under UV irradiation is examined for different ratios of Ag dopant in ZnO nanoparticles, as well as for pure ZnO nanoparticles [1]. The maximal photocatalytic efficiency is reached at a 0.32% Ag weight ratio, with an almost twice larger MB photodegradation rate than that obtained with pure ZnO nanoparticles at the same concentration.

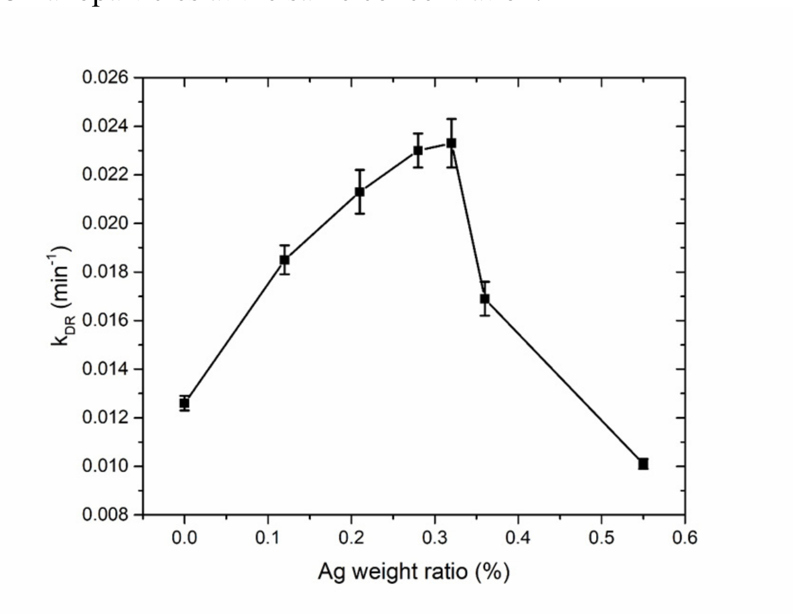


Fig. 1. Photodegradation rate dependence on Ag weight ratio in Ag-doped ZnO NP.

*This work was supported by Croatian Science Foundation HrZZ-PZS-2019-02-5276.*

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# The effects of plasma-activated phosphate buffered saline on monolayers and spheroids of cancer cells

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Conventional cancer therapies have a high level of toxicity for human body and often burden patients with severe side effect. In addition, in many cases the tumour becomes resistant to chemotherapeutic agents. Therefore, it is necessary to search for new strategies to cure cancer. One of the promising strategies is found in plasma medicine. Cold plasma has a potential to prolong patient's life and make it more valuable if the previous conventional cancer therapies fail. The main role in plasma therapy is played by reactive oxygen and nitrogen species (RONS) generated in the plasma and plasma-activated liquids (PAL) [1].

The aim of this study is to investigate the effect of indirect application of cold plasma, via plasma activated phosphate buffered saline (PBS), to drug sensitive human breast adenocarcinoma cell lines MCF-7 (kindly provided by Prof. Ján Kovář, Charles University, Prague) and the Paclitaxel-resistant sub-lines MCF-7/PAX [2,3]. MCF-7 and MCF-7/PAX were incubated in PBS treated by cold atmospheric plasma of streamer corona discharge.

We tested the effect of plasma-activated PBS (PAPBS) treated for 5 and 10 minutes added to 2D cell monolayer and 3D tumour models – spheroids. The concentration of RONS in PAPBS linearly increased with the plasma activation time. [4]. After 30 minutes of cell incubation in PAPBS, we observed the viability reduction of both cell lines. Metabolic WST-1 assay has shown a decrease of the number of viable cells depending on the plasma treatment time. The following microscopic analysis proved, that PAPBS induced processes leading to cell death in both sensitive cancer cell line and the resistant sub-line of human breast tumor cells. PAPBS also seems to inhibit the growth and induce a disintegration of spheroids.

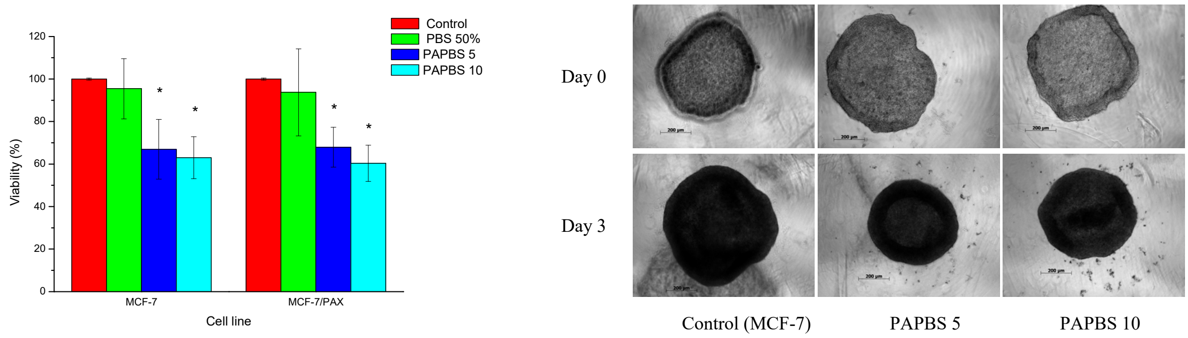


Fig. 1. Left: Effect of PAPBS 5 (treated 5 min) and PAPBS 10 (treated 10 min) on viability of MCF-7 and MCF-7/PAX measured by WST-1 assay. The significant results ($p<0.05$) are marked with *. Right: Microscopic images of the impact on MCF-7 spheroids 3 days after application of PAPBS. Scale bar = 200 μm.

*This work was supported by Slovak Research and Development Agency, grant APVV-17-0382.*

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# Plasma treatment for conditioning of non-pasteurized beverages

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Wine is one of the most popular alcoholic beverages in the world[1]. Due to the presence of alcohol and low pH, as well as compounds with antibacterial properties, wine is considered to be an unfavorable environment for the development of bacterial microflora. However, there are microorganisms that have developed certain mechanisms that allow them to adapt to the unfavorable environment, which allow them to survive the unfavorable conditions in wine at particular stages of production. Such pathogens adversely affect the physicochemical properties of the wine, deteriorating its quality. This group includes mainly *Brettanomyces* yeast, lactic acid bacteria (LAB) and acetic acid bacteria[2]. LAB is the most numerous group of bacteria in grape must and wine.

During the research, *Lentilactobacillus hilgardii* bacteria were inactivated with the use of non-thermal plasma. As a source of plasma we used dielectric barrier discharge plasma reactor operating at atmospheric pressure. A mixture of helium and oxygen was used as the working gas. The samples were processed with the given parameters by changing the plasma exposure time.

For evaluation of bacterial survival, the classical plate culture method was used, as well as flow cytometry, which allows for sorting cells and revealing various physiological states after plasma treatment. The paper presents the differences between the results of bacterial viability obtained with the use of the above-mentioned methods. The results of tests performed with the use of the classical method of counting on plates showed a significant inhibition of bacterial growth after 10 minutes of plasma treatment. However, the cytometric analysis showed about 14% of active bacteria and about 77% of moderately active bacteria. Only about 9% of the bacterial cells were dead.

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# Eradication of bacterial biofilms with atmospheric air pulsed streamer corona discharge

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Bacterial biofilms represent a predominant cause of microbial infections and impact severe economic costs in agriculture, food production, waste treatment, and manufacturing industry. Bacteria naturally exist as a biofilm within a self-generated extracellular matrix of hydrated extracellular polymeric substances. Higher resistance to antimicrobial agents, changes in microbial physiology, and toxic compounds accumulated in the biofilm matrix make the biofilm's control and eradication extremely difficult [1]. Cold plasma decontamination and sterilization have been widely studied for biological applications in recent years. Various plasma–bacteria interaction pathways and plasma effects on planktonic bacteria were proposed [2, 3]. However, much scientific attention is still required to understand the plasma–biofilm interaction as a biofilm is a very complex survival strategy for microorganisms. In this study, the influence of an atmospheric pressure cold plasma generated by streamer corona discharge operated in atmospheric air was evaluated against single– and mixed–species biofilms of Gram-positive *S. aureus*, and Gram-negative *P. aeruginosa* and *E. coli* bacteria. Bacteria viability, metabolic activity, reduction of total biofilm biomass were analyzed using colony count, crystal violet staining, and MTT assays, respectively. In addition, visualization of biofilms after plasma treatment was performed by fluorescence microscopy.

After 120 s of plasma treatment, complete inactivation of both Gram-negative (*P. aeruginosa* and *E. coli*) bacterial biofilms was achieved. Whilst Gram-positive *S. aureus* biofilms resulted in a maximum of 3.5 log reduction after 180 s. Both single– and mixed–species biofilms have undergone significant losses of biofilm biomass, suppression of metabolic activity, and removal from polystyrene and glass substrates. Additionally, the inactivation effect on dried and slightly moisture biofilms was compared. Air streamer corona discharge has shown the ability to efficiently eradicate bacterial biofilms. Only a few studies exist on the effects of cold plasma on mixed-species biofilms [4, 5]. It is crucial to investigate how cooperative and competitive interactions between different bacterial species of mixed biofilms affect the overall antibiofilm effects of cold plasma.

*This work was supported by Comenius University grant UK/206/2022 and Slovak Research and Development Agency grant APVV-17-0382* and *APVV-20-0566.*

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# Plasma polymerized oxazoline based thin films for biomedical applications

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Polyoxazolines (POx) are a promising class of polymers that have attracted substantial attention recently due to their antibiofouling properties and good biocompatibility [1, 2]. POx can be synthesized via cationic ring-opening polymerization (CROP) or by surface-initiated CROP (SI-CROP). CROP and SI-CROP are wet chemical processes using different catalysts, which have several disadvantages compared to dry processes. Recently, plasma polymerization of 2-oxazolines was used to produce robustly attached coatings on different substrates. Plasma polymerization is a one-step and substrate-independent dry process, which does not require the use of solvents or initiators and therefore it does not create liquid organic waste. POx coatings were deposited using different discharge configurations at atmospheric pressure. A suitable discharge type for plasma deposition at atmospheric pressure is atmospheric pressure Townsend-like discharge (APTD), which is a homogeneous dielectric barrier discharge (DBD) [3]. In our study POx thin films were deposited on glass substrates using atmospheric pressure plasma polymerization from 2-ethyl-2-oxazoline vapors. The plasma polymerization was performed in DBD burning in nitrogen at atmospheric pressure. The experimental set-up and experimental conditions were described in detail in [2].

The POx films were deposited at substrate temperatures 20°C, 60 °C, 90 °C, 120 °C and 150 °C and at nitrogen flow of 100 sccm through the monomer. Deposited films were imaged with scanning electron microscope MIRA3 (TESCAN, Czech Republic) equipped with secondary electron and back-scattered electron detectors as well as characteristic X-ray detector (EDX) analyser (Oxford Instruments, UK). The IR spectra of deposited films were measured by FTIR spectrometer Alpha (Bruker, USA) using a single reflection ATR module Platinum. Antibacterial tests were performed according to the ISO 22196 procedure with modifications. For the determination of antibacterial performance, gram positive Staphylococcus aureus and gram negative *Escherichia coli* were used. The mouse embryonic fibroblast continuous cell line (NIH/3T3, ATCC CRL-1658™, USA) was used for cytocompatibility test, according to the EN ISO 10993-5 standard, with modification.

All deposited films exhibited excellent antibacterial properties against all bacterial strains used for antibacterial tests. Also the deposited films were cell-repellent and they could be used as coatings with antibacterial and antibiofouling properties for biomedical applications.

*This research work was supported by the Project for the Development of the Organization „DZRO Military autonomous and robotic systems“.*

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# Non-thermal plasma and ozone disinfection of FFP2-type respirators

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The unexpected COVID-19 outbreak and subsequent rapidly increasing demand for personal protection equipment, mostly disposable face masks and respirators representing the first personal protection against infectious viral particles, resulted in critical shortages of respirators worldwide, but also in escalating environmental burden as a significant source of microplastics pollution [1]. A possible safe reuse of disposable respirators would be both environmentally and economically friendly. Decontamination of respirators for repetitive reuse can be achieved by several sterilization techniques such as dry heat, vaporous hydrogen peroxide, or germicidal UV radiation [2]. The use of non-thermal plasma (NTP) generated by numerous types of electrical discharges represents another efficient method, known for their highly efficient antimicrobial properties and decontamination effects [3].

In this study, Gram-negative bacteria *Escherichia coli* was used as a non-pathogenic model microorganism for investigations of antimicrobial properties of NTP generated by multi-electrode pulsed corona discharge under various conditions. A surface of FFP2-type respirators was inoculated with *E. coli* overnight culture using an airbrush and then dried for one hour in a laminar box. The antimicrobial effects of NTP were examined on both dry and wet respirators humidified with deionized water prior to NTP treatment. Antibacterial properties of air plasma discharges are usually assigned to ozone, therefore the effect of ozone generated by ozone generator on the decontamination of respirators was investigated separately and compared to NTP.

The outer surfaces of the respirators were successfully disinfected up to 4 log reduction without causing any microscopically visible damage to the material. Generally higher antimicrobial efficiencies were achieved for wet respirators than dry ones. The decontamination rate increased with the plasma treatment time and the plasma discharge power. Separate effect of ozone was shown to be weaker than the direct effect of plasma discharges. Our results showed a promising potential of using air plasma discharges for surface decontamination of disposable respirators.

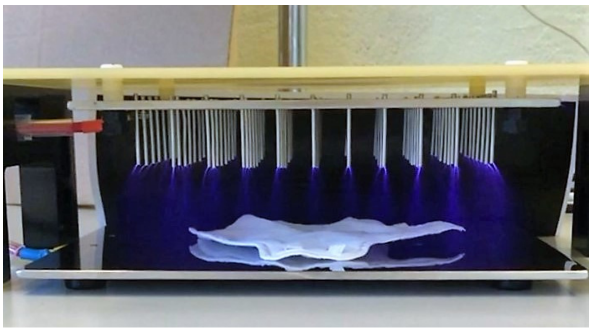

Fig. 1. FFP2-type respirator under treatment by multi-electrode pulsed corona discharge.

*This work was supported by Slovak Research and Development Agency grants APVV-17-0382 and APVV-20-0566 and Slovak Grant Agency VEGA 1/0822/21. Pulsed corona plasma source was provided by Kamea Electronics, s.r.o.*

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# Interpreting improved performance of a rotating gliding arc plasma for $N_2$-fixation: Insights from a self-consistent, fully-coupled model

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The issue of global reliance on chemical fertilisers produced *via* the Haber-Bosch (H-B) process is linked to the current climate change crisis facing us today. The process consumes 1–2% of the world's annual energy supply (and 3–5% of global natural gas consumption) while simultaneously producing 400 Mt of $CO_2$ per year, accounting for 1.2% of the annual anthropogenic $CO_2$ emission. One proposed solution, in tandem with utilising an intermittent supply of available renewable energy, is plasma-based $N_2$ fixation from air. This pathway produces NO and $NO_2$ ($NO_x$), which can be absorbed into manure and used as a nitrogen-enriched organic (NEO) fertiliser as proposed by N2 Applied [1]. The utilisation of warm plasma, such as that produced in a 3D rotating gliding arc (RGA), has recently been shown to produce record-high values of $NO_x$ concentrations, 5.5 % (EC - 2.5 MJ $mol^{-1}$) [2] when operating in the steady mode. This value was further improved in more recent work upon addition of an effusion nozzle to the outlet of the reactor, resulting in an optimum NOx concentration of 5.9 % [3]. While these results are not directly competitive with the H-B process (EC - 0.5 MJ $mol^{-1}$ $NH_3$), this result in combination with the ability of plasma reactors to use renewable energy resources clearly shows the potential of this pathway for distributed, small-scale production. To further improve our plasma reactors, we should strive to understand the behaviour of the implemented discharge. In the case of the RGA, arc dynamics and behaviour are important pieces of the complex puzzle that need to be elucidated. With this goal in mind, we have successfully developed a self-consistent, fully-coupled thermal air model of the RGA using COMSOL Multiphysics v6.0 software. Our model solves the Navier-Stokes, heat balance and current conservation equations simultaneously, under the assumption of local thermodynamic equilibrium (LTE). In addition to this, we added thermal chemistry to gain insights into the underlying mechanisms that contribute to the record-high conversions observed within the RGA reactor.

*This research was supported by the European Union's Horizon 2020 research and innovation programme "Sustainable Manure" under grant agreement No. 965546.*

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# The impact of humidity and circuit parameters in the generation of $NO_2$ and $HNO_2$ by transient spark discharge

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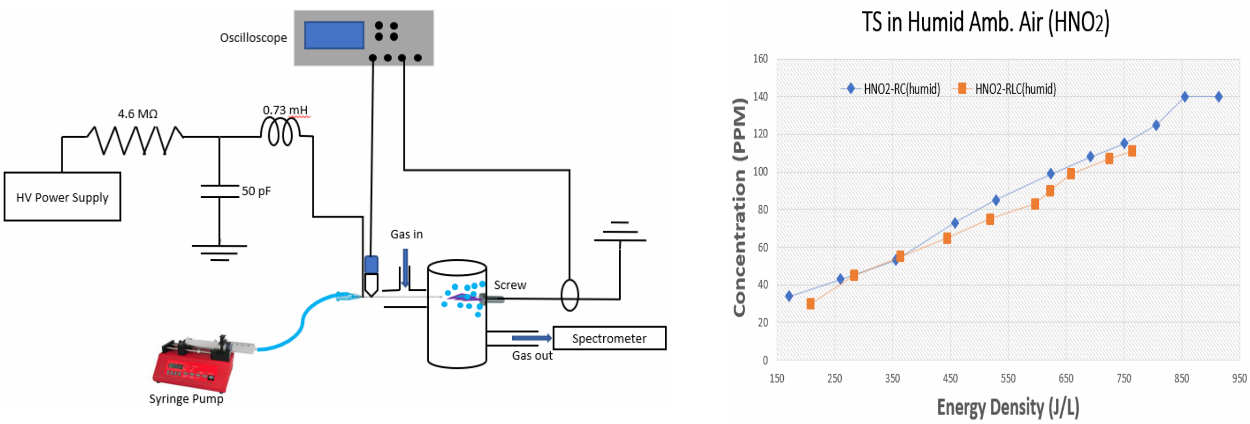
In recent years, atmospheric pressure plasma-liquid systems generating plasma activated water has become an important area of research due to many possible applications in health and agriculture sectors [1]. In this work, TS spark was generated in a closed reactor with ambient air, or humidified ambient air, with or without the water electrospray (ES). Two different driving circuits were used (Fig. 1a), RC and RLC, the latter with an additional induction coil (0.73 mH). A needle of 0.6 mm diameter was used as high voltage electrode, which was kept at a gap of 0.8 cm with the ground electrode. The needle electrode served also for deionized water inlet. The output gas was analyzed UV-Vis. absorption spectroscopic technique. The humidity was measured with humidity sensor. The humidity of ambient air was around 63%, relative humidity of humidified air was 93%, and humidity due to ES (without discharge) was observed to be 83%. When transient spark is operated with electrospray, humidity in the reactor increased rapidly to 95% due to vaporization of the micro droplets. The concentration of gaseous NO2 and HNO2 increased with the increasing input energy density (Fig. 1b). The increase of humidity has negative effect on concentration of NO2 generated by TS, with highest concentration observed in ambient air. On contrary, the increasing humidity positively influences the concentration of HNO2 generated by TS, with highest concentration observed in humidified air without ES. When TS and ES (flow rate 300 µl/min.) was operated together, the concentration of HNO2 depleted significantly in the gas phase due to rapid dissolution of HNO2 into the water microdroplets. RC circuit had overall better performance in production of higher concentration of NO2 and HNO2 in comparison to RLC circuit. In RLC circuit, higher concentration of NO is produced. These results justify the influence of humidity and circuit parameters on the production of NO2 and HNO2 by TS. These results can be used for the optimization of plasma-water systems for the generation of plasma activated water.




**(a)** **(b)**

Fig. 1. (a) Schematic of experimental setup (b) Concentration of $HNO_2$ as function of input energy density.

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# The effect of plasma on germination, oxidative stress response and DNA damage in barley

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Agriculture and food industry have to face many problems in order to ensure food for livestock and people. These include population growth, decrease of agricultural land, climate changes or plant pathogen resistance. Chemical agents used to solve problems with pathogens or germination are often not effective enough and, at the same time, are toxic for organisms and environment. A possible ecological solution could be application of cold atmospheric pressure plasma (CAPP). Plasma treatment has many positive impacts on seeds and crops, such as seed germination enhancement and seed surface decontamination [1]. The effects of CAPP vary according to plasma source, working gas, and treatment time. Therefore, the treatment conditions must be optimized to increase and accelerate germination, yields, and minimize the negative impact of plasma on seeds.

Our study is focused on CAPP effects on barley grains using diffuse coplanar surface barrier discharge, which generated plasma from ambient air, nitrogen or oxygen [2]. This experimental design allows us to compare effect of individual gases on seed germination parameters or chlorophyll levels. In case of cereals as barley, germination is almost 100%, so it is hardly possible to increase the germination. However, plasma generated from all three gases increases germination speed after short exposure, which corresponds to higher protease and glucanase activities. The exposure time also slightly increases superoxide dismutase activity, which suggests that short plasma treatment can generate reactive oxygen species resulting in oxidative stress response. Reactive species, which are not degraded fast enough, can cause severe oxidative damage of various biomacromolecules. In our study oxidative DNA damage was measured, specifically DNA single- and double-strand breaks and, at the same time, oxidative lesions. Plasma treatment suitable for germination enhancement do not cause DNA double-strand breaks, only DNA single-strand breaks and, especially, oxidative purines formation. On the other hand, short plasma exposure time does not affect the level of chlorophylls in the leaves of barley.

According to our results, plasma generated from nitrogen can cause the lowest DNA damage, and, on the other hand, has the most harmful effects on seed germination parameters.

*This work was supported by the Slovak Research and Development Agency, grant APVV-16-0216, and by projects VEGA 1/0460/21 and UK/209/2022.*

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# Genotoxic effects of non-thermal plasma generated by various sources on plasmid DNA

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Non-thermal plasma (NTP) has become a wide-spread green technology used in various fields. First biological experiments uncovered antimicrobial effects of NTP and plasma was considered as an alternative way for laboratory glass sterilization. However, plasma can be used also for decontamination of food surfaces and many studies proved positive effects of NTP on plant growth in low exposure times. Moreover, plasma has a potential in many medical applications, such as wound healing and tumor treatment. However, human cells can be negatively affected by higher doses of NTP as plasma can induce oxidative stress through the production of reactive oxygen and nitrogen species (RONS) and UV radiation. Subsequently, these agents can damage proteins, lipids and DNA in the cells. DNA damage caused by RONS can result in base modifications, cross-links and single- and double-strand breaks. In some applications, e.g. to eliminate tumor cells, DNA damage is beneficial, but in wound healing it is necessary to avoid this damage in healthy cells. Therefore, it is important to carefully examine the effects of NTP on isolated DNA and analyze the mechanisms of its action.

In our work, we decided to compare the effects of NTP generated by two plasma sources on the DNA topology of plasmid pBR322. Preliminary results suggest that increasing treatment time by NTP generated in ambient air results in the increased formation of single- and double-strand breaks. This effect is stronger in case of multi-hollow surface dielectric barrier discharge (MSDBD) compared to Robust plasma system 40 (RPS40) (plasma sources are described in detail in [1-2]). However, DNA-damaging effects of NTP were weakened by sodium pyruvate and carboxy-PTIO, scavengers of NO radical and hydrogen peroxide, respectively. These results suggest that specific particles generated by NTP could affect DNA in different ways. Understanding the molecular mechanisms of the NTP effects on plasmid DNA could lead to its safe use even in the treatment of different cells in various potential applications.

*This work was supported by VEGA 1/0460/21 and VEGA 1/0688/22 grants.*

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# Characterization of 1- and 3-pin atmospheric pressure plasma jet used for decontamination of water samples

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With growing population more and more issues arise from human influence on the environment and, therefore, on the food chain and the ability to produce enough food. The amounts of organic micropollutants (OMPs) that are released into the water bodies are huge and, in order to deal with this issue, we need more alternative green technologies that can replace (or aid) standard oxidative processes (AOPs). One of such technologies that can be used for non-biodegradable OMPs is non-thermal plasma (NTP) that operates at atmospheric pressure [1]. The NTPs created in gas phase in contact with liquid or in liquid phase are rich source of oxidative species responsible for OMP destruction [2, 3].

Here we will present pin-type atmospheric pressure plasma jet (APPJ) that uses argon as a working gas. We have investigated two different configurations: 1-pin APPJ and 3-pin APPJ. We have performed electrical characterization, temperature measurements, optical emission spectroscopy and ICCD imaging for both experimental setups. The flow of argon was kept constant in all experiments at 1 slm (1-pin APPJ) and 2 slm (3-pin APPJ). As a power supply we have used commercial sine wave resonant type power supply. In our case the resonant frequency for 1-pin APPJ was 330 kHz and for 3-pin it was 350 kHz. The discharge was created in a gas phase and was in contact with water samples in all experiments. The water samples were contaminated either with Acid Blue 25 (AB25) dye, diclofenac (DCF) or 4-chlorobenzoic acid (pCBA). In case of 3-pin APPJ we have performed treatments without mixing (water sample was in crystallizing dish) and with mixing (water sample was circulated by a pump during the plasma treatment). As expected, the removal efficacy increased with decrease in the initial concentration of the contaminants in all cases. When comparing the destruction of the DCF and pCBA in flow mode we saw that plasma removed DCF better. This can be related to the chemical structure of both compounds, whereby pCBA can only be converted by strong oxidants such as the hydroxyl radical, whereas DCF is susceptible to reaction with weaker oxidants, too.

*This work was carried out under NOWELTIES project funded by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 812880. N.S. and N.P. are funded by Ministry of Education, Science and Technological Development, grant number 451-03-68/2022-14/200024.*

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# Incorporation of ZnO nanoparticles into PVC and HDPE polymers using atmospheric pressure plasma jet

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Polymer surface modification has gained a lot of interest over the last few decades. The surface modification offers new physical and chemical properties to polymers by changing their surface properties or incorporating metal or metal oxide nanoparticles [1,2]. Nanoparticles have a significant role due to their unique magnetic, electrical, mechanical, optical, and electronic properties with respect to the bulk materials. Zinc oxide (ZnO) is one of the most widely used materials with unique physicochemical properties and direct band-gap energy of 3.37 eV, making him a good UV absorber [3]. Polymer/nanoparticle composites gain attention due to their excellent properties for applications in the food industry, biomedicine, biotechnology, optoelectronics, etc.

In this work, PVC (polyvinyl chloride) and HDPE (high-density polyethylene) polymers were treated with an atmospheric pressure plasma jet using helium as a working gas. The ZnO colloidal solution was synthesized using pulsed laser ablation in water. After plasma treatment, ZnO colloidal solution was dropped on the polymer surface, and, finally, the polymer was treated again with helium plasma. Optical emission spectroscopy (OES) was used to determine the elemental composition of plasma. All polymer samples (treated and untreated) were characterized using Fourier-transform infrared spectroscopy (FTIR), atomic force microscopy (AFM), and scanning electron microscopy (SEM). The ZnO optical properties, crystalline structure, elemental composition, morphology, and nanoparticle size distribution were studied using UV-VIS spectrophotometer, X-ray diffraction (XRD), X-ray spectroscopy (XPS), and scanning electron microscope (SEM), respectively.

*This research was supported by the Croatian Science Foundation under the project PZS-2019-02-5276.*

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# Polyphenol degradation by high voltage gas discharge plasma

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Degradation of Polyphenols in agro-industrial wastewaters is lately a very important topic because of its complex biodegradation and high phytotoxic and antimicrobial properties that make them hazardous to the environment [1, 2]. The main aim of this study was to investigate the effect of high voltage plasma treatment on polyphenols commonly present in olive mill wastewater. A model solution made of hydroxytyrosol, tyrosol, oleuropein and vanillic acid was prepared at pH values of 7 and 10. Plasma treatment parameters were set at a fixed frequency of 60 Hz, high voltage of 40 kV and 50 kV in combination with air bubbled into the liquid and treatment times 10, 20 and 30 minutes. Physico-chemical parameters (pH value, electrical conductivity, $NO_2^-$, $NO_3^-$ and $H_2O_2$) and concentration of polyphenols were determined in all samples. Degradation of polyphenols occurred in all samples, but the most efficient treatment (>90%) occurred at 50kV treatment after 20 minutes at pH 10.

The obtained results show potential for further research on the chemistry of degradation intermediates as well as a step forward to the application of high voltage gas plasma discharge on olive mill wastewater decomposition.

*This work was supported by the Republic of Croatia, Ministry of Science and Education through the European Regional Development Fund through the project (KK.01.1.1.02.0001) " Equipping the semi-industrial practice for the development of new food technologies "*

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# Plasma treatment for conditioning of juice

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The aim of the research was to investigate the effect of Cold Atmospheric Plasmas (CAP) treatment on various parameters of freshly pressed NFC (Not From Concentrate) apple juice, including on microbiological quality, physicochemical and structural properties. In addition, the conducted research will be used to assess the usefulness of CAP in extending the shelf life of fresh apple juice with minimized loss of valuable nutrients.

Currently conducted research, described by many authors, confirms the rightness of using CAP in order to eliminate unwanted bacteria and fungi from food products. However, there is little evidence of the effectiveness of the disinfection of fruit and vegetable juices. The most frequently performed experiments are based on the analysis of the effect of CAP on specific microorganisms in pasteurized juices. An interesting area, due to the prevailing trends related to healthy eating, is the possibility of extending the shelf life of unpasteurized juices.

Using a modified GildArc (GAD) reactor connected to a flow system, the potential use of low-temperature plasma technology to condition unpasteurized apple juice was investigated. The conducted experiments were aimed at determining the optimal conditions of food processing with the use of CAP.

By changing the individual parameters of the process (i.e. the type of gases used, their flow, exposure time) and the elements of the device (discharge electrodes, glass electrode covers which limiting the spread of the gases used and at the same time acting as limiters determining the distance between the end of the electrode and the tested sample), the influence of these factors was investigated to extend the shelf life of a product with a short shelf life, while maintaining its full nutritional and vitamin potential. After the tests, each sample was sent for biological tests, which determined the impact of the actions taken. The subsequent stages of the work, i.e. microscopic observation of the object, testing the presence of cell cultures or recording the occurring chemical reactions, provided information on the effectiveness of the application of a given method. Additionally, in the future, the work may be extended to the measurement of electrical parameters of the tested liquids.

# Photocatalytic water treatment by nanocomposites processed using low temperature plasma

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The photocatalysis under visible light and UV irradiation is intensively studied as a promising technology for air or water purification [1, 2]. Unfortunately, the bottleneck of photocatalytic materials decreasing their photocatalytic efficiency lies mainly in the fast recombination of photo-generated electrons and holes. However, the development of new nanomaterials can be effective way how to suppress the recombination of charges. In this work, we prepared novel photocatalytic nanocomposite coatings based on graphitic carbon nitride 2D sheets, iron oxide nanoparticles and state-of-the-art organo-silica binder (see Fig. 1) in common laboratory conditions (room temperature, atmospheric pressure) using spin-coating deposition method. The chemical and morphological parameters of the coatings have been studied by alteration of various parameters as ratio of nanomaterials and binder, duration of milling the nanomaterials, and time of plasma posttreatment performed by Diffuse Coplanar Surface Barrier Discharge (DCSBD) in ambient air atmosphere. The surface structure of the coatings was studied using Scanning Electron Microscopy (SEM) and the chemical composition by X-Ray Diffraction (XRD). The photocatalytic experiments were conducted in water reactors irradiated by visible light and UV irradiation. Formic acid was used as a test compound for polluting deionized water. The concentration of anions leaking from the formic acid indicating the efficiency of photocatalytic decomposition process controlled by coatings was monitored for 180 minutes using Ion Chromatography (IC). The leakage of metal ions and carbon compounds from coatings into water reactor during photocatalytic tests was checked by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Total Organic Carbon (TOC) measurements, respectively.

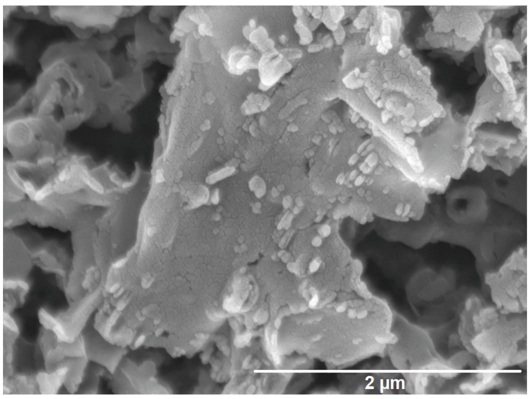


Fig. 1. 2D sheet of graphitic carbon nitride covered by iron oxide nanoparticles.

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# ABSTRACTS

## PlAgri Workshop

## Invited Lectures



## Oral Presentations

# Poster Presentations

# The effects of direct plasma treatment and indirect (PAW) treatment on physicochemical and functional properties of food

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Today trends in food technology call for techniques that only cause minimal changes and provide the „fresh-like quality" of foods [1-2]. High voltage electrical discharge plasmas are the energy efficient technology that can achieve rapid reduction of food spoilage organisms. This technology inactivates microorganisms by combinations of chemical, physical and electrical effects.

Plasma-activated water (PAW) is generated by treating water with various atmospheric plasma sources by using controllable parameters (voltage, pulses, frequencies, gases). Over the last decade due to its non-toxic label, PAW has become widely used in various applications from plant fertilizers to a disinfecting agent for microbial inactivation on food. Based on the literature, PAW treatment has been reported to be effective in inhibiting the growth of bacteria, molds, and yeasts in fresh-cut apples in a study by Liu et al. [3]. In addition, minimal or no significant change was observed in physicochemical properties (color using L, a, and b values), and antioxidant content of treated fruits and vegetables [4].

Main goal of our research was to analyze plasma effects on the properties of berry fruits after PAW treatment and direct plasma treatment. PAW was produced using argon and air as inlet gases at 30-50kV and 120Hz in gas corona discharge reactor using point to plane electrode configuration. Direct plasma treatment parameters were chosen according to the best results achieved after PAW treatment of berries. Through the manipulation of plasma chemistry of PAW, we were able to achieve microbial reduction for selected microorganisms up to 3 $\log_{10}$ CFU $g^{-1}$. Preliminary experiments of physicochemical properties of berries were not reveal adverse effects of the treatment.

*This work was supported by the Republic of Croatia Ministry of Science and Education through the European Regional Development Fund through the project (KK.01.1.1.02.0001)" Equipping the semi-industrial practice for the development of new food technologies"*

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# Applications of atmospheric plasma in the food and medical industry

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The P2ML (Projects, Plasma and Machine Learning, p2ml.org) group of the Mechanical Engineering Department of the University of La Rioja (Spain) has been executing projects related to Cold Atmospheric Plasma for more than 10 years. We started working in the automotive sector and, during the last 6 years, we have been executing projects related to the food and medical industry. We have 2 Atmospheric-Pressure Plasma Jet units with Dielectric Barrier Discharge (MPG, Luxembourg). One of them is assembled to a collaborative robot. For the food industry, we mainly apply disinfectant and ANTI-biofilm treatments using plasma in various ways: [a] Direct Plasma, [b] Plasma-Activated Water (PAW), [c] Plasma-polymerized coatings and [d] Plasma-modified atmosphere. In the medical industry, we have applied similar treatments as well as [e] PRO-biofilm coatings. Noteworthy projects in the field of food include those that were executed in the wine industry: Use of PAW for the disinfection of oak barrels and elimination of Trichloroanisoles (TCA) from wine bottle corks. In the food industry, we have used the Direct Plasma treatments for surface disinfection on cheese and food-contact items (cutting boards, knives, and chainmail gloves). We have also used frozen PAW to increase the shelf life of fish. In the field of medicine, we have developed ANTI- and PRO-biofilm coatings for the functionalization of clinical analysis plates, anti-friction coating for medical needles and a proceeding for the disinfection of face masks. We are currently working on scaling these treatments by studying the durability of the coatings (ANTI-biofilm) and the possible superficial deterioration that direct plasma treatments might cause to plastic surfaces or fibers, designing a semi-industrial prototype for PAW generation, etc.

# Non-thermal plasma and advanced oxidation processes for micropollutants removal in water

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Micropollutants (MPs) are chemical substances that occur in the aquatic environment at trace concentrations (~µg/L) [1]. Examples of MPs include pharmaceuticals and personal care products, and steroid hormones. The main sources of MPs in the environment are wastewater treatment plants. This implies that the use of treated wastewater in irrigation poses serious concerns related to increased risk of human exposure to toxic MPs via the food chain. It has recently been demonstrated that agricultural plants can uptake and accumulate MPs at levels that can reach several hundreds of µg/kg [2], exceeding the toxic level for daily intake of an adult (1 µg/day for estrone) recommended by the Food and Drug Administration [3]. Potential mechanisms by which plants uptake MPs involve adsorption and aquaporins and anion channels transport; found to govern the accumulation of 17β-estradiol, one of the most important endocrine disrupting chemicals, in wheat roots. The implementation of the new Water Reuse Regulation in the EU and the ongoing crisis related to food shortage throughout the world imply that the use of treated wastewater in irrigation will significantly increase in the not-too-distant future. Therefore, new efficient wastewater treatment technologies to eradicate MPs in wastewater are required.

In this study, the degradation of 17β-estradiol (E2) was evaluated using a nano-second pulsed non-thermal plasma system (details are shown in Fig. 1). The change of concentrations of reactive species ($O_3$, $^{\bullet}OH$, $H_2O_2$) in addition to conductivity, pH, and ORP were determined. Both conductivity and $H_2O_2$ concentration continuously increased with time while the concentrations of $O_3$ and $^{\bullet}OH$ increased to maximal values at about 10 minutes followed by decreased values. The degradation of E2 (1mg/L in DI water and spiked in real wastewater) was evaluated and it was found that 90% of E2 were degraded within 30 min reaction time in wastewater (Fig. 2) and the reaction followed a pseudo-first-order reaction kinetics with a rate constant $k_1$= 0.087 $min^{-1}$ in wastewater compared to = 0.112 $min^{-1}$ in DI water. Reaction by-products were also identified by LC/MS/MS analysis and the changes of their abundances were determined (Fig. 2).

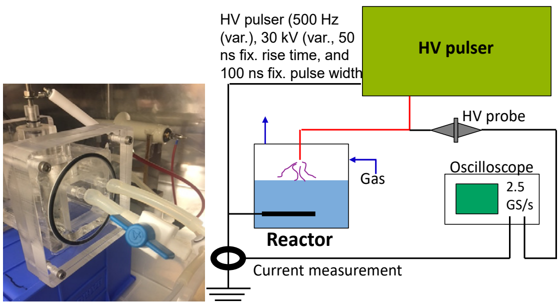


Fig. 1. Experimental set up.

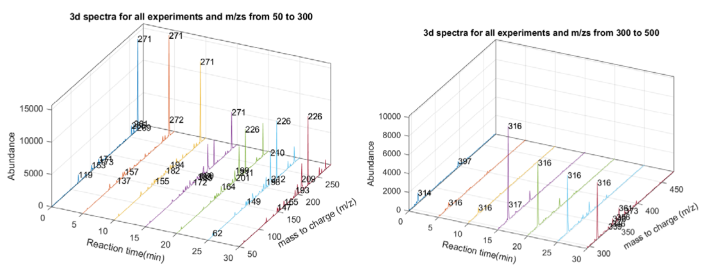


Fig. 2. Degradation of E2 (*m/z* 271) and by-products formation.

*This work was supported by the Engineering and Physical Sciences Research Council (EPSRC), UK (Grant No. EP/M017141/1).*

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# Do we need the Haber Bosch?

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The Haber Bosch process is making ammonia. The process depends on energy to make hydrogen for the catalytic synthesis of ammonia, $NH_3$. The process is energy efficient when methane is used both as energy and hydrogen source. The process is far less efficient when coal or electricity is used as energy source. Science and the fertilizer industry has concluded that 40-50% of the world population is kept alive due to the Haber Bosch. This is also why the Haber Bosch still is seen as a strategic technology for global and local food security. The paradigm shift away from fossil fuels to renewable energy and recycling of nutrients, is therefore going to be a critical shift. The blue and green Haber Bosch fertilizer technologies with low to zero $CO_2$-footprint are available. The timing and mechanism for phasing fossil out and renewable in will be critical, and we have already got a taste of the unpredictable forces being released. We have missed the opportunity of a proactive and controlled expansion of renewable energy-based alternatives, and are forced to handle a perfect storm of rising gas and fertilizer cost, combined with export restrictions and lower yields from crucial grain markets.

# Seed treatment with non-thermal plasma from the point of view of seed germination and early seedling growth

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Seeds are primary result of sexual reproduction in higher vascular plants. Thanks to the seeds, these species can reach very diverse habitats (spreading in space) and overcome various adverse environmental conditions (spreading over time). Seed germination and early growth depends on both genetic and environmental factors. Environmental factors include above all surrounding temperature, moisture availability, solar energy, gaseous concentrations, competition from surrounding plants, and predation from seed eaters. Seed germination is enhanced by both rate of water absorption and the activity of water to start biophysical processes and biochemical reactions. The seeds receive water, which triggers reactivation of metabolism, resumption of cellular respiration, translation/degradation of mRNA, and repair of DNA and proteins. Seeds are important for humanity as a food in agriculture (a source of proteins and fats), in addition, they are also a genetic resource in forestry and horticulture. Systematic experiments using non-thermal plasma to stimulate seed growth date back to the turn of the millennium. Since then, much scientific work has been done, including descriptions of seed germination and initial growth in different plant species treated with different non-thermal plasma sources. Much progress then followed in research in the disinfection of seed coats and into the internal physiological and genetic processes of treated seeds. From a practical point of view, we now find it necessary to focus on the effect of non-thermal plasma on the real yield of key agricultural crops (species sensitivity, treatment combinations, economic benefits, food safety). Some parameters related to seed germination and seedling vigour improvement via nonthermal plasma will be mentioned in the presentation.

# Impact of plasma/electric field treated seeds on germination, morphology, gene expression, and biochemical responses

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The use of cold atmospheric plasma (CAP) treatment of seeds benefits over conventional treatments due to short treatment time and low-temperature operations [1,2]. During CAP interacts with the oxygen, nitrogen, water, etc., in air, they produce various radical and non-radical species [3]. It is a general fact that reactive species like reactive oxygen and nitrogen species (RONS) can influence plant growth and development. The increased nitrogen nutrients level influences growth hormones, activation of growth-related gene expression, and other physiological processes to understand the plasma effect on seedling growth. Additionally, the presence of RONS can disrupt redox homeostasis and cause mild oxidative stress in plants during vegetative and reproductive stages.

Electron paramagnetic resonance (EPR) spectroscopy is optimal for interpreting any change in paramagnetic defect centers. The use of free radical species as precursors to identify the changes in the biological systems due to physical or chemical stress. The detection of the change in paramagnetic species before and after seed treatment is of great interest. Therefore, we used EPR spectroscopy in this study to detect the changes that occurred in seeds before and after plasma treatment. We observed that scalar DBD treatment on radish sprout seed coat increases organic free radical intensity. The weak peak at g = 4.3 represents the signal for $Fe^{3+}$, hyperfine lines belonging to the $Mn^{2+}$ peaks, and an intense sharp rise at g = 2.0 attributed to the semiquinone radical.

Further, we treated seeds that matured under heat stress with CAP and found that subsequent germination was significantly restored; genes involved in ABA biosynthesis (OsNCED2 and OsNCED5) were downregulated, whereas genes involved in ABA catabolism (OsABA8′OH1 and OsABA8′OH3) and α-amylase genes (OsAmy1A, OsAmy1C, OsAmy3B, and OsAmy3E) were upregulated [4]. CAP treatment caused sig0.5nificant hypermethylation of the OsNCED5 promoter and hypomethylation of OsAmy1C and OsAmy3E promoters, which matched their expression patterns. We suggest that CAP treatment can significantly improve the germination of rice seeds affected by heat stress by affecting epigenetic regulation.

*This work is supported by Japan Society of the Promotion of Science (JSPS)-KAKENHI grant number 22H01212. Additionally, partly supported by JSPS Core-to-Core Program JPJSCCA2019002, JSPS KAKENHI Grant Number JP16H03895, JP19H05462, JP19K14700, JP20H01893, JP20K14454, Plasma Bio Consortium, and Center for Low-temperature Plasma Sciences, Nagoya University.*

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# Cold plasma effects on plants: challenges and future in its use

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In the last decade, cold plasma production has been explored for its application in environmental science, in particular in agricultural field. Plasma, an ionized gas produced at room temperature under atmospheric pressure, generates reactive species, thus stimulating physiology, biochemical and molecular processes in plants and inactivating microorganisms. For these reasons, it is a potential tool to increase plant vigor and production, and several studies have investigated plasma-induced improvements to seed germination, plant growth, development, and reproduction. Here, cold plasma effects on plants are summarized. In particular, a focus will be carried out on challenges and future in its use mainly in agriculture field.

# Molecular response to PAW in model plant species

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Non-thermal temperature plasmas (NTPs) have rich chemistry of Reactive Oxygen and Nitrogen Species (RONS) that are formed in gas phase and, in case of water treatment, in gas/liquid interface in liquid [1]. NTPs can be applied in direct treatments of plant samples or indirectly when treated water, called Plasma Activated Water (PAW), is used in treatments. In both cases RONS (short or long-living) are responsible for triggering various mechanisms and effects in plant cells.
RONS have a dual role and a dose-dependent effect, they can regulate the normal physiological activities of plants as signaling molecules at the range of physiological concentration, and can trigger damage to lipids, proteins and DNA at too high or too low concentration. Plants integrate RONS with genetic, epigenetic and external signals to regulate developmental processes. RONS signaling is highly integrated with hormonal signaling networks, thereby allowing plants to adjust to environmental cues.

All organisms have adaptive responses to oxidative stress, with antioxidant enzymes (i.e. catalase, peroxidase, superoxide dismutase) being induced by changes in the levels of $H_2O_2$ or $O_2^{\bullet-}$, leading to the activation or silencing of genes encoding defensive enzymes and transcription factors [2]. Although various observations have led to the suggestion that cells have the means to sense RONS and to induce specific responses, the underlying mechanisms are still not fully understood. In addition, plants also have non-enzymatic systems known to remove RONS, which are important players in plant processes that use RONS-dependent signaling mechanisms [3]. In this work, we have shown, by using molecular approaches, how PAW treatment affect expression of genes coding for specialized metabolites and hormones, thus regulating development and stress responses in the model plant system *Arabidopsis thaliana*.

*This work was supported by Ministry of Education, Science and Technological Developments of the Republic of Serbia, contact number grants 451-03-68/2022-14/200007 and 451-03-68/2022-14/200024. This publication is based upon work from COST Action CA19110 - PlAgri, supported by COST (European Cooperation in Science and Technology-www.cost.eu).*

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# Plasma decontamination of food packaging material

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One of the main lessons that the COVID-19 pandemic has taught the world is the importance of decontaminating living and non-living surfaces in order to reduce the spread of diseases [1]. In this context, the problem of the global market has a major importance: goods could be transported across countries before being sold to customers with little if any attention to possible contamination of the packaging.

This work examines the relation between the decontamination efficacy of a SDBD (Surface Dielectric Barrier Discharge) plasma treatment on materials typically used in the food packaging industry and the concentrations of reactive species produced by the plasma discharge. Biological results were obtained with *Staphylococcus epidermidis* (ATCC 12228), a Gram-positive bacterium. The plasma system, developed by AlmaPlasma srl, was used to treat polypropylene contaminated sample, inactivation up to 1.60 logarithmic reduction were obtained with a 20-minute treatment. Moreover, test using clinical isolate SARS-COV-2 obtained from an RT-PCR-positive nasopharyngeal swab of a patient suffering from COVID-19 were performed to assess the efficacy of the plasma treatment against the virus. Two different operative conditions were tested varying the surface power density (SPD) absorbed by the SDBD; tests were performed varying treatment time from 10 to 40 minutes, a total inactivation was achieved for different treatment configurations.

In literature there are many papers assessing the relation between SPD and the chemical composition of air plasma [2–4]; a low SPD leads to an atmosphere enriched with ozone, while a high SPD produces an atmosphere dominated by nitrogen oxides. Optical absorption spectroscopy was performed to analyse the concentration of ozone and nitrogen dioxide during treatments. Multiple tests were performed varying the axial line of sight to observe the diffusion of ozone and nitrogen dioxide inside the treatment volume.

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# Cold plasma for bacterial decontamination: Impact of food matrix composition and relative humidity of the input gas

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Food safety and preservation are two important points of attention in the food industry. The World Health Organization (WHO) estimated that in 2010, 600 million people suffered from food-borne illnesses. This is the result of presence and growth of pathogens, viruses and/or toxins produced by e.g. molds [1]. On the other hand, it has been reported that around 1.3 billion tons of food (1/3 of the production) were globally wasted in 2007, 15 % of which because the expiration date was exceeded [2-3]. Appropriate decontamination strategies are thus inevitable, and as both consumer and industry desire minimally processed and high quality foods [4], the interest in cold plasma (CP) has increased.

The fact that CP shows a bactericidal effect has already been stated by various studies. However, as the food matrix is complex and the composition differs for all products, microbial inactivation depends on the exact product to be treated. This study investigates the degree to which the CP inactivation of bacteria is influenced by various food components in different concentrations. In this work, a Multi-Hollow Surface Dielectric Barrier Discharge (MDBD), operated at ca. 25 W, was used for the treatment. Agar plates supplemented with no, low, middle-high and/or high concentrations of protein (casein), refined oil, stripped oil, glucose, starch and NaCl were inoculated with *Escherichia coli*. Bacterial recovery from treated (30 seconds, 5 standard L/min dry air input) and untreated plates was compared. Furthermore, the same analysis was done for no and high concentrations of the same components treated under identical conditions, but with the input of air at 75% relative humidity.

All selected components induced a decrease in the CP decontamination efficiency, although this was only significant for casein, starch, stripped and refined oil ($p \leq 0.05$). For casein, the decontamination efficiency was most concentration dependent. Furthermore, inactivation of *E. coli* was clearly lower when using a more humid input gas ($NO_2$ as most abundant plasma reactive species instead of $O_3$ for dry air). In this case, a high concentration of casein did not lead to any bacterial decontamination. These are important considerations for food producers. After all, as certain biomolecules, mainly lipids, have a clear impact on the bactericidal effect, CP application for e.g. high-fat products will be considerably less effective regarding (pathogen) inactivation.

*This work was supported by The Special Research Fund of Ghent University.*

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# Effect of plasma and plasma activated water on growth media used in hydroponics

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The main purpose of this study was to investigate the effect of non-thermal plasma and plasma activated water on cultivation media used in hydroponics. The goal was to investigate if plasma or plasma activated water change the chemical composition of the medium. Oxidation of organic compounds was expected, due to the effect of reactive oxygen and nitrogen species present in plasma. The products of this plasma-liquid interaction were analyzed by HPLC with UV-VIS detector and LC/ESI-MS. Using a DBD reactor with air plasma, the treatment of diluted cultivation media was investigated. Plasma activated water, prepared by the same DBD reactor, was mixed with cultivation media.

The obtained hydroponics medium contains humic and fulvic acids, amino acids, auxins and cytokinins. Analysis of molecules of low and high molecular weight requires HPLC columns based on different working principles. Thus, high molecular humic and fulvic substances hat to be removed from the solution before the analysis of smaller molecules, such as auxins and cytokinins. Auxins and cytokinins were extracted from the solution by a solid phase extraction. Solutions were analyzed by HPLC with a UV-VIS detector. At first, the non-treated medium was analyzed to determine presented components and their concentration. After this, the medium was treated by dielectric barrier discharge, with different treatment times (2, 5 and 10 min).

Using the same DBD reactor, plasma activated water was prepared, and mixed with hydroponics medium. The final concentration of the medium in plasma activated water was 0.4 mL/L. These samples were also analyzed by HPLC and LC/ESI-MS.

According to the UV-VIS chromatograms, both plasma and plasma activated water did not have any effect on the medium composition, probably thanks to the presence of many different organic compounds. This means that we can use non-thermal plasma for the removal of biological contaminations such as bacteria from the growth media, without worrying about changing composition changes of hydroponic compounds and decreasing efficiency. Analysis of $H_2O_2$, $NO_2^-$ and $NO_3^-$ concentrations in plasma activated water was carried out using spectrometric reagents and UV-VIS spectrophotometer. Finally, plasma treated growth medium and plasma activated water were used for hydroponic cultivation of Garden cress (*Lepidum sativum*). We also studied antibacterial effect of treated media and PAW on bacteria *Esherichia coli*.

*This work was supported by COST Action CA19110.*

# Comparison of the effect of plasma activated water and artificially prepared activated water on wheat grain properties

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Recently, much attention has been paid to the application of both plasma and plasma activated water (PAW) in various areas of biological research, especially for the purposes of this article in agriculture. Although direct plasma action involves the effects of many highly reactive species with short lifetimes, the use of PAW represents the action of long-lived particles only. A number of published articles (e.g. [1]) show that the main stable components of PAW are $H_2O_2$, $O_3$, $HNO_2$, and $HNO_3$. If so, then it should be much more practical and faster to prepare PAW artificially by directly mixing of these chemicals in a given ratio. We have tried to draw attention to the otherwise rather neglected fact that there are no significant differences between the action of PAW and the artificially prepared PAW. The effect of PAW on the properties of wheat grains (*Triticum aesculentum*) was determined. The PAW exposure increased germination, length of shoot, weight of fresh and dried shoot. The length of the root and the R/S length slightly decreased, while the others changed only irregularly or not at all. Grains artificially inoculated with *Escherichia coli* are significantly decontaminated after only one hour of exposure to PAW, while *Saccharomyces cerevisiae* decontamination requires soaking for 24 hours. The small differences between PAW prepared by plasma treatment and PAW prepared by artificial mixing the active ingredients, i.e. nitric acid and hydrogen peroxide, proved to be inconsistent and statistically insignificant. Therefore, it appears that only the effects of artificial PAW may be convenient for further research.

*This work was supported in part by a COOPERATIO Institutional grant from the 1st Faculty of Medicine.*

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# CaviPlasma: A plasma source capable of application-scale generation of plasma treated water for agriculture, aquaculture, and medicine

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Up to now, the industrial applications of plasma treated water have been severely limited by the low throughput of existing plasma generators [1]. Plasma treatment of water at flow rates exceeding several $m^3$/h has been a rather theoretical consideration of massive parallelization of existing plasma sources. Only recently, the barrier discharge plasma source capable of 0.52 m3/h at 8 kW input power input was reported [2], however the high acidification (pH 3) means technical showstopper for many applications.

The newly emerged hydrodynamic cavitation plasma device called "CaviPlasma" [3] breaks the limits of existing plasma generators offering the industrial-scale flow rate of 1-15 $m^3$/h (laboratory devices) introducing peroxide-based chemistry into treated water, thus keeping the pH practically unchanged (typically within 0.5 pH difference margin). CaviPlasma combines hydro-mechanical effects of cavitation and electric discharge generated in cavitation cloud [4]. The cavitation provides reduced-pressure environment of cavities (voids) for electric discharge inception and also helps in efficient dissolving/mixing of the products in the water as the cavities collapse [5], which could be highly beneficial considering the interfacial and liquid phase processes [6].

The effects of CaviPlasma water treatment on its biocidal activity will be presented on the study of the successful remediation of cyanobacteria and algae from water [4], inactivation of pathogens collected from hospital environment surfaces or inactivation of the main rainbow trout pathogenic bacteria.

*The Czech Science Foundation is gratefully acknowledged for support of this research under project No. GA22-11456S "Complex phenomena of hydrodynamic cavitation plasma jet and their impact on advanced oxidation processes in liquids". The research is supported also by the Technological Agency of Czech Republic under project No. SS01020006. This study was carried out within the framework of research project No. QK21010030, supported by the Ministry of Agriculture of the Czech Republic. This work results within the collaboration of the COST Action CA19110.*

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# Experimental and computational study of nitrogen fixation mechanisms from (humid) air and nitrogen in pulsed plasma

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We use more reactive nitrogen than the planet can provide for us through natural means. For this reason, we have been transforming atmospheric $N_2$ into reactive (or accessible) N for the production of synthetic fertiliser since the beginning of the 1900s.[1-2] The modern environmental standards for processes of such importance and scale are not met by the current state of industry.[1-2] Thorough insight into alternative or complementary ways of nitrogen fixation are needed to develop processes which seamlessly fit in the sustainable world we are trying to realise.

$NO_x$ production from dry air in a low-power pulsed plasma jet takes place at a very low energy cost (0.4 MJ (mol N)$^{-1}$) under atmospheric pressure. Modelling demonstrates that the pulsed power and consequentially pulsed temperature are key parameters.[3] Next to the dry gases, pulsed power plasma-based gas conversion of ubiquitous humid air and humid nitrogen are of interest.[4]

Hence, insight into the influence of humidity in plasma conversion is of importance (i) for the potential of clean hydrogen introduction into molecules and (ii) in the context of further (industrial) application.

Here, we present work on a pulsed power plasma operating in humid air and humid nitrogen for the direct production of $(H)NO_x$ and $NH_3$.

In humid air, the pulsed plasma jet directly produces $HNO_2$ next to $NO_x$. The influence of humidity in the production and selectivity of $(H)NO_x$ was evaluated by means of FTIR at different flow rates (0.5 – 2 L•min$^{-1}$) and levels of relative humidity (0 – 100%). $HNO_2$ easily decomposes with the formation of $NO_x$ and $HNO_3$, i.e. base constituents of fertilisers.[1] Production of $NH_3$ from humid air was not observed here, possibly due to the interaction between gaseous $NH_3$ and $HNO_2$ decomposed into $HNO_3$, leading to the formation of solid powder ammonium salts. Therefore, experiments with the exhaust of the pulsed plasma jet bubbling through an aqueous solution were conducted in order to detect all potential species, including ionic solutes.[5]

$NH_3$ is produced directly from humid nitrogen. As our previous work suggested that $NH_3$ is formed in the gas phase [4], we mapped the gas temperature and NH production in the plasma and its evolution in the afterglow through OES for the aforementioned conditions to gain insight into how and where $NH_3$ and its precursors are formed.

This study of the low-power pulsed plasma jet for different ubiquitous gas compositions shows the potential and advantages of pulsed power plasmas for energy-efficient nitrogen fixation and the influence of humidity herein.

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# Role of metals on fixation of $NO_2^-$ in plasma-activated liquids

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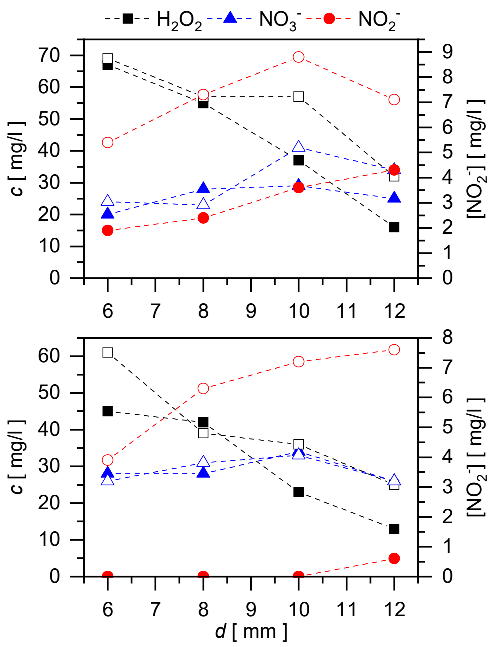
The long-lived species of plasma-activated liquids (PALs) have been identified to be the nitrate, nitrite and hydrogen peroxide. Recently, it has been shown that PAL can be used to increase the stress tolerance of plants [1], and it is further hypothesized that the created nitrate/nitrite ions can make PAL to be used as green fertilizer by providing nitrogen nutrient for plants. However, under acidic conditions the $H_2O_2$ reaction with $NO_2^-$ is very efficient, and in the case of comparable concentrations it leads to the disappearance of $NO_2^-$. In a recent study the lifetime of $NO_2^-$ could be controlled through a Fenton-type reaction [2]. The aim of the present work is to investigate the effect of metals with high reduction potential on the species life-times.

The PALs are created with the use of an atmospheric pressure surface-wave microwave discharge ignited with the help of a *surfatron* (Sairem, Surfatron 80) in a quartz tube of outer diameter 7 mm and I.D. 4 mm, using Ar gas at 2000 sccm flow rate and MW input power of 25 W [2,3]. By tuning the concentration of electrons and nitrogen and oxygen content species at the plasma-liquid interface with e.g. the treatment distance, the creation of $NO_2^-$, $NO_3^-$ and $H_2O_2$ species in the liquid can be controlled [3]. A Berzelius beaker of 35 ml filled with 32 ml of liquid is positioned below the plasma plume with the liquid surface being at 12 mm distance. In order to avoid the overheating of the quartz tube during treatments, compressed air is applied along the quartz tube with a gas flow rate of 8 slm. The metal, e.g. Mg is added in powder form to the purified water. During the 5 min treatment constant stirring is applied using a magnetic stirrer. Mg due to its high reduction potential favours the efficient reduction of $H^+$ ions into H and thus it contributes to the increase of the PAL's pH. With the increase of pH the reaction rate of $H_2O_2$ + $NO_2^-$ decreases. Figure shows the species concentrations in the plasma-activated (*i*) water (full symbols) and (*ii*) water with magnesium powder (open symbols) as a function of treatment distance right after the treatment (upper panel) and after 180 min of storage (lower panel). In the case of water with Mg powder significantly higher $NO_2^-$ concentrations can be achieved than in the case of water, and while with ageing the $NO_2^-$ disappears within 180 min in the plasma-activated water in the plasma-activated water with Mg powder its concentration is quasi-constant. During treatment the pH of water gradually decreases reaching an acidic 3.9 pH, while in the case of water with Mg powder it reaches a pH of about 6.0-7.0 depending on the treatment conditions. We can conclude that metals with high reduction potentials can be a tool to use for stabilization of PALs.




*This work was supported by NKFIH grant K-132158, AVACS project KK.05.1.1.02.0004, COST Action CA19110.*

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# Inactivation of viruses in irrigation waters

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Agriculture consumes up to 70% of global water. When contaminated, irrigation water can act as vector for the transmission of various waterborne pathogens, including viruses. Plant viruses can spread throughout the crop and destroy it, causing high economical losses and food shortages, while human viruses can adsorb to crop plants ands fruits and cause infection when consumed, posing a high health risk. This is one of the reasons why it is so important to adequately clean irrigation waters. Most of the methods used for waterborne virus inactivation have some downsides including high production of waste and toxic intermediates. Therefore, new clean and efficient methods are called for. One such promising alternative is cold plasma.

In the first part of our research, we examined the inactivation potential of atmospheric pressure plasma jet in a single electrode configuration against three different viruses: pepper mild mottle virus, potato virus Y, and bacteriophage MS2. We also investigated the mechanisms of inactivation and determined the toxicity of plasma-treated water using various methods. We successfully inactivated all three viruses after only a few minutes without introducing toxic products into the water [1-3].These encouraging results motivated us to further improve the proposed water treatment technology, through combination with hydrodynamic cavitation to allow treatment of larger volumes of water in flow mode. Hydrodynamic cavitation is a phenomenon where water vapor bubbles are formed in flowing water due to the local pressure drop. In these bubbles, low pressure plasma was generated. In this device, we treated water containing the waterborne enteric virus surrogate bacteriophage MS2. We observed reduction of the virus infectivity by more than 5 log after just a few minutes of treatment. Therefore, we demonstrated that such a combination of cold plasma and hydrodynamic cavitation has great potential as a novel, environmentally friendly tool for water decontamination.

*This work was supported by the Slovenian Research Agency (Research Core Funding No. P4-0407; Project No. L4-9325 and the Programme for Young Researchers in accordance with the agreement on (co)financing research activities for 2016-2020 No. 1000-18-0105), the Ministry of Agriculture, Forestry and Food, and Domžale-Kamnik Wastewater Treatment Plant.*

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# On the use of plasma activated water (PAW) for agricultural purposes

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According to the latest estimates of the Food and Agriculture Organization of the United Nations, the world population will rise from 6.8 to 9.1 billion in 2050. Such growth, combined with an increment in the *per-capita* income and in the urbanization, will result in a 50% increase in farm production. As a consequence, the increase in agricultural productivity should result in an increased yield and intensity of crops and a decrease of diseases which frequently affect the quality of production and the yield.[1] The traditional methods used to improve the crop cultivation are generally fertilization, irrigation, and crop protection; all these methods have economic and environmental disadvantages, and their intensive use produces a disequilibrium to the environment and the whole ecosystem.[2] An innovative approach to increase agricultural productivity and crop yield, with a lower impact on the environment and ecosystem, could be the use of plasma activated liquids (PALs).[2] They are produced from the interaction between a gas phase plasma and a liquid leading to the formation of numerous reactive oxygen and nitrogen species (RONS) [3] influencing and controlling many metabolic processes in plants.[4-5]Moreover PAW has the ability to inactivate a variety of microorganisms, due to the combined action of a high positive Oxidation Reduction Potential (ORP) and a low pH. Therefore PAW could be used in agriculture both as fertilizer and for its antimicrobial properties, being more ecological and economical than the traditional chemical resistance inducers.[4,5]

The effect of PAW on plants is complex and strongly correlated to the concentration of RONS in liquid phase, which can be influenced by several parameters such as: source type, power, type of water. In this view, two different corona plasma sources used for the treatment of tap water are presented. The first one is a scaled down source used to treat 0.5 liters of tap water for the treatment of plum trees infected by European stone fruit yellows associated with '*Candidatus* Phytoplasma prunorum' presence by endotherapy in the field. The agronomic performances in terms of stem length and fruit productions were evaluated. The second one is a scaled-up source able to treat 5 liters of tap water for hydroponic systems. Subsequently, the effect of tap water and PAW in hydroponic systems was compared evaluating plants weight, leaf area, L-A-B indexes and water use efficiency. In both cases an in-depth analysis of electrical parameters of plasma sources and chemical parameters of PAW were realized to define the most important parameters for the scale-up of corona sources used for the treatment of tap water.

*The authors acknowledge the support of the PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE 2020 titled "Sustainable Vertical Farming (VFarm)".*

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# From anhydrobiosis to germination: Effect of an air atmospheric cold plasma treatment on *Arabidopsis* seed dormancy

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Anhydrobiosis refers to the remarkable ability of some organisms as Arabidopsis seeds to survive the loss of water and enter into a quiescent state characterized by a reversible standstill of their metabolism [1]. In this state, a transitory physiological blockage of germination can set in, called dormancy. Seed dormancy can be defined as the inhibition of viable seeds germination under favorable environmental conditions [2]. Seed dormancy release in the dry state can be associated with ROS accumulation and non-enzymatic autoxidative reactions allowing oxidative signaling during imbibition resulting in germination [3].

We have designed an air plasma process relying on DBD technology that purposely releases the dormancy of Arabidopsis seeds. This treatment also stimulates seed germination in hypoxia and seed respiratory activity, suggesting that it could favour oxygen diffusion within seeds. To understand the effects of plasma on seed germinative properties, we have investigated the biological processes triggered by the treatment from the dry state until germination through oxidative reactions, anatomy changes and gene regulation.

Confocal microscopy has been achieved to study and quantify the oxidative reactions occurring following seed treatment. Besides, X-ray microtomography has been carried out on dry seeds to decipher the impact of plasma treatment on physical structure, pores network and oxygen diffusion within seed tissues. In parallel, transcriptomic experiments have been performed to study the regulatory pathways of gene expression linked to the breaking of dormancy in Arabidopsis seeds by plasma treatment from the dry state until 24 hours of imbibition. The results of all these diagnostics are correlated and discussed to highlight the mechanisms ruling dormancy release by cold plasma treatment.

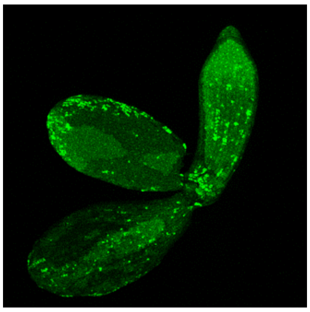

Fig. 1. Image of ROS fluorescence in an Arabidopsis seed embryo by confocal microscopy.

Fig. 2. Reconstruction images of microtomography experiment of an Arabidopsis seeds: 3D volume (left), 3D pores network (right).

*We acknowledge SOLEIL (Saint-Aubin, France) for provision of synchrotron radiation facilities and we would like to thank ANATOMIX team. We would like to thank the transcriptomic platform POPS of IPS2 (Gif-sur-Yvette, France) for the transcriptomic experiments.*

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# Cold plasma treatment effect on the germination and seedlings growth of durum wheat genotypes

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Two types of cold atmospheric plasma (CAP) sources are used for treatment of seeds in this work: microwave plasma torch and underwater diaphragm discharge. In the case of surface-wave-sustained (SWD) plasma torch the treatment is in the active discharge zone. This results in high concentrations of short-lived active particles together with electromagnetic field and UV radiation at plasma-sample interface. The effect of cold plasma seed treatment on the germination and growth of durum wheat was studied.

As a result of this study the most appropriate combinations of plasma source, discharge parameters and duration of treatment, positively affecting seed germination and seedling growth at three durum wheat cultivars are identified. This research was conducted with seeds of three durum wheat variety. All used variety are created in Field Crops Institute – Chirpan, Bulgaria – the older variety Progress, the standard variety Predel and one of the newest varieties Kehlibar. The treatment of the seeds was carried out in the Plasma laboratory at Sofia University, and the germination of the seeds and the cultivation of the plants was carried out at the Field Crops Institute - Chirpan.

During the experiment the effect of the seeds treatment on germination, germination energy and indicators related to the initial growth of seedlings was investigated. It was found that the treatment with both sources (the plasma torch and the underwater plasma discharge) have the greatest positive effect for specified discharge conditions. The variation in germination energy, shoots length and root length after the cold plasma treatment of seeds is mainly due to the interaction between genotype and treatment variant and to a small degree due to genotype.

*This work was supported by COST Action CA19110 - Plasma applications for smart and sustainable agriculture*

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# The persistance of effects of seed treatment with cold plasma, vacuum and electromagnetic field: 7-years of observations on Norway spruce

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We investigated the effects of pre-sowing Norway spruce (*Picea abies*) seed treatment with physical stressors – low-pressure cold plasma (CP, 2, 5 and 7 min), electromagnetic field (EMF, 5, 10 and 15 min) and vacuum (7 min) on seed germination and plant performance. We had reported earlier [1], that all applied seed treatments stimulated emergence in the substrate, except CP (7 min) having negative effect. After the second vegetation season, 17 month-age seedlings grown from CP (5 min) and CP (7 min) treated seeds, characterized by negative effects on either the germination *in vitro* rate or yield, had 50–60% larger height and 40–50% increased branching in comparison to the control.

Here we report that effects of pre-sowing seed treatments persisted at least for 7 years, as estimated by changes in the morphometric traits and amounts of secondary metabolites in spruce needles. In 39-month period after sowing plants grown from vacuum and CP (5 min) treated seeds were higher (by 26 and 17%, respectively) and had larger (by 33 and 21%, respectively) number of branches, as compared to the control. Significant differences in the number of trichomes on needles was determined among experimental groups, e.g., in CP (7 min) treated group the number of trichomes was almost twice as higher as in control group. Individual phenolic compounds, including stilbenes, flavonoids, simple phenylpropanoids and the precursor shikimic acid were identified and quantified in the extracts of sprouting and mature Norway spruce needles by liquid chromatography-mass spectrometry. The obtained results showed that CP and EMF treatments increased concentration of shikimic acid and quercetin in mature needles, whereas the concentration of stilbenes was lower in treated groups as compared to the control. The performed sequencing of fungal DNA in seedling needles revealed 116 different fungal taxa. The lowest diversity of fungal taxa was in the control needles, while the highest diversity was found in samples exposed to EMF 10 min - 77 fungal taxa. There were 54 fungal taxa in the CP 2 min, 44 in the EMF 5 min, 20 - in the CP 5 min samples. Analysis of the similarity of fungal taxa shows that there were 9 common fungal species in all samples, of which 7 are the most commonly found among all identified fungal taxa. The highest number of unique (only for that effect in samples) fungal taxa was found in EMF10 exposure samples - 30 fungal taxa. Among these fungal taxa, there were no plant pathogenic. Pathogens such as *Ophiosphaerella agrostidis*, *Alternaria alternata*, which were significantly more abundant in the affected needles, were not detected in the control samples.

The last morphometric and biochemical analyses were performed 85 months after sowing and revealed that effects of pre-sowing Norway spruce seed treatments on growth, antioxidative capacity and secondary metabolism are retained for more than 7 years of perennial plant growth.

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# Cold plasmas application on onion bulbs

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Applications of cold plasmas in the field of agriculture became a hot topic during the last decade. The most common application is on seeds with the goal to improve their germination and to reduce plant pathogens on their surface. Unfortunately, there is no available study about cold plasmas application on the other planting kinds like bulbs or tubers. The presented research shows the first results filling this unknown territory.

The onion bulbs (cultivar Sturon without any surface treatment) were used as the material. Five sets of onions were prepared for this experiment. Bulbs were treated directly by corona discharge for 20 and 80 seconds (a half of time from each side with respect to the corona discharge orientation). The indirect treatment was realized by plasma activated water (PAW) prepared in a small batch DBD reactor (details see in [1]) for 2 minutes using distilled water. The PAW composition in this system is shown in abstract of Z. Kozáková included in this Book of abstracts Bulbs were immersed in PAW for 6 or 24 hours and then, planted immediately. The non-treated bulbs and bulbs immersed in distilled water for 24 hours were used as references. Small field experiments were realized at 24 locations across the Czech Republic to cover majority of soil and climate conditions. Each treatment version contains 40 bulbs in total at each location (4 replications by 10 bulbs).

The crop was studied just after the harvest with respect to plasma application. Overall results showed a negligible effect of the corona discharge application and the shorter PAW application, but the 24-hours PAW application induced the crop increase of about 5%. These are general data; the situation was strongly dependent on the location as it was expected.

Besides the biomass yield, content of volatile compounds in fresh onion juice was measured by Proton Transfer Reaction Time of Flight Mass Spectrometry (PTR-TOF). The obtained results did not show significant changes in aromatic compounds. However, all data were also strongly dependent on the location.

The last studied parameter was number of onion shells. The overall results did not show any significant differences among all variants. Finally, changes of onion quality after the longer term storage were monitored. Also, in this case, no significant changes among variants were observed (for three locations stored at the same cellar room, only), both in quality (number of unspoiled bulbs) and aromatic compounds composition (by PTR-TOF).

The presented data are from the first year of the field experiment. Thus, conclusions are very preliminary because all open air processes are strongly dependent on weather conditions. The field experiment is continuing this year. New data from the second season will be presented, too.

*This work was carried out under the COST Action CA19110 frame.*

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# The evaluation of cold plasma effect on morphometric and biochemical parameters in *Stevia rebaudiana* by principal component analysis

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*Stevia rebaudiana* contains natural low-calorie sweeteners steviol glycosides (SGs) and other secondary metabolites, mainly flavonoids and cathechins, that have various beneficial effects on health [1]. Cold plasma (CP) is known to improve various plant properties by the hormesis effect on seeds [2]. We have recently reported for the first time that CP treatment of stevia seeds tremendously increases SGs biosynthesis and have negative effect on total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA) [3]. Stevia plants grown from seeds are heterogenous in the morphological traits, the composition, and concentrations of bioactive phytochemicals. Therefore, the elucidation of the relationship between plant morphometric and biochemical traits could add to the prediction of CP-induced stimulation of SGs biosynthesis.

The aim of this study was to analyse the effect of stevia seed treatment with CP (2, 5, 7 min) in individual plants on morphometric and biochemical parameters and to carry out the principal component analysis (PCA). Atmospheric pressure dielectric barrier discharge (DBD) plasma device was used for seed treatment. Morphometric parameters (plant height, dry weight, leaf number) and biochemical parameters (SGs, TPC, TFC, AA) were assessed in 12-week-old stevia plants. The most abundant and commercially important SGs found in stevia, rebaudioside A (RebA) and stevioside (Stev), were separated and quantified using high-performance liquid chromatography. TPC, TFC, AA were measured spectrophotometrically.

Even though CP had no effect on morphometric parameters, it increased RebA and Stev, whilst decreasing TPC, TFC, and AA in treatment groups. PCA revealed that dry leaf mass inversely correlated with AA, TPC and Stev, but along with plant height it showed a positive correlation with RebA, TFC, and RebA/Stev ratio. Plant height and the number of leaves negatively correlated with Stev and the total amount of SGs. PCA didn't reveal separate clusters of treatment groups.

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# Effect of plasma activated water, its chemically equivalent solutions and arsenic stress on growth, development of selected seed and plants

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Atmospheric plasma has shown a promising potential in various agricultural applications as being utilized to stimulate germination of seeds and modulate growth of plants. The plasma produces various gaseous nitrogen and oxygen reactive species (RONS) that dissolve in water and produce plasma activated water (PAW) [1]. The study presents an overview of the results on PAW generated by a transient spark discharge (TS) operated in ambient air in contact with water on germination of seeds (wheat) and plants (wheat and lettuce) in *in vitro* and in vivo conditions, and on maize corns and young seedlings and confronted with the effect of As stress in several different treatments. The discharge was generated in a reactor with water circulating through the discharge zone.

For wheat (*Triticum aestivum* L.) [2] and letuce (*Lactuca sativa* L.) [3] water uptake, germination, various growth parameters, and vigor indices, as well as photosynthetic pigments, content of soluble proteins and activity of antioxidant enzymes were studied and evaluated with respect to the type of the PAW and its activity both in vitro and in vivo conditions. The effect of the PAW was correlated with the PAW activity, i.e. its chemical composition and concentrations of the RONS ($H_2O_2$, $NO_2^-$ and $NO_3^-$). Its effect was also compared to chemically equivalent solutions of $H_2O_2$ and/or $NO_3^-$ of various concentrations to assess their individual and combined role in the process of plants stimulation by the plasma activated water. The $NO3^-$ in the solution mainly contributed to the increase of dry weight, photosynthetic pigment content, photosynthetic rate, decrease of SOD activity, and overall better visual appearance of plants. The $H_2O_2$ in the solutions had usually negative effect on the development of plants. Although it induced an increase of dry weight, it did not contribute to photosynthetic pigment content and photosynthetic rate and even increased SOD activity.

For maize corns (*Zea mays* L.) germination and growth, and root development of young maize plants was investigated. Antioxidant enzymes activity of the seedlings and changes in the root cell walls lignification and suberisation was evaluated. Two pre-treatments were established, “priming PAW” and “rolls PAW”, with corns imbibed in the PAW and then watered daily by fresh water and PAW, respectively, and compared with a control. PAW improved the growth parameters only in the young seedlings; however, in continuous hydroponic cultivation, plants also achieved positive changes in the dry biomass accumulation. The primary root morphology also changed due to PAW treatments, which significantly influence the plant nutrients uptake. Different pattern of plant response to the subsequent As treatment was found. Plants from different PAW treatments reacted to the As stress by elevating their antioxidant capacities depending on PAW pre-treatment.

*The work was supported by Slovak Research and Development Agency APVV-17-0382 and APVV-20-0566, and by Scientific Grant Agency VEGA 1/0822/21 and COST Action CA19110.*

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# Effects of plasma treated water on seed germination and growth of blue lupine (*Lupinus angustifolius L.*) plants under abiotic stress

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Cold atmospheric pressure plasma has the potential to be applied in pre-harvest processes as a sustainable technology for seed and plant treatment.

In the present work, the chemical changes of plasma treated water (PTW) generated by a gliding arc discharge and a pin-to-liquid discharge as well as their biological effects on germination and plant growth of blue lupine (*Lupinus angustifolius L.*) were investigated. Germination of seeds, soaked in plasma treated water, was determined in correlation with different treatments times of water. Moreover, growth parameters of drought-and flooding stressed plants treated by spraying PTW, as well as the content of photosynthetic pigments, soluble proteins and activity of antioxidant enzymes were determined relative to the control groups. Additionally, effects of plasma treated water on survival of bacterial cells and spores were studied to compare the PTW effects on both, prokaryotic and eukaryotic systems.

The results show that plasma treated water is harmless for plants and seeds at the tested treatment time and has the potential to enhance lupine germination, depending on the concentration of reactive oxygen and nitrogen species. While survival of bacterial cells were strongly affected by PTW, bacterial spores were still vital. The treatment of lupine plants with plasma treated water may stimulate and affect the biomass and the metabolism. Moreover, plasma treated water does not amplify abiotic stress reactions of plants.

# Development of experimental system for plasma irradiation effects on plants using *Marchantia polymorpha*

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Utilizing the plant growth-promoting effects of low-temperature plasma have attracted much attention to improving agricultural productivity [1-4]. Although growth promotion in response to plasma irradiation has been reported in many cases, the molecular mechanism of the plasma irradiation effect is still largely unknown [5, 6]. One of the factors hindering the elucidation of the molecular mechanism is natural variation in plants. Plasma irradiation of seeds is often used for plasma irradiation effects on plants, while the seeds have large genetic diversity and is highly dependent on the condition of parental plants and seed storage [7]. *Marchantia polymorpha* (*M. polymorha*) is one of the model plant species which is actively studied in molecular biology. Here we examined air scalable dielectric barrier discharge (SDBD) plasma irradiation results in growth inhibition and promotion of *M. polymorpha* gemmae. They were put on wet filter paper to prevent drying damage. It was set 3 mm under the SDBD device and was treated for 10 s. The humidity was set 45 %rh. Plasma-irradiated gemmae were cultured for 10 days on agar medium under continuous white light of approximately 40 μmol $m^{-2}$ $s^{-1}$ at 22 $^{o}$C, 45 %rh. After 10 days of cultivation, the change in the fresh weight of gemmalings were 17.1, 20.4, 24.7, 17.2, 14.0, 14.6, and 8.1 mg at applied voltage of 0, 1.64, 4,72, 6.48, 6.80, 7.52, and 7.80 kVpp, respectively. The weight increased at discharge power 0.47 W. Further increase in the power results in the decreased weight of gemmalings compared with control. It was suggested that SDBD plasma irradiation at low power promoted the growth. The response of *M. polymorpha* to the plasma irradiation was highly reproducible. The gemmae are convenient tissue that can be easily obtained, enable a unified genetic background, and have relatively little physiological variability [8]. The whole-genome sequence has been completed and many techniques for molecular experiments, such as transformation and genome editing methods, were established [9]. The *M. polymorpha* experimental system is suitable for the molecular biological study of plasma irradiation effects.

*This work was supported by KAKENHI JP20H01893 and the Plasma-Bio Consortium project.*

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# Application of plasma and pulsed electric field for the treatment of microalgae

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The potential of microalgae to help tackle challenges such as greenhouse gas emission and the increasing demand for food, bioactive compounds is already well established. However, the most algae valuable compounds are accumulated inside the cell [1-3]. Therefore, various chemical, biological, and physical pretreatment methods have been employed to disrupt the cell. One of the methods used to disrupt the algae cell and selectively extract valuable compounds are pulsed electric field (PEF) treatment [2]. The plasma treatment of microalgae is another technology used to increase the efficiency of extraction of valuable compounds [3]. The aim of work was to investigate the influence of plasma and PEF treatment parameters on the extraction yields of valuable compounds from marine and freshwater microalgaes.

The different technologies were used for the treatment of *Chlorella vulgaris* (freshwater microalgae cultivated in BG-11 medium) and *Porphyridium purpureum* (marine algae cultivated in F/2 medium). The gliding arc discharge plasma treatment was used. The distance between the "knife-edge" type electrodes and surface of the algae suspension was 30 mm. The plasma treatment of the algaes suspensions was done by air plasma at various power supply output voltages ranging from 50 V to 250 V at frequency of 270 kHz. The duration of plasma treatment was 300 s. PEF treatment consisted of 10 μs long 1-10 pulses at frequency of 1 Hz. Applied electric field strength varied from 23 kV/cm to 25 kV/cm. Afterwards pH, optical density, conductivity, chlorophyll a and soluble protein concentration were determined. More precisely, microalgae suspensions were incubated for 24 hours at room temperature and protein concentration was measured using ROTI®Quant universal assay kit. Chlorophyll a content (as indirect algae viability indicator) was measured after 5 day using ethanol extraction method. Then, the absorbance of samples was determined at 630 nm, 645 nm and 663 nm wavelengths using a UV-visible spectrophotometer.

Experimental results indicated that the chlorophyll a content, cell density, pH values, conductivity and viability of *Chlorella vulgaris* and *Porphyridium purpureum* microalgaes strongly depends on the plasma and PEF treatment parameters. The increase of the plasma discharge voltage reduced the protein extraction values. The extracted protein concentration from *C. vulgaris* was lower compared to non-treated algae, when plasma treatment was performed at 130 V or higher discharge voltages.

*This work was partly supported by the Research Council of Lithuania under Grant P-MIP-22-257.*

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# Preparing for large scale use of cold plasma discharges: Pitfalls and challenges

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Cold plasma electric discharges are the basis for the development of various devices commonly known as cold plasma reactors. In the column of the electric discharge, active species (electrons, ions, excited particles, photons, etc.) with a short lifespan (100 μs) are generated and they can lead to the formation of metastable radicals responsible for the desired effects.

Previous investigations performed in various laboratories worldwide have shown a broad spectrum of effects induced by such reactive species. In accordance with the multiple possibilities of application, a wide variety of cold plasma reactors using different types of electric discharges (Corona, GlidArc, DBD) and power supplies (DC, AC or pulse) have been designed for laboratory research or small-scale use. The transition to the industrial use of cold plasma discharge-based equipment is not easy to achieve, modular treating units being required instead of singular high-power reactors. The treatment facilities should be appropriate to each intended purpose in terms of selectivity, performance and efficiency.

The identification of these specific requirements still represents challenges for all specialists working in this field as they should focus on various aspects: the most effective reactive species for a given purpose, treatment duration, possible restrictions regarding the energy density, discharge power or density of reactive species, avoidance of negative effects for the target to be treated (i. e. living tissues or cells, seeds, plants, inanimate surfaces, etc.). All these aspects are linked with the frequency value of the power supply or other reactor parameters.

Secondly, it is necessary to identify the performance requirements for cold plasma reactors that involve the characterization of these devices from different points of view such as: evaluation of the power of useful electric discharge and the specific quality indicators; defining the nominal parameters of the cold plasma reactor module; optimization of the reactor module in terms of nominal operating parameters; defining the size of the reactor in accordance with the proposed purpose (for example, to obtain a maximum amount of plasma activated water in a given period of time).

Another important challenge refers to the choice of the power supply in accordance with the treatment requirements, specifying the value of the no-load voltage, the electric current, the current frequency and other operating conditions. The correct choice of the type of power supply requires the identification of suitable criteria to be identified.

Other aspects that will be discussed are related to the choice of direct cold plasma treatment or indirect treatment using plasma-activated water or other plasma-activated liquids (media, saline, etc.), respectively the use or not of the post-discharge effect. It is therefore clear that the transition from laboratory to large-scale application of cold plasma treatments requires a reorientation of research in order to achieve conclusive answers to all these challenges.

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# Influence of radish (*Raphanus Sativus L.*) seed coat color on plant secondary metabolites and response to cold plasma treatment

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The polymorphism of the plant seeds helps the plant to survive. Research has shown that each color of the seed has a different germination, amount of secondary metabolites, phytohormones and a response to cold plasma (P. Attri et al. 2021). However, it is not known yet, whether the nutritional value of sprouts and secondary metabolites depend on the color of the seed and seed plasma treatment. This study aimed to estimate the dependence of the effects of radish seed color and irradiation with atmospheric cold plasma (CP) on morphophysiology, and amount of secondary metabolites in the *Raphanus sativus* sprouts.

Fujitaseed and Nichinou cultivars seeds were separated by the color to grey and brown. A part of the selected seeds was left as control and another part was treated for 3 min with a scalable dielectric barrier discharge (DBD). Then a relationships between the seed color and seed germination, sprouts morphometrics, sprouts antioxidant activity and metabolite levels were estimated. Additionally, it was studied how seeds DBD plasma treatment affects analyzed parameters of sprouts depending on the seed color.

Results showed that while most of the analyzed sprouts parameters differed between grey and brown seeds, the parameters' ratio between the seeds color mostly depended on cultivar. The color of the seed coat had no effect on seed germination (%), however germination half-life (Me) and germination uniformity (Qu) was different between grey and brown seeds, while the difference was depended on the cultivar. Morphometric measurements showed that sprouts grown from brown seeds had longer (10.79 - 12.98%) and heavier (13.87 - 20.16%) shoots than sprouts grown from grey seeds, but root length and weight differences were more dependent on the cultivar. The antioxidant activity of the sprouts, the total amount of phenolic compounds, flavonoids and carotenoids also depended on the cultivar, but not on the color of the seed. However, sprouts of brown seeds had 11,7 – 34,5 % higher content of chlorophyll a and 26,7 – 28,2 % of chlorophyll b than sprouts of light seeds.

Seeds treatment by DBD plasma for 3 min had tendency to increase seeds germination, but effect on the germination kinetics was cultivar depended. DBD plasma had no effect on the shoots' length, weight and roots' length of either brown or grey seeds radish sprouts, but roots' weight was decreased (12-19% depending on cultivar) by DBD treatment. From secondary metabolites only the total phenolic compound content was reduced (12,04 – 12,16 %) by plasma in sprouts of grey seeds in both cultivars, while in sprouts of brown seeds it did not had effect. Other secondary metabolites were not effected in Nichinou seeds sprouts, but there was response to 3 min plasma treatment in Fujita seeds sprouts: antioxidant activity was increased (62% in grey and 13% in brown seeds sprouts, compared to control), flavonoids and carotenoids levels were increased (by 25% and 49%, compare to control), chlorophyll b was decreased (by 24%) only in brown seeds sprouts.

To conclude, the seeds germination and its kinetics, as well sprouts and their secondary metabolites differ between seeds color, but the differences are cultivar depended. The response to DBD plasma treatment is also cultivar depended, but plasma has tendency to increase seed germination and to decrease the total phenolic compound.

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# Analysis of the reactive species in the gas-plasma–water interactions

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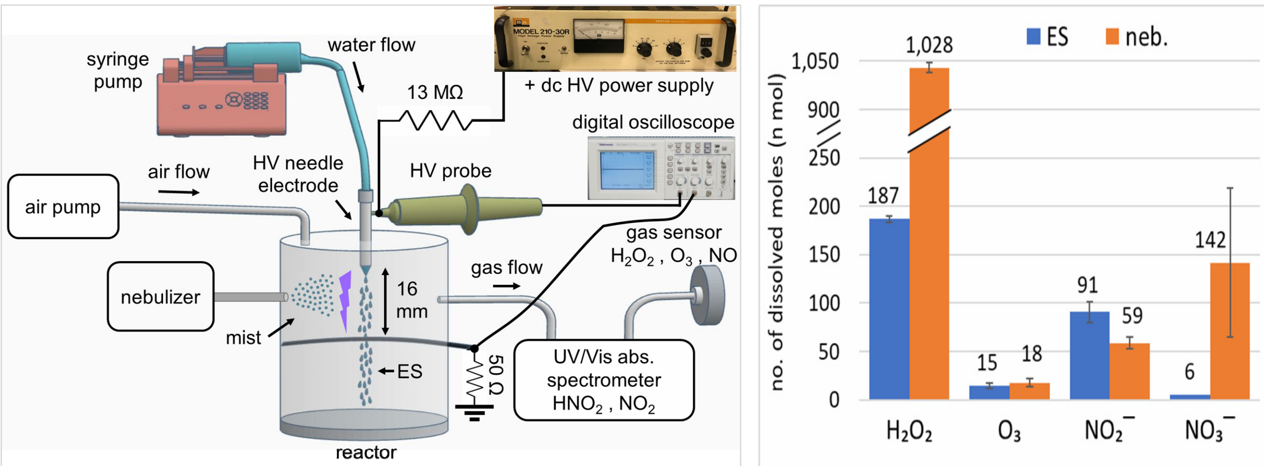
Atmospheric air plasma produces a mixture of reactive oxygen and nitrogen species (RONS). The plasma–water interaction enables the transport of RONS to the liquid phase, which is significantly enhanced by converting bulk water to microdroplets [1]. Having different Henry's law solubility coefficients, the expected solubility of various RONS is very different. Here, we aim to verify the applicability of Henry's law coefficients under strongly nonequilibrium conditions characteristic of plasma–water interaction, with water in the form of microdroplets. This work can lead to optimized designs of plasma–water interaction systems for multiple applications in biomedicine, environment, and agriculture.

Fig. 1(a) shows the schematic diagram of the experimental setup. RONS are created by streamer corona discharge in direct contact with water microdroplets. The solvation of RONS in water is compared in two types of microdroplets, charged microdroplets produced by electrospray (ES), and nebulized non-charged microdroplets. Fig. 1(b) shows the total molar number of the dissolved aqueous RONS. The amount of $H_2O_{2(aq)}$ and $NO_3^-{}_{(aq)}$ (mainly from gaseous $HNO_3$) dissolved in the nebulized microdroplets is around 1 order of magnitude higher compared to that in the ES microdroplets. We assume that $H_2O_2$ and $HNO_3$ solvation in nebulized microdroplets is enhanced by their smaller size, providing larger plasma–water interface area. On the other hand, the production of $NO_2^-{}_{(aq)}$ (mainly from $HNO_2$ [2]) is higher in the charged ES microdroplets.




(a) (b)

Fig. 2. (a) Schematic of the experimental setup, (b) Amount of dissolved aqueous RONS.

*This work was supported by Slovak Research and Development Agency APVV-17-0382 and Comenius University grant UK/141/2022.*

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# Changes in hemp growth and content of cannabinoids after seeds treatment with cold plasma, vacuum, and electromagnetic field

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One of the most important issues of sustainable agriculture is to increase crop productivity and yield without the use of pesticides. To achieve these goals, intensive research is currently being carried out on the use of cold plasma (CP) and electromagnetic field (EMF) in agriculture. It is shown that such treatments increase the agricultural performance of different crops, improve seed yield, resistance to disease and stress, accelerate germination and seedling growth.

This study compared the response of two varieties of hemp (Cannabis sativa), ‘Futura 75’ and ‘Santhica 27’, to seed treatment with low-pressure condenser-type CP, dielectric barrier discharge CP (DBD), and EMF was compared. Vacuum treatment was used as an additional control for low-pressure CP treatment. Changes in morphometry and cannabinoid content in leaves and inflorescences of plants have been studied.

The results showed that the effect of seed treatment with stressors on plant growth in the field depends on the variety of hemp. They confirmed previously obtained data [1] (Ivankov et al., 2020) that CP suppresses, and EMF promotes the growth of 'Futura 75' varieties (EMF 3 minutes group weight 49% and inflorescence weight were 58% higher compared to control). CP inhibited the growth of 'Santhica 27' plants, but DBD plasma significantly stimulated it. The positive effect of EMF was smaller compared to the effect on 'Futura 75' variety. Vacuum treatment of the seeds slightly improved the growth of both varieties and increased the cannabinoid content. Treatment protocols suitable for increasing CBD production in 'Futura 75' leaves have been identified: V3 and EMF2, in inflorescences - V2, V3 and DBD2; To increase CBD / CBG production in 'Santhica 27' leaves: V3, DBD1, DBD2, EMF2, in inflorescences - DBD1, DBD2, EMF3. There were no correlations between stress-induced changes in plant growth or changes in cannabinoid levels and changes in trichome density.

Stressor treatments of hemp seeds had a significant effect on the amount of cannabinoids in plant leaves and inflorescences. The effects depended on the hemp variety, and the dynamics of change were complex.

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# Investigation of an atmospheric pressure pin-type plasma jet for water treatment - optical diagnostics and temperature measurements

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In the increasingly important field of plasma agriculture, atmospheric pressure plasmas have proved their excellent potential for the elimination or reduction of organic contaminants and sterilization of microorganisms [1,2]. The exposure of aqueous solutions to plasma induces many reactions occurred in the gaseous phase and introduces reactive species in aqueous phases. One of the most commonly detected chemical species in gas plasma is OH radical because it plays a key role in the reactions of degradation of organic pollutants from water [3]. Another important factor that should be controlled during the plasma wastewater treatment to avoid the evaporation of harmful substances is the gas temperature. From the point of view of plasma physics, it is essential for future applications that the plasma processes and the effects of treatment are correlated, which can only be done by accurate and thorough diagnostics of the plasma sources.

Atmospheric pressure plasma jet (APPJ) in pin-electrode configuration operating in contact with liquid sample has been investigated by two different measurement techniques for gas temperature measurement. Firstly, the gas temperature above the liquid was determined by using the optical emission spectroscopy (OES) method for measurement based on partially rotationally resolved emission from hydroxyl (OH) radical. Another goal was to employ an active method - Rayleigh scattering laser spectroscopy which allows spatially resolved gas temperature measurements and to compare obtained results with OES measurements. Since the plasma jet generates intense streamer discharge, such a detailed analysis of the emission spectrum of plasma radiation and precise measurement of gas temperature was necessary for the potential application in biotechnology and wastewater treatment. Finally, as earlier studies have shown that OH radicals formed in the plasma play an important role in the degradation of pollutants dissolved in water, the absolute values of OH radicals were recorded by laser-induced fluorescence (LIF) technique.

The recorded spectra showed intense emission of OH radicals at wavelengths around 306 nm for discharge generated in pure Ar and an admixture of Ar and synthetic air. Preliminary results showed that the temperature in the plasma core reached values of up to 300°C. Also, it was obtained that the intensity of the LIF signal increases with the increase in the power deposited in the plasma, whether the discharge was in contact with distilled water or tap water.

*This work was supported by a STSM Grant from the COST Action CA19110 PlAgri, supported by the COST Association (European Cooperation in Science and Technology) and the Ministry of Education, Science and Technological Development, grant number 451-03-68/2022- 14/200024.*

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# High flow rate plasma activated water generation using dielectric barrier discharge reactor

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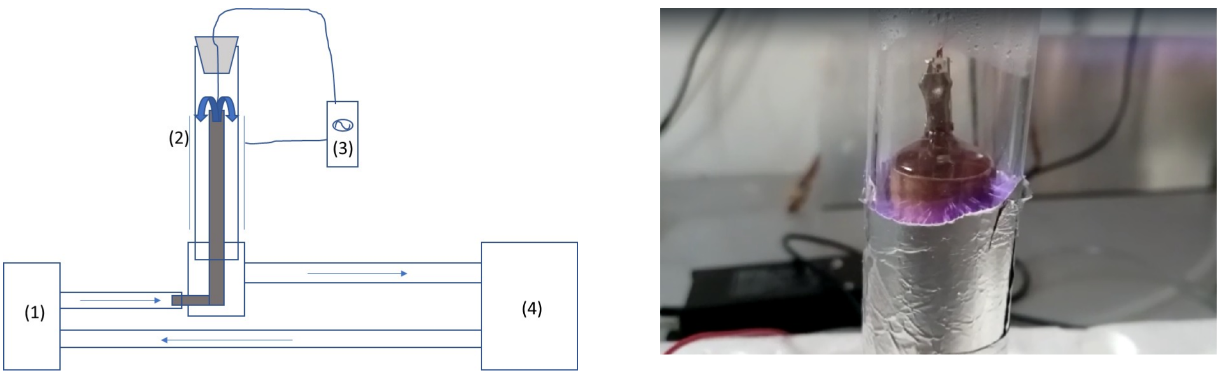
In recent years, research of plasma-liquid interactions has been motivated by a growing field of plasma applications, such as plasma medicine, agricultural applications, water treatment, etc. However, the complex and highly dynamic plasma-liquid interface opens many challenging, fundamental and interesting scientific questions, e.g. modeling and diagnostics of plasma-liquid interactions, different physicochemical processes, and cost-efficient/high-performance or standard reactor design for each application [1]. In this work, a novel Dielectric Barrier Discharge (DBD) reactor for the production of Plasma Activated Water (PAW) in bulk quantities is introduced. The DBD reactor can be used to produce PAW with a high flow rate up to 2.5 l/min. In addition, a recirculation of water is possible to achieve higher concentrations of reactive species, such as $NO_2^-$, $NO_3^-$, and $H_2O_2$ in PAW. The reactor has a form of a coaxial cylinder which enables water to flow through the central electrode upwards until the springhead. Water then flows down from the top of the central electrode and interacts directly with plasma microdischarges in the gap space (Fig. 1). Water flow rate is changed using a 12V water pump and a DC generator between 5-2.5 l/min. A quartz tube with 2.2 mm thickness, 36 mm external diameter and 25 cm length was used as the dielectric. A copper tube with dimensions 22×200 mm was used as the central electrode and the gap space was around 5 mm. A high voltage neon transformer (10 kV, 35 KHz) was used to generate the microdischarges in the reactor.




(a) (b)

Fig. 3. (a) Plasma activated water setup: (1) Water pump, (2) Coaxial DBD reactor, (3) Neon transformer, (4) Water container. (b) Water springhead and microdischarges in the DBD reactor.

In the initial experiments, one liter of water was recirculated in the reactor for 1, 5, or 15 min with a flow rate 0.5 l/min. Reactive species concentrations in PAW were measured using colorimetric indicator papers and absorption spectrophotometry with specific reagents. The concentration of $NO_2^-$, $NO_3^-$, and $H_2O_2$ after 15 min treatment reached around 12, 23, 10 mg/l, respectively.

*This work was supported by Slovak Research and Development Agency APVV-17-0382. S.K. was supported by National Scholarship Program of SAIA.*

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# Characterization of plasma activated water prepared in different plasma systems

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Interaction of non-thermal plasma with water produces so called plasma activated water (PAW). Activation leads to the formation of reactive species with short lifetime (hydroxyl, oxygen and hydrogen radicals, excited electrons, etc.) as well as long lifetime (hydrogen peroxide, nitrates, nitrites, peroxynitrite, ozone, etc.) [1]. Due to the high oxidation potential of these oxygen and nitrogen species (RONS), PAW provides antimicrobial and antifungal properties. Increased content of nitrogen also contributes to the stimulation of seed germination and plant growth.

With respect to different environment in which plasma is created, composition of plasma activated water varies in concentration of reactive species as well as in other chemical and physical properties. Therefore, it is essential to choose appropriate plasma system for PAW preparation to ensure desired PAW composition. For sterilization effects, higher concentration of hydrogen peroxide and nitrites is required. On the other hand, increased production of nitrates is suitable for plant growth stimulation as a biofertilizer.

In this work, PAW prepared in three plasma systems was characterized by physical and chemical changes (pH, conductivity, RONS). Colorimetric methods joined with UV-VIS spectrometry were utilized for the detection of hydrogen peroxide, nitrates and nitrites in PAW prepared from distilled or tap water. The first system generating plasma above water surface was based on the dielectric barrier discharge (16 kV; 11 kHz; 36 W) with the liquid electrode. In the second system, plasma was generated directly in the liquid by high frequency voltage (16 kHz; 50 W) applied on the main pin-hole based electrode [2]. In the third system, gaseous products from the ozonizer (maximal power of 30 W) operating in synthetic air were bubbled into the liquid. Activation time was 2 minutes in all systems. Results are compared in Fig. 1.

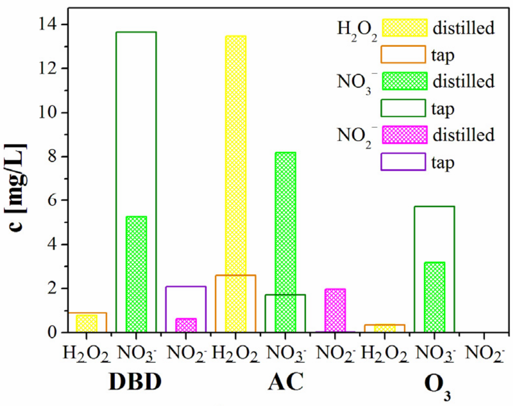


Fig. 1. Comparison of reactive species concentration (hydrogen peroxide, nitrates and nitrites) in plasma activated water prepared by three plasma systems from distilled and tap water: DBD – dielectric barrier discharge with the liquid electrode, AC – pin-hole based high frequency discharge in liquid, $O_3$ – bubbling of gas products produced by the ozonizer.

*This work was carried out within the frame of COST Action CA19110.*

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# GAD impact on selected foods

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Fresh, cold-pressed juices are an increasingly popular and constantly growing segment of the market. One of the popular and nutrient-rich products in this sector is carrot juice. According to the National Nutrient Database for Standard Reference, 100 g of carrots are a valuable source of vitamins. In addition to its many nutritional benefits, 100 g of carrot juice is only about 40 kcal.

Fresh juices are unstable products and require appropriate storage and transport conditions. The production and logistics related to one-day juices are becoming a particular challenge. In order to maintain the parameters of their quality, extend the shelf life without the use of thermal methods of fixation; Research is still being carried out on the application of new physical and physicochemical methods such as plasma technologies, sonication, pascalization and alternating electric fields. Such works have an economic, ethical and ecological dimension, as they ensure a safe and more durable product with high nutritional values, at the same time they allow to reduce the amount of waste, costs related to their disposal and avoid unnecessary food waste.

The aim of the research work was to investigate the effect of plasma treatment on the juice of two carrot varieties, Belgrado and Nerec. The freshly squeezed juice was treated with low-temperature non-thermal plasma generated in a gliding arc reactor. Thanks to the use of a plasma reactor operating at atmospheric pressure, we were able to check the effect of the plasma treatment time on various physical properties of the carrot juice, such as: temperature, PH, ORP or dynamic viscosity. The conducted research will be used to study the effect of plasma treatment on extending the shelf life of carrot juice.

*We would like to express our gratitude to "VICTORIA CYMES" Sp. z o.o., ul. Kołobrzeska 43, 78-600 Wałcz, Poland for new research opportunity. The research work was supported from the Program Operacyjny Inteligentny Rozwój 2014-2020 („Proinnowacyjne usługi dla przedsiębiorstw" Poddziałanie 2.3.2 „Bony na innowacje dla MŚP" w ramach II Osi priorytetowej" Wsparcie otoczenia i potencjału przedsiębiorstw do prowadzenia działalności B+R+I").*

# Examining the impact of non-thermal plasma on lipid model systems

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Non thermal plasma is a mild processing technology for food preservation, but its impact on food quality and interaction with food components especially lipids are not well explained. Fatty acid methyl esters were considered as basic lipid model systems and treated by non-thermal dielectric barrier discharge plasma. The plasma operational parameters consisted of exposure time, gas composition, humidity and flow rate, voltage/power and plasma-sample distance.

Plasma-induced effects were firstly examined by monitoring classical lipid oxidation indicators such as peroxide value (PV) and p-anisidine value (p-AV), which increased parallel with the intensity of the treatment and the degree of unsaturation of the fatty acid methyl ester studied. Increases in the formation of specific volatile oxidation products as function of treatment intensity and unsaturation degree were also noticed using SPME-HS-GCMS analysis in combination with stable isotope dilution. The formation of non-volatile oxidation products such as epoxy and hydroxy fatty acid methyl esters were limited. In addition, plasma generated in air affected oxidation status and oxidative stability. Among all the plasma reactive species, ozone, dinitrogen pentoxide and nitrogen dioxide were considered as the most relevant species to induce primary lipid oxidation. The formation of lipid carbonyls, regarded as secondary oxidation products, was attributed to ozonolysis of unsaturated lipids.

# PAW properties for agriculture: What we want?

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Numerous chemical processes can occur in the plasma-produced reactive liquids, also called as Plasma-Activated-Water (PAW), and this chemical reactivity depends on many parameters. Primary on the used plasma source and working gases/atmosphere, however, very significantly also on the chemical composition of exposed liquid, its pH value, temperature, concentrations of chemical species in liquid, etc. Several transient reactive oxygen and nitrogen species such as OH•, $O_2^{\bullet -}$, NO• and $NO_2$• radicals, peroxynitrite may be produced in plasma-treated liquids through post-discharge processes [1-3]. These species have highly cytotoxic properties and cause prolonged biochemical and antibacterial activity of plasma-treated solutions. However, they are difficult to measure due to their short lifetimes and fast disproportionation in the plasma/liquid systems. It is clear that such short-lived species require in situ and fast measurements and achieving this presents challenges. Because of the complexity of the reactions, giving rise to both stable and non-stable intermediates and reactions products, detailed characterization of aqueous chemistry in PAW is one of the key issues in order to control the properties of PAW and its use in specific applications including agriculture. The latter task brings, however, another important question: PAW of which properties do we want to generate? Do we want produce water with "stored" radicals but time limited chemical/cytotoxic activity (i.e., mixture $H_2O_2$, $NO_2^-$, $H^+$), then how to stabilize such PAW, or do we want to produce rather fertilizer (i.e., to fixate nitrogen into water in form of $NO_3^-$)? This needs to be considered in choice of design/optimization of suitable plasma source, i.e., whether to use „hot" plasma source of RNS or rather „cold" plasma source of ROS/RONS. These aspects need to be addressed and will be discussed in this contribution.

*This work was supported by the Czech Science Foundation (project No. 19-25026S).*

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# Microwave plasma torch treatment of *in vitro* plum (*Prunus domestica* L.) plants

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Microwave plasma torch generated in Argon is used for treatment of *in vitro* nodal segments of plum (*Prunus domestica* L. 'Kyustendiska sinya') plants. The aim of the study is to observe the effect of cold atmospheric plasma (CAP) treatment on the explants and the development of the plants obtained from them. The requirements to the plasma source for this treatment are low temperature, easy operation, low price and high concentration of active splashes in order to provide fast and efficient treatment. Two types of cold atmospheric plasma sources are used for treatment in this work: microwave plasma torch and underwater diaphragm discharge. Microwave plasma torch is the source fulfilling most of the requirement for treatment with its wide range of operating conditions. The plasma torch is produced by surfatron type wave launcher exciting electromagnetic wave travelling along the plasma–dielectric interface. Cold atmospheric plasmas have an effect on the treated objects by combination of UV radiation, MW EM field, electrons, ions and radicals together with the heating. The proper discharge conditions need to be chosen in order to provide stimulating not harmful effect on the explants. The plasma temperature in the chosen regime of operation does not exceed 40 degrees and during the treatment there is not thermal damage. The results of CAP treatment are compared to the development of non-treated explants. The development of the plants has been observed more than 2 years after treatment. Treatment on nodal segments of *in vitro* cultivated plum plants performed demonstrates that although *in vitro* plants are very delicate they can be treated with cold atmospheric plasma without being damaged. Plants, treated with plasma with suitably selected plasma parameters, not only survive the treatment but also develop better than the non-treated plants.

*This work was supported by COST Action CA19110 - Plasma applications for smart and sustainable agriculture.*

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# Comparative evaluation of different plasma reactors for PFAS degradation

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PFAS (Per- and PolyFluoro Alkyl Substances) are synthetic compounds which have been applied since the 1950s in the production of surfactants, lubricants, water-repellent textiles, fire-fighting foams and many other products of common use. They are characterized by high chemical and thermal stability and are thus very resistant to natural degradation processes. They are nowadays widely distributed in the environment and generate high concern due to their negative effects on human health [1]. Traditional methods for water treatment have proven to be ineffective in PFAS removal, so they are currently adsorbed on ion exchange resins or granular activated carbon. These processes, however, transfer the pollutant from water into a sorbent, which can be regenerated for a limited number of times, thus producing new wastes that need in turn to be properly treated and disposed of. Water treatment by means of plasma is instead capable to activate both highly efficient advanced oxidation and reduction processes, thus allowing to combine PFAS removal with their permanent destruction [2].

However, ozone and OH radical, which are generally among the most important reactive species in the case of air plasma applied to water treatment, do not react with perfluoroalkyl substances, because these compounds lack both C-H bonds and C-C $\pi$ bonds which are the typical sites for attack onto organic compounds by OH radicals and also ozone in the case of C-C $\pi$ bonds [3]. The reactive species which are instead able to attack PFAS are positive ions and free and hydrated electrons [4-6]. Moreover, long chain PFAS are characterized by surfactants properties: they accumulate on the water surface and are more effectively degraded as the contact between plasma and the liquid is more extended. On the contrary, short chain PFAS, which are present as water contaminants on their own but also form as products during the degradation of long-chain PFAS, have not these same surfactant properties and require different optimized experimental conditions. Considering these principles and the previous solutions reported in the literature, our group is investigating different electrodes shapes, configurations and positions to generate a plasma, in air or in argon, able to mineralize these recalcitrant compounds. In Figure 1 a Radial Plasma (RAP) discharge in argon is shown as an example. It is produced between a pointed edge high voltage tungsten electrode and a ring ground electrode partially submerged in the liquid, i.e. placed at the liquid/gas interface [7]. The results obtained with this and other plasma systems will be shown, compared and discussed.


Fig. 1. Photograph of RAP discharge in argon.

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# Atmospheric-pressure plasma for seed germination of lentils and mung bean and its effect on nutritional value

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Germinated seeds are foods that have traditionally been consumed in a large number of diets, and in recent years these products have acquired great importance due to the change in diet that is taking place in today's society. Mung bean (*Vigna radiate*) and Lentil (*Lens culinaris*) are important leguminous crops, widely cultivated and consumed, and rich in nutrients such as protein and iron. Many factors can result in a considerable loss in production, nutritional value and economic yield of these foods, especially microbial diseases and physiological deterioration. Various methods such as physical intervention technologies and chemical addition have been developed to achieve considerable improvement in agricultural efficiency. In these sense, different plasma-based technologies have been studied as an alternative to the existing techniques with ecologically safe, economical and effective ways to improve seed performance and crop yield. The present study aimed at exploring the effects of non-thermal atmospheric plasma on seed germination of lentils and mung bean, and on nutritional value of germinated seeds.

A novel large gap pin-to-plate nonthermal atmospheric plasma powered by an AC Supply (Leap100; PlasmaLeap Technologies) was used to treat lentils and mung bean seeds. It consists of an 88-pin stainless steel electrode, paired with a flat stainless-steel ground plate that has an electrode matched resonance frequency of 30-125 kHz, a discharge frequency of 50-3000 Hz with the power range from 50 to 400 W and a discharge gap of a maximum of 55 mm. The pins are arranged in a slightly convex manner, with pins being closest to the ground plate at the center at a distance of 40 mm with a gradual increase to 55 mm at the outer edge. The air gap between the pin electrode and the ground plate serves as the sample treatment area, with all seed samples in the current study being placed in the center. Seeds were treated at a fixed discharge voltage of 250 V, discharge frequency of 1000 Hz and duty cycle of 54 µs during an exposure time of 5 minutes. The study evaluated the germination rate of both seeds and the content of polyphenols, flavonoids, vitamin C, soluble and total nitrogen of sprouts.

Plasma-treated seeds showed a significant increase on germination rate compared with controls for both, lentils and mung bean seeds. The germination time was reduced by 20%, where the time to reach 100% germination was 10 and 14 h less for plasma-treated mung bean and lentils seeds, respectively. Additionally, plasma treatment did not affect the content of polyphenols, flavonoids, vitamin C, soluble and total nitrogen on germinated seeds.

According to the results obtained, non-thermal atmospheric plasma treatment of seeds contributes to stimulating their germination without greatly altering the nutritional value, and thus providing a green and effective mean to accelerate the growth cycle of seeds.

# Control of *Listeria innocua* biofilms on food industrial surfaces with plasma-based technologies

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Biofilms are considered an important source of microbial contamination in the food industry and they can contribute to the persistence of both pathogenic and spoilage bacteria. The effectiveness of multiple plasma-based innovative disinfection technologies was evaluated against biofilms of *Listeria innocua* developed on stainless steel surfaces for 6 days at 12 $^{o}$C, conditions that are especially relevant in the food industry. Thus, the biofilm-removal efficacy of direct atmospheric air plasma (AAP), plasma activated water (PAW) and the combination of PAW with airborne acoustic ultrasound (AAU) was tested. It was also evaluated the ability to reduce *L. innocua* biofilm formation of a previously developed coating applied with an atmospheric-pressure plasma jet system on stainless steel and whose anti-biofilm activity was based on preventing bacterial adherence to the surfaces through physico-chemical modifications[1]. PAW was generated by the activation for 15 min of 100 ml of sterile distilled water with a thermal plasma beam system (Diener electronic GmbH & Co.KG, Ebhausen, Germany) operating at 20 kHz with compressed air and a condenser connected to an external refrigerating system to maintain the activated gas at ambient temperature. The AAP treatments were performed with a multi-pin (11 x 8) plasma system (Leap100, PlasmaLeap Technologies, Dublin, Ireland) using atmospheric air at a fixed discharge voltage of 40 kV and varying the discharge frequency (500 and 1000 Hz) and exposure time (2, 3, 4, 5 and 8 min). For the combined treatment of AAU and PAW, the biofilms were first treated for 15 min with an airborne ultrasonic system (Pusonics S.L., Madrid, Spain) operated at a frequency of 26 kHz and energy density of 10 W/cm$^2$, and then exposed to PAW for 15 min.

The results showed that the highest inactivation efficacy was obtained with the combination of AAU and PAW, followed by PAW alone, with barely no inactivation observed with AAP. Biofilm exposition for 15 and 20 min to PAW immediately after its generation resulted in log reductions of 1.4 ± 0.2 and 2.44 ± 0.1, respectively. On the other hand, AAP treatment resulted in only 0.7 ± 0.1 log reductions after 5 min. Although the AAU treatment alone did not show antimicrobial effect against *L. innocua* biofilms, the combination with 15 min of PAW treatment resulted in a higher inactivation effect than the one obtained with PAW alone, showing reductions higher than 2.6 log units (detection limit of the method). Additionally, the anti-biofilm coating allowed a reduction of biofilm formation by 68% compared to the uncoated stainless steel.

The results contribute to the available knowledge on some of the multiple existing anti-biofilm plasma-based technologies, which will facilitate their transferability to the industry.

*This work was developed within the frame of a STSM supported by the COST ACTION 19110 - PlAgri.*

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# Direct and indirect effects of cold atmospheric plasma on green moulds

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*Penicillium digitatum*, one of the biggest postharvest pathogens of citrus fruits, causes an irreversible damage in the form of green progressively spreading moulds resulting in food deterioration and thus high economic losses. Moreover, common commercial methods of its control are limited to use of postharvest chemical fungicides which can lead to environmental and health issues and also to antifungal resistance [1]. Therefore, attempts to find suitable, sustainable and safe ways to provide food safety and to extend the shelf life of citrus fruits are of a great interest.

Cold atmospheric plasma (CAP) with its well-known antimicrobial nature is considered as a novel potential "greener" technology for fungi inactivation. Although the eukaryotic fungi are more resistant to antimicrobial agents than prokaryotic bacteria, several studies showed, that cold plasma treatments act rapidly in inactivation of moulds on various types of food such as grains, nuts, spices, fruits and vegetables, meat, etc. reviewed in Ding et al [2]. CAPs act on multiple levels in fungal inactivation by causing permeabilization of the cell wall leading to efflux of cytoplasm and thus leakage of organelles, DNA damage of spores or disruption of mycotoxins [3]. It was confirmed that ozone and UV radiation are not dominant plasma components responsible for the inactivation of *P. digitatum,* contrary to reactive oxygen species (O, OH, $O_2$) [4]. However, the mechanisms of CAP action and its components has not been still described in detail. Hashizume et al. investigated effects of oxygen radicals on inactivation of *P. digitatum* spores and reported oxidative degradation of organelles and cell wall, and decreased number of spores [5]. Guo et al. investigated the effect of plasma activated water (PAW) on kumquats infected by *Penicillium spp.* and showed 3.3 log reduction with no significant changes in colour, ascorbic acid content, total flavonoids, and total carotenoids and no serious nitrate and nitrite residues on their surface [6].

In this work, we used transient spark (TS) discharge for inactivation of spores of *P. digitatum*. TS discharge was used in two ways: 1) for a direct treatment of *P. digitatum* spore suspension and 2) for generating PAW used for indirect treatment of spore suspension. Different conditions of plasma treatment were tested, e. g. discharge power, treatment time, and carrier gas. PAW was tested for its pH, conductivity, oxidation-reduction potential and reactive oxygen and nitrogen species (RONS) content, i.e. concentration of $H_2O_2$, $NO_2^-$, and $NO_3^-$. Overall, we analyzed direct plasma treatment, post-treatment delay effect and indirect effect of plasma by PAW on spore suspension.

*This work was supported by Slovak Research and Development Agency grant APVV-17-0382* and *APVV-20-0566.*

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# AgriPlasma - Non-thermal air plasma treatment of multispecies swards seeds for reduction of greenhouse gas emissions

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The maintenance of grasslands for animal forage is essential in countries with large ruminant livestock herds. However, many grasslands are mono sward. These require high chemical fertilizer input for optimum yield as forage for ruminants, which contribute to high greenhouse gas emissions and methane profiles, which are significant contributors to climate change. The agricultural sector seeks alternative approaches that can reduce GHG emissions and reduce reliance on artificial Nitrogen application. Incorporating diversified and multi-species swards (MSS) in the grasslands for ruminant forage can reduce GHG emissions concurrently improve ruminant health. Moreover, MSS with diversified root systems can enhance soil nutrition. However, despite the potential advantages presented though use of MSS, the germination capacity and rate of the different grass, herb and legume constituents can present a challenge with mixed growth performance. Therefore, this AgriPlasma study focuses on developing a green and sustainable seed priming technology to address limitations and enhance the performance of multi sward species comprised of selected grass, herbs, and legume seeds. An atmospheric Dielectric Barrier Discharge (DBD) reactor was developed which uses air as its input. The plasma process parameters of treatment duration and input voltage were optimised against key seed performance parameters. The electrical characteristics and measurement of key reactive species of the generated plasma is presented. The germination capacity, rate, growth and modifications in the chemical and surface properties of the plasma-treated multi-sward species comprising six independently treated seed types, were determined as described in Los et al [1].

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# Growth of plasma-treated wheat seeds under realistic conditions

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For the field experiment, 4 different cultivars of wheat seed were used: Alixan, Sofru, Genius, and Nexera 88. Wheat seeds were treated uniformly without burning or blackening in RF industrial scale plasma reactor to investigate growth rate changes under realistic conditions. Each treatment was performed on a total of 3 kg of wheat seeds (each cultivar) under different plasma conditions: oxygen plasma in E-mode was used and discharge power and the treatment time varied. Five samples, each 3 kg of seeds, were treated differently: seeds without treatment, seeds treated with fungicide Redigo Pro, seeds treated with eco-layer *Bacillus subtilis* (0,5 % suspension) + macroalgae, seeds pretreated with plasma and then with *Bacillus subtilis* (0,5 % suspension) + macroalgae and seeds treated only with plasma. Fungicide Redigo Pro controls all main seed- and soil-borne wheat and barley diseases, whereas *Bacillus subtilis* is a soil-based probiotic, a commercial biological seed treatment that helps to protect the seeds from fungus, insects, and nematodes after planting.

The 3 kg of each sample was divided evenly into three replications, with 0.5 kg of seeds planted for each replication at two different locations across Slovenia. Germination, growth, and product yield were observed and monitored over the 2021 growing season. Overall, no statistically significant difference in the germination and growth of wheat seeds was found between control, different cultivars and any of the four treatments. This is likely due to the already near-100% germination rate of the wheat cultivars used in the study, the use of the fungicide Redigo Pro and *Bacillus subtilis* suspension. However, a slight difference in the yield of harvested wheat was found between the control, different cultivars and the 4 different treatments. This difference in yield could be due to the genetic predispositions of each cultivar to weather conditions, plasma treatment and many other possible reasons.

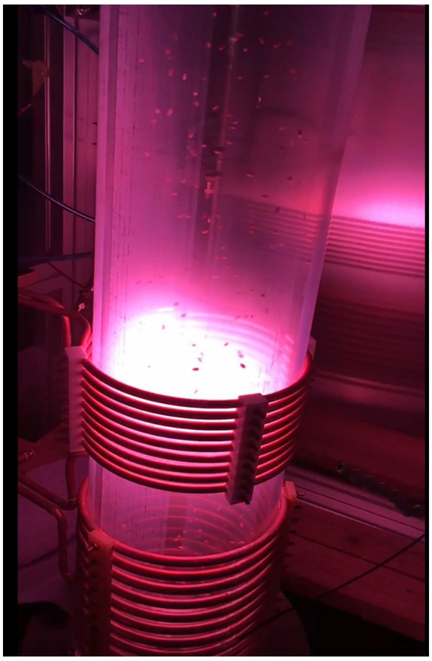

Fig. 1. Industrial-scale RF plasma reactor for the treatment of seeds.

*This work was supported by the Slovenian Ministry of Economic Development and Technology [grant number C2130-19-091009 (Plasma Grain)].*

# Changes of soil properties after plasma activated water application

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Plasma activated water (PAW) applications in agriculture deals into two mail directions. The first one is application on seeds with aim to improve their germination and early phase of groove. Less investigated is PAW application on plants as leave fertilizer and antibacterial/antifungal agent. PAW is applied by spraying on leaves [1] and some part also terminates on soil. However, effects on plants are generally positive, there is leak of knowledge what will happen with soil affected by PAW application. Soil itself is very complex system combining inorganic and organic compounds with huge number of living organisms and all of these components can be affected by PAW. Unfortunately, it is not known yet, if this effect is positive, neutral or negative. The aim of this work is extension of our recent study [2] on different soils.

13 different soils were selected for this comparative study. PAW was prepared from distilled water using DBD system with liquid electrode (details see in [2]). Water was applied on the soil samples surface twice per week (10 ml per dose to 90 g of the soil samples pressed by 5 atmospheres in Kopecky's rolls), whole experiment was designed for 8 weeks. Samples were kept in dark closed room without with no air flow. All was carried out in twelve replication divided in two groups; 6 for PAW and 6 for distilled water as reference.

Tap water absorption of three samples from each group was tested by saturation on the glass covered by a filter paper with continuous tap water delivery The absorption was measured at selected times by sample weighing. In next step, the fully water saturated soil samples were put on the dry filter paper (4 layers) to monitor water holding capability, this was measured at selected times by sample weighing.

Soil from the second group of samples was removed from the rolls individually, dried and stored at ambient laboratory conditions up to their processing. An amount of 10 g of the soil was shaken with 25 ml of boiled distilled water or with 25 ml of 1 M solution of potassium chloride for 1 minute. The colloid solution was kept in a closed vessel for 24 hours at laboratory temperature. After that, the solution was shaken again for 1 minute and solution pH was measured. The pH values measured using distilled water reflect free proton concentrations in the soil, while pH values measured using the KCl solution also reflect bounded protons in the sorption system of the soil.

The final part of experiments was focused on the long term PAW influence on the soil biological activity. Measurements were carried out in the special soil incubator for 6 weeks that relates to two years under the outdoor conditions. 50 g of dried soil ware saturated to 60% of their retention capability before incubation start.

The obtained results show that PAW application is strongly soil dependent and thus further experiments will be needed to be able generalize results about PAW application.

*This work was carried out under the COST Action CA19110 frame.*

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# Plasma treatment decreases the concentration of elements in pericarp of buckwheat grains

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Common buckwheat (*Fagopyrum esculentum*) is becoming a more and more popular crop as important food, rich in high-quality proteins, fibers, lipids, essential minerals, vitamins, and antioxidants. It is an excellent alternative as a gluten-free source of nutrition for gluten-intolerant people and people with celiac disease [1], [2].

Cold or non-thermal plasma (CP) technology is actively developing in the field of agriculture. It has great potential in grain decontamination and is a method for priming grains to improve plants' germination, yield, and/or stress resistance. Thus, the use of CP technology could benefit the production of popular crop species, such as buckwheat [3].

We investigated the impact of plasma treatment on pericarp morphology with scanning electron microscopy (SEM) and localization of elements in grains tissues (pericarp, aleurone, endosperm, and cotyledons) with proton-induced X-ray emission spectroscopy (PIXE). The impact of CP on grain germination, water contact angle and water uptake of seeds was also assessed.

The CP treatment of grains caused no visible morphological changes on the grain surface. However, a decrease in water contact angle, and increase in water uptake was detected. The speed of grain germination was slower compared to untreated seeds. However, the total number of germinated grains was similar to the control. The CP treatment caused a decrease in concentration of some elements in pericarp compared to untreated ones. Although statistically, there were no differences in potassium (K) concentration in pericarp, aleurone, and cotyledons, a trend of lower concentrations of K in tissues of CP treated samples was seen. The distribution of Mn in untreated buckwheat grains is presented in Figure 1.

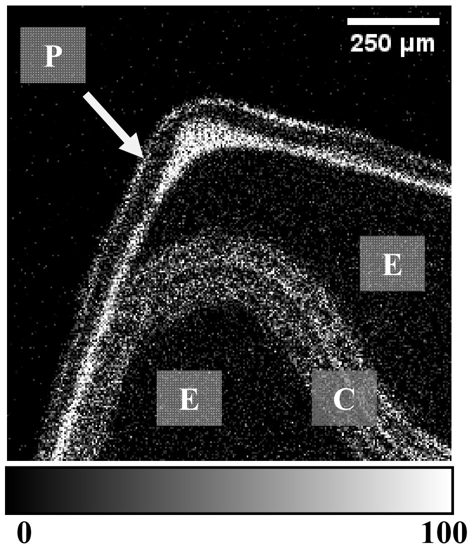


Fig. 1. Localisation of Mn in buckwheat grain cross-section. Different grain tissues are marked with letters: pericarp (P), Endosperm (E) and cotyledons (C). Color scale represents concentration of Mn in mg kg$^{-1}$ of dry weight.

*This work was supported by Slovenian Research Agency (ARRS): Young research grants (Pia Starič), program groups (P2-0082, P1-0112 and P1-0212) and project V4-2001.*

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# Bactericidal properties of aminoacids modified by plasma treatment in physiological buffered saline solutions

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Nowadays, cold atmospheric plasma in contact with liquid media have been utilized in different fields such as medicine, agriculture, food processing, wastewater treatment, etc. Bactericidal, cytotoxic, oxidative/reduction or nutrient properties of plasma treated (activated) liquids are given by the gas phase plasma supplied reactive species that induce the activity of these liquids and by the composition of these liquids. Typically, liquids from tap water, through different growth media to wastewater contain both inorganic components and organic biomolecules that are targets for the plasma induced reactive species and may affect the post-discharge properties of these solutions.

We investigated the chemical effects of cold plasma on the model solutions of aminoacids (AA) in physiological buffered saline solutions (PBS) and their bactericidal activity in post-plasma treatment time. We used the atmospheric-pressure COST reference plasma jet supplying O atoms that was shown to induce the formation of hypochlorites ($OCl^-$) by reaction of O atoms with chloride ions [1]. Such plasma activated PBS induced a strong bactericidal effect on *E. coli* bacteria due to formation of cytotoxic $HOCl/OCl^-$. While in the presence of AA in the solution led the formation of $OCl^-$ to the subsequent chlorination of AA following by the formation of organic chloramines. The bactericidal effect was at first hindered due to formation of non-toxic monochloramines. However, based on the type of the AA present in the solution the longer plasma treatment times or acidification of the solution (pH ~ 3) enhanced significantly the observed bactericidal effect. The toxicity of these plasma modified AA solutions was ascribed to the post-discharge chemical reactivity represented by the further formation of organic dichloramines and their decay while producing cytotoxic intermediates [2].

This study represents another step to the understanding of the effects of cold plasma on the behavior of the plasma-liquid systems with the more complex solution.

*This work was supported by the Czech Science Foundation (GA19-25026S).*

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# Air plasma exposure of *Tagetes erecta* seeds: Gas phase FTIR monitoring and effects on germination dynamics

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Plasma exposure of seeds, plants, soil and water gains increasing attention in agriculture and plant biology, as a new technique to modify the germination rate or the development and productivity of plants [1]. A joint effort across the Europe and broader, in the form of a recent action from the European Cooperation in Science and Technology (COST), CA19110 - Plasma applications for smart and sustainable agriculture, is focused to study the use of plasmas for treating food and packaging, combining expertise from multiple research groups: physics, chemistry, botanic, biology, agriculture, engineering and food technology. The understanding of plasma assisted generation of reactive oxygen and nitrogen species (RONS) is an essential component in controlling the reproducibility of biological effects [2].

In this respect, we describe here some diagnostic and monitoring tools applied in the study of atmospheric pressure air plasma during exposure of *Tagetes erecta* seeds. The seeds were hosted by a glass petri dish, which plays also the role of the dielectric in a dielectric barrier discharge (DBD) setup, 5 mm discharge gap. A large disc electrode and a mesh electrode are connected to an AC power supply at 50 Hz and 13 - 16 kV peak-to-peak amplitude. A pulsed plasma is generated in air at atmospheric pressure, the treatment duration being 2 or 5 minutes. The discharge is feed with dry air at constant flow rate and then the exhaust gas is passed through a single pass gas cell. A Fourier-transform infrared spectroscope (FTIR, Jasco 4700) technique was used to acquire spectra in the range 4000 $cm^{-1}$ – 800 $cm^{-1}$, integrated over 2 minutes, before, during and after plasma exposure. The following reactive species were identified: $HNO_2$, $N_2O$, $N_2O$ overlapped with $HNO_2$ and $O_3$. The absorbance values and the observed differences for all treated seeds lots are discussed in this work. Moreover electrical and optical diagnosis of air plasma is used in order to determine the histograms of discharge current intensity, as well vibrational and rotational temperatures. Immediately after plasma exposure, the seed were placed in Petri dishes (25 seeds/plate) and controlled volumes of distilled water, 0.1 M and 0.05 M NaCl solutions respectively are added, at 22 °C, in a light incubator. After 6 days of observation, specific germination dynamics parameters were calculated.

Plasma monitoring in real time, applied for multiple parameters, during exposure of seeds represents a strong approach to improve the reproducibility and to control the biological effects. Our preliminary observations emphasize the positive effect of the atmospheric pressure plasma on *Tagetes erecta* seeds germination capacity in both saline and non-saline solutions.

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# GAD impact on selected properties of bread

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The growing demands of consumers regarding the quality and safety of consumed products create a demand for new techniques in the food industry. One of them is plasma treatment. It can effectively inactivate a wide range of microorganisms, including spores and viruses. The effect of plasma on various microorganisms can be selective, which means that the plasma can damage only selected pathogenic microorganisms without damaging the host, or it can activate different metabolic pathways in different organisms.

In recent years, many types of non-thermal plasma at atmospheric pressure have emerged, including corona discharge, micro-hollow cathode discharge, sliding arc discharge (GAD), single-atmosphere glow discharge, dielectric barrier discharge (DBD), plasma spraying, plasma needle , and an atmospheric pressure plasma jet (APPJ) for biodecontamination of contact surfaces with food and biological material, as well as the food itself, from various bacteria and molds. This paper presents the results of tests carried out with the use of the GAD reactor. On their basis, the influence of low-temperature plasma on the properties of bread was assessed.

Cold plasma is a new technology that has attracted the attention of scientists around the world. It was originally developed, inter alia, in order to improve the adhesive properties of polymers and to introduce innovations in various fields of electronics. In the last decade, cold plasma has also found application as a tool for the non-thermal processing of food products.

The GAD plasma reactor is a compact and flexible source of non-thermal plasma. Stable operation of the GAD plasma projector can be achieved through careful configuration of the power system. The generated plasma approaches the equilibrium state due to an appropriately optimized current value, and the gas temperatures with active ingredients can be lowered to a selected limit value depending on the selected application by controlling the gas flow rate. The biggest advantage of this type of reactor over other designs is the possibility of working under atmospheric pressure while maintaining a relatively low temperature and a wide work zone with many types of substrate gases and their mixtures. Miniaturized GAD does not require the addition of expensive noble gases such as helium or argon.

GAD is preferred because of its low equipment and operating costs, high efficiency in deactivating various microorganisms, and working under atmospheric pressure with low surface gas flow, which helps to increase plasma efficiency. In addition, cold plasma generates a number of active species including radicals, ions, excited molecules and ultraviolet (UV) photons that play a key role in the microbial inactivation process.

# Long term effects in dwarf bearded iris (*Iris reichenbachii* Heuff.) calli metabolism induced by plasma treatment

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The Plasma Agriculture is a new field of plasma applications where non-thermal (cold) plasmas (NTPs) operating at atmospheric pressure are used as a tool in biotechnology for genetic manipulation of plants, for micropropagation, for studies of plant metabolism and cellular development or a commercial production of natural products that cannot be chemically synthesized. NTPs have rich chemistry of Reactive Oxygen and Nitrogen Species (RONS) that are responsible for triggering various mechanisms and effects in plant cells, such as the induction of somatic embryogenesis, higher and faster seed germination, better water uptake or have an anti-bacterial and anti-viral effects, etc. [1-3]. In the current study plant undifferentiated compact tissue (calli) of Balkan endemic dwarf bearded iris (*Iris reichenbachii* Heuff.) was treated using a RF plasma needle device operating with He as a working gas. The flow of He was kept constant at 1 slm and the power deposited to the plasma was below 2 W. The plasma needle was positioned 3 mm above the callus surface enabling direct contact between the active plasma volume and the surface of the sample. We induced significant morphological alterations in structure of non-embryonic calli that could be attributed to the enhanced cell division of the plant cells at the surface of the calli that was in contact with plasma. The differentiation of the calli cells was stimulated by reactive species created in gas phase of NTP. The morphological changes were then followed by the significant long term alteration in specialized metabolite content in derived calli types. Our results implicate that direct plasma treatment could serve as a significant elicitor of the production of specific metabolites in dwarf bearded iris calli.

*Supported by Ministry of Education, Science and Technological Developments of the Republic of Serbia, contact number grants 451-03-68/2022-14/200007 and 451-03-68/2022-14/200024. This publication is based upon work from COST Action CA19110 - PlAgri, supported by COST (European Cooperation in Science and Technology-www.cost.eu).*

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# CESPC-9

| TIME | MON (Sep 5) | TUE (Sep 6) | WED (Sep 7) |
|---|---|---|---|
| 8 15 | **Opening ceremony** | | |
| 8 30 | **D. Lacoste** | **G. Primc** | **P. Lukeš** |
| 9 05 | T. Nitsche | Z. Kelar Tučeková | N. Škoro |
| 9 25 | C. Ndayirinde | R. Menthéour | M. Schmidt |
| 9 45 | C. Verheyen | J. Khun | A. Sainz García |
| 10 05 | Coffee break | Coffee break | Coffee break |
| 10 30 | **E. Marotta** | **A. Bogaerts** | **B. Mitu** |
| 11 05 | Z. Machala | M. Becerra | I. Topala |
| 11 25 | J. Pawłat | R. Cimerman | K. Sgonina |
| 11 45 | T. Nishime | T. Vazquez | Lunch |
| 12 05 | Lunch | Lunch | |
| 13 00 | | | Excursion |
| 13 30 | **C. Canal** | **T. Homola** | |
| 14 05 | K. Wende | M. Mozetič | |
| 14 25 | F. Tampieri | M. Gherardi | |
| 14 45 | S. Kyzek | S. Sihelník | |
| 15 05 | Coffee break | Coffee break | |
| 15 30 | **Š. Matejčík** | **H. Kersten** | |
| 16 05 | K. Korytchenko | S. Van Alphen | |
| 16 25 | | E. Morais | |
| | | | |
| 18 30 | Dinner | Dinner | |
| 19 30 | | | Gala dinner |
| 20 00 | | **Poster session** | |

# PlAgri

| TIME | THU (Sep 8) | FRI (Sep 9) |
|---|---|---|
| 8 20 | **Opening ceremony** | |
| 8 30 | **Intro 1** | **Intro 4** |
| 8 45 | **T. Vukušić-Pavičić** | **S. Di Lonardo** |
| 9 15 | **F. Alba-Elías** | F. Krčma |
| 9 30 | | R. Žūkienė |
| 9 45 | F. Capelli | K. Hensel |
| 10 00 | K. De Baerdemaeker | S. Bousselmi |
| 10 15 | Coffee break | Coffee break |
| 10 35 | **Intro 2** | **M. Milutinović** |
| 10 50 | **C. Tizaoui** | |
| 11 05 | | K. Koga |
| 11 20 | L. Čechová | L. Marcinauskas |
| 11 35 | V. Scholtz | E. Hnatiuc |
| 11 50 | J. Čech | **Closing ceremony** |
| 12 05 | Lunch | |
| 13 30 | **R. Ingels** | |
| 14 00 | E. Vervloessem | |
| 14 15 | K. Kutasi | |
| 14 30 | A. Filipić | |
| 14 45 | R. A. Bisag | |
| 15 00 | Coffee break | |
| 15 20 | **Intro 3** | |
| 15 35 | **B. Šerá** | |
| 16 05 | **P. Attri** | |
| 16 35 | J. August | |
| 16 50 | P. Marinova | |
| 17 05 | V. Mildažienė | |
| | | |
| 18 30 | Dinner | |
| 20 00 | **Poster session** | |