3rd TRAINING SCHOOL PIAGRI COST ACTION CA19110

„Plasma applications for smart and sustainable agriculture“

PLASMAS FOR PLANT AND FOOD PROCESSING

11-14 June 2024, Faculty of Natural Sciences, Vytautas Magnus university, Kaunas, Lithuania

COST European Cooperation in Science & Technology · CA19110 · Vytautas Magnus University · Lithuanian Energy Institute · Lithuanian Research Centre for Agriculture and Forestry

PROGRAM

🛠 - practical; 🚜 - field

Tuesday, June 11	**Session 1: Plasma /*auditorium 159*/**	
9:00-10:00	**Registration**	
10:00-10:15	**Opening TS3**	
10:15-11:15	Achim von Keudell Ruhr University Bochum, DE	Introduction to plasma science for life science application
11:15-11:45	**Break**	
11:45-12:30	Liutauras Marcinauskas Lithuanian Energy Institute; Kaunas University of Technology, LT	Diagnostics of reactive oxygen and nitrogen species generated by plasma sources
12:30-13:30	**Lunch break**	
13:30-14:15	Achim von Keudell Ruhr University Bochum, DE	Chemistry in plasma treated liquids
14:15-14:45	**Break**	
14:45-16:45	**Poster session /*the foyer to the auditorium 159*/**	
17:00-19:00	Transportation by bus from campus to VMU Botanical Garden. Social program and excursions. (19:30 - transportation by bus to city center)	
Wednesday, June 12	**Session 2: Seeds and plants /*auditorium 159*/**	
9:00-9:15	***Registration***	
9:15-10:15	Christophe Bailly Sorbonne University, FR	Main issues of seed biology in a changing world
10:15-11:00	Christophe Bailly Sorbonne University, FR	Advances and prospects in the understanding of seed germination and dormancy
11:00-11:30	**Break**	
11:30-12:15	Vida Mildažienė Vytautas Magnus University, LT	Do plasma effects on seed germination predict effects on plant growth and productivity?
12:15-13:30	**Lunch break**	
13:30-15:30	Jūratė Žaltauskaitė, Rasa Žūkienė, Vida Mildažienė Vytautas Magnus University, LT	🛠Estimation of plant stress response ***Laboratories 158 and 160***
15:30-16:00	**Break**	
16:00-18:00	Aušra Marcinkevičienė Vytautas Magnus University, LT	🚜Experimental Field studies. ***Agricultural experiment station, Rapsų str. 7, room 302***
Thursday, June 13	**Session 3: Plasma-plants-microorganisms /*auditorium 159*/**	
9:00-9:15	***Registration***	
9:15-10:15	Jure Mravlje	The biology of plant-microbial interactions

	University of Ljubljana, SI	
10:15-11:00	Jure Mravlje University of Ljubljana, SI	A brief overview of decontamination of buckwheat grains using cold plasma
11:00-11:30	**Break**	
11:30-12:30	Danas Baniulis Lithuanian Research Centre for Agriculture and Forestry, LT	Effect of cold plasma treatment on seed microbiome and plant-microbial interactions
12:30-13:30	**Lunch break**	
	Laboratory and field practicals	
13:30-14:15	Rasa Žūkienė Vytautas Magnus University, LT	Plan your plant experiment ***Room 431***
14:15-15:30	Liutauras Marcinauskas Lithuanian Energy Institute; Kaunas University of Technology, LT Lina Ragelienė**,** Vytautas Magnus University, Kaunas, LT	PAW generation and analysis of chemical composition ***Laboratories 513 and 517***
15:30-16:00	Transportation to forest plant nursery in Jonava district (refreshments in the bus)	
16:00-18:00	Vida Mildažienė Vytautas Magnus University, Kaunas, LT	How persistent are effects of seed treatments on plant growth? Excursion to Norway spruce collection
Friday, June 14	**Session 4: Plasma for food /*auditorium 159/***	
9:00-9:15	***Registration***	
9:15-10:15	Silvia Tappi University of Bologna, IT	Cold plasma application for food stability and functionality
10:15-11:00	Silvia Tappi University of Bologna, IT	Application of cold plasma for the functionalization of food ingredients
11:00-11:45	Rasa Žūkienė Vytautas Magnus University, Kaunas, Lithuania	Cold plasma-induced changes in *Stevia rebaudiana* growth, content of steviosides and phenolic compounds
11:45-12:30	**Break**	
12:30-13:30	**CLOSING REMARKS**	

ABSTRACTS

3rd PlAgri Training School CA19110

Plasma applications for smart and sustainable agriculture

Abstracts are positioned in alphabetic order by the surname of the presenting author.
The number of abstract means poster number in the poster session.

1. The use of atmospheric pressure plasmas for the control of antibioticresistant bacteria in the agri-food sector

***Z. Aliyeva*, T. Maho, P. Guillot, C. Muja**
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Bacteria with antibiotic resistance have evolved the capacity to withstand antibiotics, leading to the ineffectiveness of these drugs against them. In the treatment of bacterial infections, antibiotics target specific bacterial structures or functions, either eliminating the bacteria or inhibiting their growth. However, as some bacteria can acquire resistance through genetic mutations or by obtaining genes from other sources, the food industry faces a significant concern about potential consumer exposure to antibiotic-resistant bacteria through contaminated food, posing challenges for human health. Freshly cut raw fruits, particularly leafy greens, pose an elevated food safety risk due to potential microbial contamination, including antibiotic resistance genes.[1] This study aimed to explore the effectiveness of atmospheric pressure plasma in biologically decontaminating food, with a specific focus on its impact on antibiotic-resistant bacteria (ARB). In this research, I use atmospheric pressure plasma, specifically employing a Dielectric Barrier Discharge (DBD). Cold plasma technology is a promising environmentally friendly solution for decontamination in the agri-food industry.

This study explores the potential of atmospheric pressure plasma for decontaminating leafy vegetables, focusing on its impact on antibiotic-resistant bacteria (ARB). The goal is to assess the effectiveness of plasma in mitigating ARB contamination on leafy greens.
This study utilized salad and spinach as models for leafy greens, investigating their native bacterial communities through classical microbiology techniques. Antibiotic resistance profiles were characterized using both microbiological and molecular methods. The impact of DBD plasma treatment on bacterial load and antibiotic resistance was assessed in a closed reaction chamber, employing classical microbiological methods and molecular techniques in treated and untreated samples.

Acknowledgement: This work was supported by the Occitanie Region, France.

References

[1] G. A. Francis, A. Gallone, G. J. Nychas, J. N. Sofos, G. Colelli, M. L. Amodio, and G. Spano, Journal, Volume 52, 595-610 (2012).

2. Effects of low-pressure and dielectric barrier discharge plasma on seed germination, morphology, and amount of rebaudioside A in *Stevia rebaudiana* plants

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Stevia rebaudiana, also known as sweet stevia, is a perennial entomophilous plant belonging to the Asteraceae family. It is widely recognized for its diterpene glycosides, known as steviol glycosides (SGs). Among these, stevioside (Stev) and rebaudioside A (RebA) are the most abundant, together accounting for 90% of all SGs found in sweet stevia leaves. Since SGs are naturally occurring and require minimal amounts to achieve a sweet flavor (being 100–300 times sweeter than sugar), they serve as an excellent organic, calorie-free alternative to synthetic sweeteners. The flavor attributes of stevioside are inferior to those of rebaudioside A. Therefore, one of the main goals of this study was to increase the amount of RebA relative to Stev. To achieve this, seeds were treated with plasma generated by dielectric barrier discharge (DBD) and capacitively coupled (CC) systems for 2, 5, and 7 minutes before sowing. Prior studies from our group have shown that cold plasma (CP) treatment can increase germination, improve plant growth, enhance plant yield, increase SGs production in stevia plants, and reduce the need for antifungals and other chemicals [1, 2]. CP was chosen for its ability to induce plasma chemical reactions on the seed surface while being less damaging to heat-sensitive compounds. The positive effects of CP on seed germination and other physiological processes in growing plants are associated with generated reactive nitrogen species (RNS) and reactive oxygen species (ROS), and in the presence of water, superoxide anions, hydrogen peroxide, hydroxyl radicals, and peroxynitrite anions. These radicals function as signaling molecules in eukaryotic cells. By treating Stevia rebaudiana seeds with CP, we successfully achieved higher RebA concentrations in the Shug 6-A3 cultivar, but we did not achieve similar results in the Shug High A3 cultivar. We observed a statistically significant CP effect in the DBD 2 group, where RebA concentration increased by 81.5%. Surprisingly, in the DBD 5 group (same Shug A-3 cultivar), Stev increased by 189.1%. Other groups in both cultivars showed a tendency to increase Stev while decreasing RebA. Seed germination, both in vivo and in vitro, did not show significant improvement. It is notable that sweet stevia's germination rate is low even in the control group, where it can reach a maximum of 20% in the best cases. Morphological tests showed no significant changes in the mass of leaves and plant height, although a statistically smaller number of leaves and stem nodes were observed in the Shug A-3 DBD 5 group.

References:

[1] Judickaitė, A., Lyushkevich, V., Filatova, I., Mildažienė, V., & Žūkienė, R. (2022). The potential of cold plasma and electromagnetic field as stimulators of natural sweeteners biosynthesis in stevia rebaudiana bertoni. Plants, 11(5), 611.
[2] Pauzaite, G., Malakauskiene, A., Nauciene, Z., Zukiene, R., Filatova, I., Lyushkevich, V., ... & Mildaziene, V. (2018). Changes in Norway spruce germination and growth induced by pre-sowing seed treatment with cold plasma and electromagnetic field: Short-term versus long-term effects. Plasma processes and polymers, 15(2), 1700068.

3. Enhancing Food Safety and Longevity through Plasma-Deposited Functional Coatings on Packaging Materials

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The global food industry faces a significant challenge in balancing food safety and shelf-life extension while minimizing waste. Innovative solutions are essential to address these issues simultaneously, especially as food waste contributes to environmental degradation and economic losses. Functional packaging emerges as a promising approach, offering a way to enhance food preservation and safety without compromising quality. By integrating advanced materials and technologies, such as low-temperature plasma (LTP) processes, functional packaging can play a pivotal role in mitigating food waste, efforts which will further progress the deployment of plasma technology across the food industry. My research project focuses on the use of plasma technology to deposit functional coatings on food packaging materials.

In my first PhD year, I have successfully designed and constructed a dielectric barrier discharge (DBD) plasma system capable of polymerizing thin-film food packaging materials (Fig. 1). Additionally, I have developed a plasma system for generating nanoparticles, paving the way for antimicrobial coatings. These advancements demonstrate theapplicability of plasma processes in creating innovative packaging solutions to enhance food safety and preservation.

The choice of possible precursors is wide ranging and is tailored to minimize both environmental burden and detriment to consumers. Plasma polymerized hexamethyldisiloxane (HMDSO) shows great promise in producing uniform coatings with good adherence to many conventionally used packaging materials. My preliminary results using Raman scattering to assess the nature and uniformity of plasma deposited polymeric films has shown excellent promise, Fig. 2.

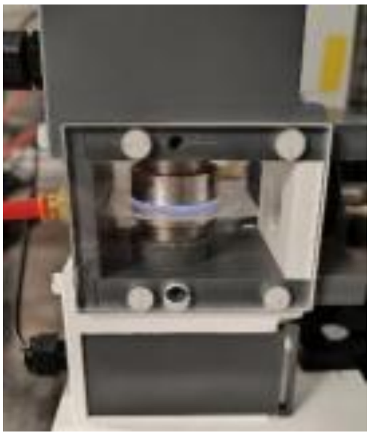

Fig. 1. Image of developed DBS system.

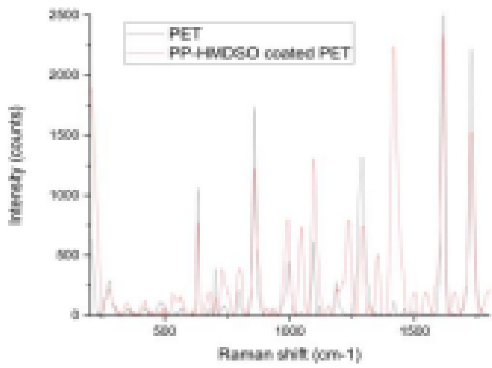

Fig. 2. Raman spectrum of HMDSO coated and uncoated PET substrate

Moving forward, my research will focus on synthesizing a range of functional coatings and assessing their efficacy in extending the shelf life and enhancing the safety of packaged foods. This will be achieved by generating functional nanoparticles (e.g. Ag) and embedding them within a deposited polymer matrix. To fully understand and optimize the underlying physical and chemical processes various plasma diagnostics (e.g. Optical Emission Spectroscopy (OES), Laser Induced Fluorescence) will be combined synergistically with surface analysis techniques. Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and X-ray Photoelectron

Spectroscopy (XPS) will be used to link the structural and compositional attributes of the deposited coatings to fundamental plasma parameters such as electron density and radical concentration. Ultimately, the antimicrobial properties of the coatings will be evaluated, aiming to establish a direct correlation between plasma-engineered materials and their performance in food preservation.

4. Plasma Activated Water Effect on Growth of *Cannabis sativa* L. Under Heavy Metal Contamination

Ludmila Čechová[1,2], Daniel Holub[2,3], Lucie Šimoníková[4], Pavlína Modlitbová[2], Karel Novotný[4], Pavel Pořízka[2,3], Zdenka Kozáková[1], František Krčma[1], Jozef Kaiser[2,3]

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Contamination of the environment with heavy metals such as cadmium or lead is a worldwide issue. Phytoremediation, or bioremediation is an environmentally friendly way of soil decontamination by means of planting plants which have hyperaccumulating properties. This means that they accumulate toxic metals mainly in their leaves and roots and have higher tolerance to the toxic effects of heavy metals [1]. Non-thermal plasma-based technologies may be a helpful tool in the process of phytoremediation. Plasma activated water (PAW), prepared by a contact of gaseous plasma with water, contains reactive oxygen and nitrogen species, which enhance the growth of plants [2].

One of the plants with a potential for phytoremediation is *Cannabis sativa* L. In a short-term toxicity test, *C. sativa* was grown in a medium which consisted of PAW and different concentrations of cadmium or lead. The dielectric barrier discharge with a liquid electrode (generating the non-thermal plasma above the water surface) was used to prepare PAW. Concentrations of reactive oxygen and nitrogen species (RONS) such as hydrogen peroxide, nitrates and nitrites were determined colorimetrically by the UV-VIS spectrometry. It was found that PAW enhanced the growth of *C. sativa*, especially in the case of lead contamination. Furthermore, the bioaccumulation and distribution of these metals as well as the influence on the nutrient composition of plants was studied by the Laser-induced breakdown spectroscopy (LIBS). LIBS is a multi-elemental analysis technique capable of analysing a broad range of elements. Its biggest advantage is the possibility of fast bioimaging and determination of the exact distribution of elements [3]. The data were processed statistically and in the form of elemental maps. It showed that PAW did not have an effect on bioaccumulation properties of *C. sativa* and that cadmium bioaccumulation disturbed distribution of magnesium. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used as a complementary method to determine the exact concentration of elements both in plants and solutions.

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[2] Puač, N., Gherardi, M., Shiratani, M. (2018). *Plasma Processes Polym.* 15, 1700174. doi: 10.1002/ppap.201700174

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5. The impact of *Pinus sylvestris* seed treatment with low-temperature plasma on content of phenolics, flavonoids and activities of antioxidant enzymes in seedlings of different half-sib families

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In my PhD studies, I am investigating how pre-sowing seed treatment with physical stressors (low-temperature plasma and electromagnetic field) change the accumulation of biologically active compounds in different half-sib families of Norway spruce and Silver birch needles/leaves [1-3]. However, abiotic and biotic factors are expected to increase the frequency of many stressors for trees in the future due to climate change. Biologically active compounds, such as secondary metabolites, antioxidant enzymes, photosynthesis pigments, and sugars, play an essential role in plant defense mechanisms, in order to reduce the damage of biotic and abiotic stress.

We hypothesized that the application of innovative technologies, including both physical methods (seed treatment with low-temperature plasma and electromagnetic field) and genetic approaches (trees genetic selection), could increase trees resistance to stress. To determine this, we assessed the total phenolic content, total flavonoid content, chlorophyll a, chlorophyll b, carotenoids, sugars, and the activity of antioxidant enzymes, such as catalase, peroxidase, ascorbate peroxidase, glutathione reductase, superoxide dismutase activity in different half-sib families of Norway spruce and Silver birch at one and two years old age. All analyses performer using spectrophotometric method. Our studies revealed that trees exhibit a dual response to seed treatment. In some half-sib families, defense mechanisms activated through growth parameters, including increased height parameters, photosynthesis pigments and sugars content. Conversely, in other half-sib families defense mechanisms are activated through the antioxidant system: increased activity of antioxidant enzymes. It is expected that seed treatment with low-temperature plasma and electromagnetic field can be used as a tool to enhance the accumulation and activity of biologically active compounds in the needles/leaves. This could impact the plants' defense against both biotic and abiotic stressors in the future. However, aside from multifactorial and systematic changes occurring at different levels of structural organization (including proteomic and metabolomic), a strong dependency on trees genetic properties is significant.

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3. **Lučinskaitė I.,** Laužikė K., Žiauka J., Baliuckas V., Čėsna V., Sirgedaitė-Šėžienė V. 2021. Assessment of biologically active compounds, organic acids and antioxidant activity in needle extracts of different Norway spruce (*Picea abies* (L.) H. Karst) half-sib families. Wood Science and Technology, 55: 1221–1235.

6. Degradation mechanism of micropollutants in water by O atom produced by atmospheric pressure He/O2 plasma jet

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The presence of micropollutants in water bodies poses a threat to aquatic organisms, plants, microorganisms and humans. Nonthermal plasma-based advanced oxidation process (AOP) is a promising technology for effectively removing hazardous micropollutants from water. The potential of the O atom, produced during this process, was investigated in this work. An atmospheric pressure plasma jet (APPJ) was employed to treat the water containing 4 different organic micropollutants (atrazine, carbamazepine, 1,7-α-ethinylestradiol and bisphenol A), and their degradation efficiencies treated by APPJ under different experimental conditions were determined. By manipulating the composition of the carrier gas, it was possible to achieve different exposures of reactive oxygen species (ROS, O atom and O3) under the different experimental conditions. The degradation efficiencies of the four micropollutants after treatment under conditions A (feed gas: 0.6% of O2 in He) and B (feed gas: 1.5% of O2 in He) were 95-99% and 55-80%, respectively, after 480 s of treatment. The degradation of micropollutants was mainly attributed to the O atom, and the contribution of O3 was very limited. A comprehensive investigation was conducted to study the degradation pathways of atrazine (ATZ) induced by O atom, being the most recalcitrant micropollutant in this study. The ATZ degradation mainly occurred on its branched chains and the two primary degradation routes triggered by the O atom were dealkylation and dechlorination. After APPJ treatment, the toxicity of ATZ contaminated water was reduced by 6-10 times. This work elucidates the reaction mechanism between O atom and micropollutants (including reactive species contents, reaction rate constants, degradation pathways, etc.), thus increasing the understanding of the degradation of micropollutants by O atom during the AOP.

7. Non-Thermal Atmospheric Plasma: a cutting-edge technology for the valorisation of food industry by-products through the production of biofertilizers

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Department of Food Hygiene and Technology, University of León, 24071, León, Spain

Food waste management presents a substantial global challenge, with up to 20% of the food available in the European Union being wasted. Approximately 24% of this waste occurs during the food processing and manufacturing phase. The waste generated as by-products in the food industry carries substantial potential for valorization due to its nutrient-rich composition. This

contributes to the circular economy approach, where valuable materials, previously labeled as waste, are reintegrated into the supply chain to create new products. Traditionally, nitrogen fixation, essential for agricultural productivity, has relied on energy-intensive industrial methods such as the Haber-Bosch and Ostwald processes, resulting in notable CO_2 emissions. While the Birkeland-Eyde process, utilizing arc plasma, provided an alternative, its energy efficiency remained moderate. Recent progress in non-thermal atmospheric plasma technology (NTAP) holds potential for efficient and localized nitrogen fixation, particularly significant in agricultural environments. The main goal of this research is to assess the feasibility of using NTAP to convert waste from vegetable food processing industries, particularly from tubers such as beets and potatoes, into high value biofertilizers. Additionally, the study seeks to evaluate the microbiological risks associated with using waste from plant-based food processing as organic fertilizers.

The research will firstly focus on identifying and characterizing various wastes and by products from the processing of red beet and potato for their transformation and valorization through sustainable processes to obtain biofertilizers. The principles of the food waste hierarchy will be employed to select the most suitable wastes and valorization processes. To achieve this, we will conduct microbiological and physicochemical characterization of diverse by-products from both the red beet and potato processing industries. Following the initial characterization, the assessment of NTAP technologies serving as an innovative process for nitrogen fixation, facilitating the production of biofertilizers with higher ammonia and nitrate content will be conducted. This approach will be tested employing different NTAP configurations, including bubble, frit, and catalyst reactors, operating at 180 V, 54 µS, 1500 Hz, for 30 minutes, using air as the working gas at a flow rate of 0.8 L/min. Once the optimal NTAP treatment is defined, the impact of the generated biofertilizers on seed germination capacity and plant growth will be assessed through pot or greenhouse experiments. The assessment of the microbial inactivation capacity of the evaluated NTAP treatment for biofertilizer production and the stability of the resulting products is of paramount importance to ensure their quality and safety. In this research, the primary biological hazards associated with the reuse of the studied waste as biofertilizers will be identified by conducting a qualitative risk assessment regarding its potential impact on public health. This study will integrate data derived from microbiological characterization of waste (by-products) and the final organic fertilizers along with information gathered from a systematic review of existing literature.

After conducting an initial investigation on liquid waste streams originating from potato processing industries, upon applying plasma treatment using the catalyst reactor resulted in an increase in the pH values and oxidation-reduction potential; however, no changes were detected for the electrical conductivity. On the other hand, there was a significant increase in the concentration of the various ions studied compared to their presence in the liquid before treatment. This increase was particularly remarkable in the case of nitrites, with an 80-fold increase, and in the case of nitrates, with a four-fold increase.

It can be anticipated that NTAP treatments will yield favourable outcomes, resulting in elevated levels of nitrates and nitrites, alongside other essential components, thereby enhancing the fertilizing properties of the product. This research aims to elucidate the effectiveness and feasibility of employing NTAP to valorise liquid waste streams from the food industry. By offering a more sustainable and energy-efficient method for nitrogen fixation, this technology has the potential to significantly improve food waste management and fostering sustainable agricultural practices.

8. Non-thermal atmospheric plasma: A possible solution for water scarcity in fresh produce industry

***Francés, Á.**, Oliveira, M., González-Raurich, M., Prieto, M., Álvarez-Ordóñez, A·, López, M.*

Department of Food Hygiene and Technology, University of León, 24071, León, Spain

Nowadays, food systems are facing a serious problem of water scarcity, particularly in Southern European countries, where water shortages are expected to increase in the near future. The food industry largely depends on water, and in most cases drinking water, the latter representing 75% of the water consumed in this sector in the European Union. Fresh water is particularly critical for the maintenance and disinfection of processing areas, as well as in many processing operations.

In the production of fresh produce, significant amounts of water are used across various processing stages, especially during washing. Consequently, this process generates considerable volumes of wastewater, presenting an intriguing opportunity for its reuse. In recent years, there has been a noticeable search for innovative and sustainable technologies for water reconditioning with the objective to enable the reuse of water in the processing operations, thereby leading to a reduction in overall water consumption. The aim of this study is to assess the efficacy of non thermal atmospheric plasma (NTAP) in disinfecting microbial contaminants present in fresh-cut produce washing wastewater. Additionally, the study seeks to evaluate NTAP's capability in removing pesticides and other chemical contaminants that may have accumulated in the water. The ultimate goal is to enable the safe recirculation of this treated water within the washing operation or other processes, thereby mitigating the risk of cross-contamination in fresh produce.

In this research, specialized equipment designed to replicate the real conditions of an industrial fresh produce washing tank will be used. During washing, the water will be circulated through a plasma bubble spark discharge reactor (using several combinations of voltage and discharge frequency) with a defined flow rate, subsequently, the treated water will be reintroduced into the processing tank. Before and after NTAP treatment, we will carry out a microbiological and chemical evaluation, including the measurement of physico-chemical properties (pH, oxidation reduction-potential and electrical conductivity). Once the optimal NTAP treatment is defined, the impact of the water reuse on the organoleptic characteristics and nutritional value of fresh-cut produce will be evaluated for its potential application in real industrial conditions.

Preliminary results showed that NTAP generated at 1600 Hz and 175 V efficiently reduced artificially inoculated *Listeria monocytogenes* – one of the most relevant bacterial hazards associated with fresh-cut produce – (around 3 logarithmic reductions) within a 5-min treatment. However, we anticipate that alternative plasma generation conditions will effectively reduce the accumulated microbial load and chemical contaminants. Among these conditions, the ones necessitating the lowest energy cost and the shortest activation time will be selected. Additionally, the reconditioned water is expected to not significantly affect the organoleptic characteristics and nutritional value of fresh-cut produce during washing. This expectation is based on observations from previous experiments using the aforementioned reactor, where the physico-chemical values of tap water did not change markedly after treatment.

To sum up, this study aims to validate the use of NTAP in the fresh cut produce industry as an effective and sustainable strategy for the reuse of water, while public health and environmental quality remain protected.

9. Enhancing Seed Germination and Growth using Plasma-Activated Water (PAW): A Preliminary Study

***Mustafa Ghulam*, Bozena Sera**
Comenius university in Bratislava, Slovakia

The application of plasma-activated water (PAW) for seed priming in agriculture, PAW derived from non-thermal plasma treatment, has garnered attention for its ability to promote seed germination and early seedling growth through the effects of reactive species.

My study aims to investigate the efficacy of PAW treatment in enhancing seed germination uniformity, rate, and yield. Through an exploration of the physical and biochemical changes induced by plasma treatments, including endospore weakening, surface alterations, and physiological enhancements, the research seeks to optimize PAW application methods for safe and effective seed treatment. Furthermore, the program will evaluate the early growth characteristics and induced resistance of plants derived from plasma-treated seeds. Parameters such as leaf number, root branching, biomass production, and resilience to pathogens and abiotic stresses will be assessed to elucidate the benefits of PAW treatment on overall plant vigor and health.

Decontamination of seeds from seed-borne pathogens without compromising seed viability is also a critical aspect of the study. By identifying treatment conditions that balance pathogen elimination with improved germination rates, the research aims to develop a comprehensive approach to seed priming, enhancing crop health and productivity. My proposed doctoral study aligns with the PlAgri Working Group's focus on innovative agricultural technologies and sustainable farming practices. Through interdisciplinary collaboration and cutting-edge research methodologies, the study seeks to contribute to the advancement of agricultural science and address key challenges in global food security.

10. Enhancing Antiviral Performance: Incorporating Silver Nanoparticle-Polymer Nanocomposite into 3D-Printed Protective Covers

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Efforts to combat microorganisms involve altering surfaces and developing antimicrobial coatings. The direct application of biocidal substances onto surfaces facilitates effective modification without affecting their bulk properties. Recent progress includes the creation of polymer-solvent-active material nanocomposites, which offer diverse antimicrobial effects depending on concentrations and compound compositions. Additionally, the utilization of 3D scanning and printing technology allows for the production of intricate, geometrically shaped, and flexible protective coatings to mitigate the spread of microorganisms on frequently touched surfaces. In this study, silver nanoparticles (AgNPs) synthesized via photochemical methods using AgNO3 and Irgacure 819 are integrated into a PVB polymer matrix, resulting in an AgNP-PVB nanocomposite. This nanocomposite is applied as a thin-film coating on custom protective covers produced with the Artec Space Spider 3D scanner (Artec 3D, Luxembourg) and Form 3 stereolithographic 3D printer (Formlabs, USA), utilizing Flexible 80A resin (Formlabs, USA). The reconstruction algorithm for the 3D model is developed using Matlab (MathWorks, USA). To assess the antiviral properties, 10-well substrates are 3D printed from Flexible 80A polymer. Eight wells are coated with AgNP-PVB-A (AgNP concentration – 500 ppm) and AgNP-PVB-B (AgNP concentration – 200 ppm) nanocomposites, while the remaining wells are left empty to evaluate the properties of the printed cover itself. The filled wells are incubated with the test solution for 24 hours, whereas the ninth and tenth wells serve as controls for one-step qRT-PCR analysis after the same duration.

The antiviral test results using the AgNP-PVB-A nanocomposite coating reveal an average cycle threshold (Ct) value of 30.78 ± 2.00 in the test wells compared to 25.92 ± 0.04 in the control wells. Meanwhile, the Ct values decrease to 28.22 ± 0.88 (test) and 24.65 ± 0.40 (control) when utilizing the AgNP-PVB-B nanocomposite coating. The AgNP-PVB-B control test Ct values closely resemble those of the Flexible 80A polymer (25.27 ± 1.41 (test) and 24.61 ± 0.11 (control)), indicating the intrinsic antiviral properties of the printed coating itself. The 3D printed door handle cover exhibits uniform thickness without cracks or bulges. This research contributes to the development of durable antiviral coatings aimed at inhibiting the transmission of infectious diseases in various environments. In conclusion, this study demonstrates the creation of AgNP-PVB nanocomposite coatings with different concentrations (AgNP concentration – 200 ppm and 500 ppm) on 3D printed protective covers, showcasing effective antiviral properties with Ct values of 30.78 ± 2.00 and 28.22 ± 0.88, respectively. Additionally, an algorithm for reconstructing the 3D model of the cover from the 3D scanned model has been developed and validated.

11. Effects of Reactive Species Generated by DBD Plasma-based Reactor on Germination of Peanut Seeds

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Atmospheric pressure cold (APC) plasma has applications in various fields, including health, farming, food preservation, and manufacturing [1]. In the agricultural sector, plasma treatment enhances seed germination, and growth and modifies the surface of seeds [2]. The current study investigated APC plasma treatment’s impact on peanut seed physicochemical and functional characteristics by altering their surface properties. Seeds were pretreated for 0, 10, 20, 30, 40, and 50 s using an APC discharge system operated at a voltage of 9 kV and frequency of 20 kHz. A change in seed germination length, moisture content, seed permeability, water uptake, and colour parameters are analysed after APC plasma treatment. At a particular time of APC plasma treatment, boosted seedling germination is found and drastic growth is observed. Increased water uptake by the seeds, improved nutritional absorption, and increased seedling growth are the favourable benefits of APC plasma treatment [3].

Field emission Scanning electron microscopy (FESEM) evaluated the morphology of seeds, which revealed that the APC plasma treatments etched the seed coat, increasing the peanut seed's wettability. Surface etching produced by plasma species increases the conductivity of the seed coat and reduces the contact angle on its surface. This research examined how low-temperature atmospheric-pressure plasma affected germination rates, water activity, and water content. The effects of DBD in cold atmospheric plasma on the wettability, imbibition, and germination of peanuts were studied. Findings suggest that higher water intake by the seeds, and improved nutrient absorption, would have been responsible for increased germination growth.

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12. Effects of seed treatment with non-thermal plasma and plasma activated water on *Lactuca sativa* germination, early growth and biochemical processes

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The escalating demand for agricultural products necessitates the exploration of sustainable agricultural technologies. These technologies should prioritize yield and quality improvements while minimizing environmental impact of chemically intensive practices for fertilisation and pest control.

The aim of our study was to examine and compare the effects of combinations of the pre-sowing seed treatment of low-pressure cold plasma (CP), vacuum, atmospheric dielectric barrier discharge (DBD) plasma and imbibition in plasma activated water (PAW) on agricultural performance of two lettuce (*Lactuca sativa*) cultivars (cv. 'Pearl Gem' and cv. 'Cervanek'). We estimated changes in seed germination, early growth, and secondary metabolite content in 7-days-old seedlings. A gliding arc discharge (GAD) plasma was used for PAW generation. Water for plasma activation was modelled from the deionized water by adding several selected salts present in tap water (Ca^{2+}, Mg^{2+}, Mn^{2+} chlorides and $KHCO_3$). Studies of PAW composition showed that added HCO_3^- ions stabilized PAW pH levels in a plant-friendly zone (pH 6.9-7.4) without affecting the generated H_2O_2 level.

The effects of seed treatment with different plasma generator types as well as effects of PAW were cultivar-dependent. Seed treatments did not change 'Pearl gem' germination kinetics and only CP (5 min) treatment had a weak positive (2%) effect on the germination rate of 'Cervanek' seeds. Seed treatments promoted the early (7 days) growth of 'Pearl Gem' seedlings, but both DBD and CP plasma treatments had negative effect on sprout length in 'Červanek'. Compared to the control, the antioxidant activity in 'Pearl Gem' seedlings was significantly increased in the CP3 group only (9%), and the total phenolic compound content of 'Cervanek' seedlings was significantly increased by vacuum (31%) and by DBD 3 min (28%). However, the CP 5 min treatment reduced this value by 11%. All treatments had positive effect on the amounts of photosynthetic pigments in 'Pearl Gem' seedlings. DBD 5 min increased amount of Chl a by 50 % and Chl b by 34 %. Treatment with V5, DBD3 and CP5 increased the photosynthetic pigment content of 'Chervanek' leaves, but DBD5 and CP3 decreased it.

13. Changes of secondary metabilites induced by seed treatment with cold plasma (CP) in *Stevia rebaudiana* Bertoni

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The most economically important source of natural low-calorie sweeteners - steviol glycosides (SGs) is *Stevia rebaudiana* Bertoni. Among them, stevioside (Stev) and rebaudioside A (RebA) are the most abundant. Presowing seed treatment with cold plasma (CP) was shown to increase SGs biosynthesis/accumulation up to several folds. In addition to SGs stimulation, the CP-induced increase of antioxidant activity was observed as well. This study aimed to evaluate the universality of two types of CP-induced biochemical changes in stevia by comparing SGs, total flavonoid content (TFC), total phenolic content (TPC) and antioxidant activity (AA) changes in two cultivars. Seeds of stevia cultivars – SHUG A36 and SHUG HIGH A3 (HYBRID) were treated for 2, 5 and 7 min with two different CP equipment types – capacitively coupled (CC – CC2, CC5 and CC7 groups) and dielectric barrier discharge (DBD – DBD2, DBD5 and DBD7 groups) plasmas before sowing.

Both CC and DBD treatments stimulated SGs production; however, to different extents. In HYBRID CP5 induced the highest increase of 10% in RebA+Stev concentration, in SHUG A36 the highest increase was observed in DBD5 group by 1.9 folds. The overall tendency to increase Stev and decrease RebA concentrations compared to control was observed in all groups. CP did not affect TFC and TPC except for SHUG A36 DBD5 group where the increase in TPC concentration by 1.6 folds was observed. AA was stimulated in both cultivars by both plasmas. The highest AA increase was observed in DBD5 groups – by 1.7 folds in SHUG A36 and by 2.4 folds in HYBRID. In conclusion, seed treatment with two types of CP induced SGs concentration increase due to Stev biosynthesis stimulation which, in the case of DBD, correlated with the increase in antioxidant activity and TPC concentration rather than TFC in both cultivars.

14. Engineering Antibacterial Plasma Polymerized Polyethylene Glycol/ZnO Nanocomposite Coatings for Extending Pork Sausage Shelf Life

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I'm excited to share my participation in the course on "Applications of Plasma in Agriculture and Food Processing," aligning with the topics discussed in Working Group 5. My research, focused on the application of plasma processes and technologies in the food industry, delves into the creation of antibacterial plasma polymerized polyethylene glycol (PEG)/ZnO nanocomposite coatings to extend the shelf life of pork sausages. This research stems from the imperative need for eco-friendly techniques in manufacturing food packaging films with antimicrobial properties.

Utilizing an atmospheric-pressure aerosol-assisted plasma deposition method, PEG coatings incorporating varying concentrations of zinc oxide (ZnO) nanoparticles were deposited on polyethylene (PE) substrates. The optimization process involved fine-tuning plasma operating parameters, such as input power, monomer flow rate, plasma head movement, and plasma substrate distance, to achieve a PEG matrix with optimal water stability for controlled ZnO release over time.

Comprehensive physical and chemical characterizations of the PEG/ZnO nanocomposite coatings were conducted using advanced techniques such as atomic force microscopy, scanning electron microscopy, energy-dispersive X-ray spectroscopy, X-ray photoelectron spectroscopy, Fourier-transform infrared spectroscopy, and water contact angle analysis. Antibacterial efficacy against Escherichia coli and Staphylococcus aureus was assessed in accordance with ISO 22196 standards. Results revealed the deposition of uniform conformal PEG/ZnO nanocomposite coatings at optimized plasma parameters, displaying a chemical composition similar to typical PEG polymers. Morphology analysis showed a homogeneous dispersion of ZnO nanoparticles in the PEG matrix, even at high concentrations. The coatings also exhibited robust antibacterial effects against S. aureus and E. coli, with enhanced activity at lower ZnO NPs size.

To simulate real-world scenarios, plasma-coated PE was employed as packaging material for sterilized pork sausages inoculated with a mix of Listeria monocytogenes and Lactic Acid Bacteria strains, which were then vacuum-packed. Bacterial growth was monitored during storage at 7 °C, revealing robust antibacterial effects, particularly against lactic acid bacteria. However, interactions with the food matrix suggested the necessity for higher ZnO concentrations to achieve significant antibacterial activity.

The study also addressed safety concerns by investigating ZnO release into food simulants, confirming that it remained below specific migration limits. Overall, this research contributes valuable insights into the development of effective and safe plasma-deposited coatings with potential applications in enhancing food preservation and safety.

15. Evaluating role of dielectric barrier discharge plasma on nitrogen dynamics in slurry and digestate to optimise it as fertilisers in agriculture

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Ammonia (NH3) losses from use of animal slurry and digestate in agriculture is a cause of environmental concern and a loss of valuable resource. If the NH3 loss can be minimised, it can tackle these challenges and the nitrogen from NH3 can be utilised as a valuable source of renewable fertiliser for crops. With escalating price of natural gas, which is directly influencing fertiliser prices, alternative solutions are urgently needed. This project is timely to tackle this challenge as it entails using plasma technology to treat air and convert it into Reactive N Species (RNS) such as nitric oxide (NO) and create an acidic environment which minimises losses of NH3. However, what is less known is the detailed chemistry of what happens when these N species interact with the animal slurry or digestate liquor.

The aim of this project is to assess the role of dielectric barrier discharge (DBD) plasma and its impact on nitrogen dynamics in slurry and digestate to meet crop requirements. Dielectric Barrier Discharge (DBD) Plasma, a form of non-thermal plasma, establishes a discharge between two dielectric barrier-separated electrodes (Peng et al., 2017). Acknowledged for its low operation and maintenance costs, it offers the widest range of operational pressures among plasma technologies, spanning from 5 to 105 Pa (Wang et al., 2011).

The incorporation of a dielectric barrier promotes a strong electric field and leads to the production of an array of reactive species (such as ·O, ·OH, O_2^+, H_3O^+, O_2^-, O_3^-, and ·N). These reactive species are capable of breaking chemical bonds or initiating various chemical reactions, ultimately leading to the breakdown of complex organic molecules within digestate and slurry. This project involves a design of tailor made coaxial DBD reactor which consists of an inner and outer quartz tube to which tungsten copper sheet will be wrapped around serving as the ground electrode. The inner electrode which is tungsten rod will be placed inside the inner quartz tube and connected to an alternative current (AC) to generate electric field necessary for plasma formation. Selective synthesis of NOx from atmospheric air using DBD can inhibit the loss of NH_3 (Chen et al., 2023) and cause a breakup of complex carbon molecules in digestate and slurry which will make it more available as substrates for microbes which in combination with pH reduction can influence N dynamics.

This project attempts to summarise the key knowledge gaps related to DBD interactions with slurry and digestate and how pH variation further impacts on its physico-chemical properties. This will ultimately inform on the impact of plasma induced acidification on the mineral fertiliser efficiency (MFE) of slurries and digestates.

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16. Vapor phase deposition of thin films for food packaging application

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In recent years, many innovative food packaging techniques are being developed due to changes in production processes, retail practices and growing consumers demands of healthier, higher-quality and with a longer shelf-life food products.

Cold plasma surface modification of materials, particularly thin film deposition processes, can offer a good strategy to address these requests. Since these processes are carry out at room temperature, they can be used for the treatment of thermolabile materials such as natural and artificial polymers, or to deposit biomolecules or microorganism. These characteristics make these processes less environmental impactful and extremely attractive for industries[1].

The purpose of this PhD project is the deposition of inorganic, organic and composite thin film by means of different kind of cold plasmas to improve barrier properties of food packaging or to obtain active coating to control the release of metals, metal oxide and bioactive molecules like essential oils. Enhance barrier properties means to better preserve the food matrix shielding external factors that can impact negatively on food quality like oxygen and moisture responsible of common degradation processes such as lipid oxidation, microorganism growth and enzymatic browning. Active packaging is a recent type of packaging, characterized by the incorporation of bioactive or chemoactive molecules. This technology allows to increase the shelf-life of products and their quality by minimizing or replacing the addition of additives directly to the food[2].

Three deposition methods will be investigated and applied both individually or in combination: 1) Plasma enhanced-chemical vapour deposition (PE-CVD)/sputtering processes to obtain barrier coatings and composites made up by a matrix and nanoparticles of metals or the corresponding oxides, which will release ions with an antibacterial function; 2) Atomic layer deposition (ALD) to deposit metal oxides barrier layers or active layers (e.g. Al2O3); 3) Aerosol assisted atmospheric pressure PE-CVD for depositing composite coating containing essential oils. The deposited films will be characterized by means of different techniques and their effectiveness will be tested using permeability measurement, release tests, evaluation of antimicrobic and antioxidant activity, and shelf-life tests.

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Keywords: Cold plasma, active food packaging, deposition, barrier layer

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17. Investigation of the possibility of using cold atmospheric pressure plasma for sterilization of freshly squeezed citrus juices

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Currently, widely utilized pasteurization methods might result in a deterioration of the nutritional properties of juices as a result of high temperatures impact. Therefore, it is necessary to look for new, cheap, and effective sterilization methods. One of the promising method is using cold atmospheric pressure plasma (CAPP) for this purpose. Extending the shelf life of juices might be a result of the generation of reactive oxygen and nitrogen species (RONS) during proper juice treatment with CAPP. RONS can be involved in the reduction of number of microorganisms that naturally occur in juices.

Here, we present the preparation of freshly squeezed citrus juices, i.e. grapefruit, orange and mandarin. As a CAPP source, we have used pulse modulated radio frequency atmospheric pressure glow discharge (pm-rf-APGD)-based system. The influence of plasma treatment of citrus juices on changes in the physicochemical properties of the samples such as pH and conductivity was examined. Determination of saccharide content - Brix measurements and concentration of ascorbic acid were also examined to check the change in nutritional properties as a result of CAPP treatment of juices. Moreover, the determination of the number of colony forming units of the microorganisms for untreated and CAPP-treated juices, was examined in order to assess whether this method can be used for their sterilization. To select the optimal conditions for plasma treatment of juices, multivariate optimization of its working parameters was used.

The obtained preliminary results are promising, which gives hope for the development of an effective method of sterilizing citrus juices in the future, which can replace the methods previously used in the food industry.

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18. The Effect of Bisphenol A Degraded by Non-Thermal Plasma on Yeast

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Bisphenol A (BPA) is a toxic chemical compound used as a monomer in the production of plastics, especially polycarbonates and epoxy resins, which are traditionally not recycled and go to landfills [1]. BPA can leak from the materials to the environment, including beverages, food etc. Bioactive levels of BPA were detected in water, soil, air and living organisms [2-5]. It is a known endocrine disruptor linked to numerous diseases in humans, including cancer, in which it can induce resistance to chemotherapeutics [6]. Slowly, BPA is being banned by regulatory agencies to produce certain items, but its production is still predicted to grow in the near future [1]. Recent studies show it is possible to degrade BPA by non-thermal plasma, which could be potentially applied in wastewater treatment plants [7-8].

We investigated the BPA degradation by plasma and the impact of the plasma-treated BPA solutions on the model organism *Saccharomyces cerevisiae* to verify whether the produced intermediates and long-lived reactive oxygen and nitrogen species are not more harmful to the cells than BPA itself. The effects of two non-thermal plasma sources, transient spark and dielectric barrier discharge, were analysed. We measured cell survival and intracellular oxidation by flow cytometry. Preliminary results indicate that immediately after the degradation of BPA, the cytotoxicity of the plasma-treated solutions is higher compared to the positive control, represented by untreated BPA solution. In the following weeks post plasma treatment, however, the tested parameters improve. The BPA content in the plasma-treated and untreated solutions was measured by HPLC (High-Performance Liquid Chromatography). In the future we plan to test the plasma treated solutions on plant models and human cells.

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19. Effect of plasma activated water on lattice plant growth and seed germination

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Cold plasma produced by atmospheric pressure air discharges contains a variety of gaseous reactive oxygen and nitrogen species (RONS) [1,2]. When plasma comes in contact with water, the RONS dissolve and affect the chemical composition of the water, generating plasmaactivated water (PAW) [1,3]. The long-lived liquid RONS (H_2O_2, NO_2^-, NO_3^-) in the PAW may function as nutrients or as signaling molecules in plant metabolism, opening up potential uses in a variety of agricultural applications. We studied the effect of PAW generated by transient spark with water electrospray (TS-ES) [1] and Fountain Dielectric Barrier Discharge (FDBD) [3] on lettuce (*Lactuca Sativa*) seed germination and plant growth. The schematic diagram of the plasma systems is shown in figure 1. Tap water was used for activation by plasma and irrigation of plants in soil. Immediate measurements were taken to determine the concentrations of H_2O_2, NO_2^-, and NO_3^- in the PAW after plasma activation. Different parameters of PAW including pH, OxidationReduction Potential (ORP), Total Dissolved Solid (TDS), and conductivity are measured in this study. After 8 and 12 weeks the plant growth parameters, number and quality of leaves, fresh and dry weight of plants, photosynthetic pigment (chlorophyll) content, and other parameters were evaluated.

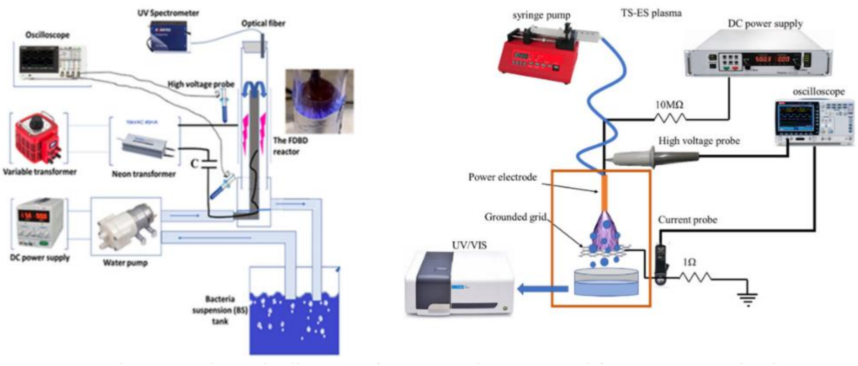

Figure 1. schematic diagram of FDBD and TS-ES used for tap water activation.

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20. Impact of Plasma-Activated Water on Germination potential and Early Seedling Development of Radish

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The main objective of the current research is to examine the impact of plasma-activated water (PAW) on radish seed germination. The surface dielectric barrier discharge as a cold plasma source was utilized to generate Plasma Activated Water (PAW)[1]. Preliminary studies explored multiple process factors, including applied voltage, frequency, treatment duration, and volume of water, in relation to the physico-chemical qualities of the produced PAW. In order to create PAW, we exposed deionized water to SDBD based plasma discharge for 5, 10, and 15 minutes. We observe that the $NO^{2-}$and NO^{3-}concentration are both significantly greater than the control for 15 minutes plasma treated water. The impacts of PAW on radish seed germination potential have been studied at different water activation time. Plasma activated water (PAW) had a notable impact on seeds germination and seedling growth. PAW-irrigated seeds imbibe faster than deionized water irrigated ones. PAW irrigation boosted germination metrics including final germination rate, mean germination rate, germination index, and growth parameters like shoot/seedling length and vigor index. These outcomes indicated that cold plasma reactors could improve seed germination and plant growth, however, each seed's plasma treatment time must be optimized. The plasma species created an etched surface, which increased the seed coat's capacity to absorb water. In the course of the germination phase, the reactive oxygen and nitrogen species (RONS) impacted the development of the seeds and subsequent seedlings[2], [3]. Recently developed PAW utilization in the field of agriculture could be a great eco-friendly and economical option for fertigation. There remains plenty of scope for commercial level expansion.

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21. Plasma processes for the enrichment of water with reactive species

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I am Alexander Mouzakis and I am a new PhD student in the National Technical University Of Athens (NTUA), Greece. Under the supervision of associate professor Georgios Kokkoris, I will study plasma processes for the enrichment of water with reactive species.

Until now, nitrogen based chemicals and fertilizers have been produced by the Haber-Bosch process. Due to the high operational costs and operation parameters (high pressure and temperature), the Haber-Bosch process leaves a significant ecological footprint (CO_2 and CO emissions and excessive energy consumption). Many alternatives have been proposed, with one of the most promising the use of low temperature plasma for nitrogen fixation.

In particular, the aim of the thesis is to develop an atmospheric pressure plasma reactor for the enrichment of water with NOx with high efficiency and throughput. The plasma activated water (PAW) will be used in agricultural applications (fertilizers, increase the growth rate of plants). A plasma jet reactor is already available for the enrichment of water which it will optimized and eventually scaled up for the maximum efficiency and throughput of NOx. Moreover, a study of production of reactive species in the water will be implemented. To be more specific, the mass transport mechanisms from the gas to the liquid phase will be studied (computationally and experimentally) and the chemical kinetics in water. Furthermore, the production of the plasma generated species will be investigated.

The results of the research mentioned above will lead to the design and development of a new plasma reactor with the optimal parameters for the enrichment of water with NOx.

22. Developing an advanced plasma-water spray system for RONS-rich droplet production in seed treatment

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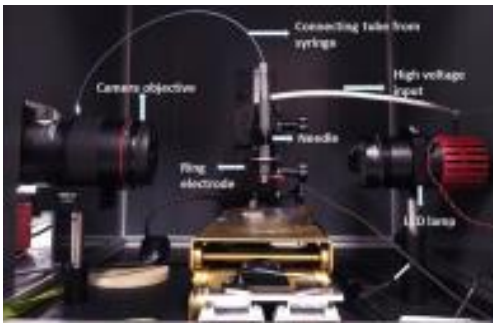
Cold plasma technology is emerging as a crucial player in seed treatment for various purposes, including seed germination, preventing microbial contamination, and promoting plant growth. The utilization of cold plasma in seed treatment can enhance seed growth uniformity, reduce germination time, and protect seeds from pests and microbial infections, ultimately improving overall plant growth and food yield. The existing methods include physical treatments such as ultraviolet light, gamma irradiation, and hot water soaking, which can certainly affect seed quality, causing damage to the cells. Chemical treatments involving chemical compounds and pesticides leave traces of eco-unfriendly toxic residues, and these methods are labour-intensive and time consuming. Recently, the use of direct plasma treatment and plasma-treated water has shown great potential as alternatives for seed treatment. The unique feature of plasma species is their low penetration depth which prevents structural damage, and importantly, these species are naturally occurring and tend to recombine shortly after treatment, unlike toxic chemical residues that persist for longer periods and potentially affect crops and soil. Compared to direct plasma treatment, the use of plasma-treated liquid has advantages in terms of scale-up, flexibility and repeatability. Water plays a key role in unlocking the pathway to pass reactive species into the cell layers of seeds, triggering the metabolic activity of the seeds.

This research aims to develop and optimize a plasma-spray system for producing charged droplets enriched with tailored concentrations of reactive oxygen and nitrogen species for seed treatment. The unique combination of a plasma and water spray system involves potential optimizations through careful monitoring, achieving a balance between configuration, electrical parameters, and liquid parameters. This ensures the formation of a stabilized spray mode through plasma discharge. The focus is on customizing the configuration to produce reactive species under particular discharge and spray regimes, tailoring their concentrations according to specific application requirements. For instance, a precise concentration of reactive oxygen species is essential for seed germination and antimicrobial activity, while a higher concentration of reactive nitrogen species can serve as a potential fertilizer for plant growth. Thus, the combination of plasma and liquid microdroplets is gaining attention as a novel approach that maximizes the flexibility of plasma treatments while maintaining the efficiency of short-lived reactive species.



Plasma - water spray system setup

Acknowledgement: This work is supported by the ANR-23-CE04-0003 PLASMASOL project

23. Application of plasma technology in decolorization, deodorization, and modifying techno-processing functionality and taste of proteins from side streams

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The global effort to minimize agro-industrial and food waste is continuously increasing. Protein is one of the most intensively investigated from such side streams. Several advanced and sustainable techniques have been applied as pre- and/or treatment in obtaining high yield of protein. The most common issues recently arise in terms of its quality including color, taste, and odor, as well as techno-functionality in terms of its application in the food and pharmaceutical industry. The obtained proteins often lack solubility, tend to obtain color, odor, and taste of its original sources which make it less suitable in further utilization. Plasma technology has been applied in several aspects of food processing.

The study aims to investigate the impact of plasma process technology in modifying the techno-functional properties as well as in decolorization, deodorization, and taste of protein obtained from side streams. Plasma technology will be applied in two different processes including as pre-treatment on protein extraction and treatment on proteins obtained. Firstly, plasma will be designed for protein extraction for wastewater and biomass of side streams in comparison to microwave, ultrasound, and high pressure as well as their combination with plasma process. Secondly, plasma will be applied as a treatment on the obtained proteins.

The investigation will be made on how the plasma affects the yield of proteins and how the plasma affects the color, odor, taste and techno-functional properties such as solubility, water holding capacity, oil holding capacity, emulsion capability and emulsion stability, and foaming capability and stability. The obtained results will be used for comparison with several treatments which are not involving plasma technology. The study will provide an insight to the industry how the advanced food processing can be optimized in generating a high desirability of proteins from side streams.

24. Influence of non-thermal plasma (NTP) on honey-based beverages properties

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Food processing helps to preserve nutrients that might be lost during storage. Currently, the most common methods used for food preservation are thermal processing and thermal sterilisation (Sruthi et al., 2022). However, it should be noted that food ingredients such as antioxidants, minerals, pigments, and vitamins are sensitive to process parameters, leading to e.g. loss of organoleptic properties. As a result, novel techniques for food processing without direct heating are becoming increasingly searched, enabling high-quality products to be manufactured without loosing nutritional properties. A remedy to this drawback might be the application of non-thermal plasma (NTP). NTP-based food processing is an innovative technique with a wide range of uses in microbial decontamination, enzyme inactivation, and functional modification (Sruthi et al., 2022).

Plasma sources such as dielectric barrier discharge (DBD), corona discharge (CD), and atmospheric pressure plasma jet (APPJ) were applied to treat food products such as smoked salmon, dried laver, breast chicken fillets, blueberries, shrimp, and kumquat fruits, in order to understand their antimicrobial effect (Ucar et al., 2021). Due to the lack of studies, related to the deep the knowledge in the character and application of pulse-modulated radio-frequency atmospheric pressure glow discharge (pm-rf-APGD), there is a need to further research on this type of discharge uses in food chemistry. We predict that application pm-rf-APGD might effect on nutritional, microbiological and physicochemical properties of honey-based beverages. For this reason, we prepared acacia, maple, and multiflower honey-based solutions and subjected them to pm-rf-APGD treatment. Afterward, we analysed their properties such as total content of polyphenols and Brix (as a nutritional properties), the pH and conductivity, (as physicochemical properties). In addition, shelf-life analysis and determination of nitrites and nitrates were also carried out. In the research, spectroscopic analyses were also performed using attenuated total reflectance Fourier transform infrared (ATR FTIR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, and optical emission spectroscopy (OES). To know the influence of the plasma used on the analysed honey-based solutions, the results of the untreated and pm-rf-APGD-treated honey-based solutions were compared with each other. We truly believe that presented approach after in vivo test might be a tempting alternative for food preservation, including the production of pm-rf-APGD honey-based solutions.

Acknowledgement: The presented studies were co-founded by National Science Centre (Poland) in the frame of Opus 23 project (UMO-2022/45/B/ST8/02410).

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25. Achieving selectivity in reactive species compositions in PAW by different reactor geometries in transient spark and DBD plasma setups

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Plasma-liquid interactions have become of great interest due to the wide range of cold plasma applications where these interactions occur frequently. Plasma activated water (PAW) is obtained by treating water with plasma, whereby various reactive species are transferred from the plasma to the water. The simultaneous generation of ROS and RNS in PAW makes it difficult to assess their individual effects. While some species may be beneficial for certain applications, others may have adverse effects [1]. For example, ROS are essential in advanced oxidation processes for wastewater treatment [2,3], whereas RNS are not essential and may hinder the effectiveness of PAW. Therefore, tailoring the production of ROS and RNS for different PAW applications is crucial for the targeted implementation of plasma technology.

In this study, we present the selective production of RONS in PAW generated by fountain dielectric barrier discharge (FDBD). Furthermore, we also present the tuning of the RONS composition in PAW generated in a transient spark (TS) discharge with electrospray (ES) system. The selectivity in the FDBD system was achieved with different inner electrode tubes (ceramic and copper) and with and without cooling system of the reactor. The Cu setup without cooling system resulted in a non-linear rising and falling trend of H2O2 concentration, while the Ce setup showed a linear increase, resulting in significantly higher H2O2 concentration in PAW. Conversely, the FDBD with cooling system did not produce H2O2 but showed significantly higher concentrations of NO_2^- and NO_3^-.

TS was used in direct and indirect contact with ES in a closed reactor with humid and dry synthetic air as input gas. When TS and ES were separated, PAW rich in NO_2^- only was produced in higher concentration in humid synthetic air than in dry synthetic air. Furthermore, when TS and ES were in direct contact, both H2O2 and NO_2^- were produced and the ratio NO_2^-/ H2O2 increased with the increase in input energy density. The importance of selectivity in RONS production was further investigated by treating methylene blue dye in the FDBD setup. The FDBD setup without cooling and ceramic tube achieved higher degradation rate due to higher H2O2 concentration than the copper tube and cooling setup. The FDBD with cooling system resulted in nitration of methylene blue due to high content of reactive nitrogen species.

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26. Identification of reactive oxygen and nitrogen species in PAW through DNA damage observation

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Non-thermal plasma has potential applications in various fields, such as medicine, agriculture and food industry. However, before applying such treatment, it is important to understand the mechanisms behind the effect of non-thermal plasma on living organisms and DNA.

In my master thesis, I focused on identifying reactive species that damage DNA when pBR322 plasmid istreated with RPS40 (Robust Plasma System 40) and MSDBD (Multihollow Surface Dielectric Barrier Discharge) plasma sources. Water containing the plasmid was treated with plasma generated in air by both sources and plasma generated in oxygen and nitrogen by the MSDBD. In addition, we analyzed the contribution of UV radiation to the genotoxic effect of the non-thermal plasma. DNA damage increased with exposure time for all working gases and sources, with plasma generated in air by the MSDBD showing the most significant genotoxic effect. Using selected scavengers of reactive oxygen and nitrogen species, we identified nitrites, nitrates, peroxynitrous acid and hydroxyl radicals as the major particles causing DNA damage under our experimental conditions.

This simple cell-free method can be used to determine reactive particles generated in PAW and could be used as a type of chemical analysis of the plasma treated solutions. However, with my PhD. thesis, I would like to expand to a multicellular organism such as Caenorhabditis elegans. Currently, there is little information available about the effect of non-thermal plasma on this particular model organism and our group aims to contribute to this topic. In addition to transcriptome analysis of C.elegans´ genes after plasma treatment, I would like to clarify which reactive particles have an important role in transcriptional changes and how do they affect the whole organism.

27. Interactions of cold plasma treatment on growth and waterlogging stress tolerance of RGT planet barley seeds

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Barley, one of the important cereal crops often encounters challenges caused by abiotic and biotic stressors. Waterlogging is one of the stressors that results in a substantial yield loss in barley ranging from 10 to 50 % depending on the genotype, growth stage, and duration of waterlogging conditions. The prevalence and intensity of waterlogging stress may increase in certain regions depending on the climatic conditions. To address these challenges and improve stress resilience, we are investigating cold plasma technology as an alternative to conventional seed priming techniques. Recent studies have shown promising results in improving seed germination and stress resilience towards cold and drought stressors with cold plasma treatment. In this study, we are exploring the resilience towards waterlogging stress in RGT planet barley seeds with cold plasma treatment.

An atmospheric Dielectric Barrier Discharge (DBD) reactor which uses air as its input is used for the current study. The plasma process parameters including treatment durations of 10, 30 and 60 s and input voltage 50, 60 and 70 kV were used to analyse the seed key seed performance parameters like germination rate, seed vigour index, shoot length and root length as described in Los et al., (2019). The treatment voltage and processing time were optimised from the obtained results and used for further studies on seedling growth and waterlogging stress resistance studies. The waterlogging stress was introduced on day 15 after seed germination for 10 days and the plant growth, electrolyte leakage, photosynthetic pigments concentration, proline content and plant biomass were measured.

The results might present a comprehensive understanding of the interactions between cold plasma treatment and the germination and growth of RGT planet barley seeds. Furthermore, this might offer a potentially sustainable alternative to current chemical priming techniques. This could pave the way for innovative strategies in crop management under waterlogging stress conditions.

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28. Investigating the potential of cold plasma treatment to modulate the plant associated microbiome and stimulate germination in *Arabidopsis thaliana* seeds

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Seeds, a crucial element for agriculture and food security, face growing challenges. Rising global population and climate change threaten agricultural yields, while drought and high temperatures negatively impacting crop productivity and water availability [1]. Atmospheric cold plasmas (CAPs) offer a promising technology to improve seed quality and contribute to a more sustainable agriculture. These weakly ionized gases can modify the biological and physicochemical properties of seeds, promoting germination, breaking dormancy, and enhancing resistance to environmental stresses [2].

Our study explores the application of CAPs on *Arabidopsis thaliana* seeds, as a model system. We aim to evaluate the impact of plasma treatment on:

• Germination at suboptimal temperature
• Seed decontamination

• Seed microbiota

We focused on the effects of plasma exposure on seed germination at suboptimal temperatures. We found that a 15 min treatment increased germination at 20°C by 20 % for the Lansberg ecotype. We investigated the effectiveness of short treatments (5 to 30 minutes) on seed decontamination. Seeds participate in the transmission of microorganisms from one plant generation to another and therefore constitute the initial inoculum of the plant microbiota [3]. Some of these microorganisms can be pathogenic. Our results showed that a 15 min treatment could inactivate pathogenic microorganisms of heavily contaminated seeds, while a 5 min treatment was sufficient for less contaminated batches.

We are currently using metabarcoding approach to analyze the microbiota and evaluate the changes induced by CAP treatment in the microbial communities of seedlings. The ITS regions for fungi and V4 regions for bacteria are used as molecular markers to determine the species and their relative abundance before and after different treatment durations (5, 15 & 30 min). This work aims to establish the foundation for optimizing CAPs as an innovative approach to breaking seed dormancy, decontaminating seeds, and shaping seedling-associated microbial communities. This research has the potential to contribute to the implementation of more sustainable agricultural practices and securing the global food supply.

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29. Comparative analysis of plasma treatment technologies on seed germination and early growth of *Triticum aestivum* L. cv. Glosa

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Plasma treatment is a promising technique for enhancing seed germination and plant growth due to its potential to improve seed viability and reduce pathogens load and is increasingly considered as an alternative to the traditional pre-sowing seed treatments in the agriculture field. The present study evaluates the effects of two plasma technologies on the germination and early development of *Triticum aestivum* L. cv. Glosa seeds. The seeds were treated at INFLPR either in a pulsed voltage DBD (Dielectric Barrier Discharge) reactor (PV-DBDR) or in RF plasma fluidized bed reactor (RF-PFBR) and analyzed in term of growth parameters at USAMVB. The Petri plates were kept at 22°C, half day light program in growth chamber. The biometric indices refer to number of germinated seeds, roots number, roots length and sprouts lengths. For germination, the control had the lowest rate – 92.5%, similar with RF-PFBR (7 minutes), followed by RF-PFBR (10 minutes) - 95% and PV-DBDR - 100%. The average roots number was the lowest at control – 4.13 and the highest was for the PV-DBDR – 4.49. The control also had the lowest roots average length

– 136.95 mm, while RF-PFBR (7 minutes) had the highest value - 167.63 mm. For the sprout's length, the same evolution was observed, with the control showing the lowest growth - 28.72 mm and the RF-PFBR (7 minutes) with the highest growth 36.56 mm. These preliminary results show that both plasma technologies have a positive effect on seeds germination and growth, when compared with untreated seeds, the PV-DBDR having the best impact on seeds germination and number of roots and the RF-PFBR (7 minutes) having the best impact on early plant growth, on roots average length and sprouts average length. For optimum results, it is mandatory that the seeds are homogeneously treated by plasma flux. Further investigations are intended, with different levels of energy and power.

Keywords: *Triticum aestivum*, plasma treatment, seed germination, root growth, plant height, DBD reactor, RF-generated plasma

Acknowledgement. This work is part of program Ader 2.1.7. - Research on the use of plasma technologies in pests and diseases of stored agricultural products and evaluation of its effects on seeds and plants, for sustainable and quality productions

30. Influence of plasma treated water on the plant growth and vitality

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The main element of plant nutrition is nitrogen. Major source of the inorganic nitrogen taken up by the roots of plants is nitrate. Sources of nitrate are generally derived from inorganic minerals by a high energy-consuming chemical process. That results to higher prices of fertilizers and chemical process is not verry environmentally friendly [1]. This means that there is a need of finding new alternative ways of preparing fertilizers. The plasma activated water (PAW) provides an alternative opportunity for nitrogen supply to plant by fixing the nitrogen available in the air into the water using plasma. Treatment of water with plasma produces a mixture of nitrate, nitrite, and hydrogen peroxide as its major constituents [2].

Radish (*Raphanus sativus*) plants were grown in pots filled with soil for 30 days. Plants were treated by plasma activated water prepared by pin-hole electrode generating plasma directly in liquids with air flowing into discharge. Pots were divided to 4 variants based on the PAW application: PAW prepared from distilled water (PAW DW), PAW prepared from tap water (PAW TW), foliar application of PAW on leaves and watered by tap water (PAW F) and control group watered by tap water (TW). Concentration of nitrate, nitrite and hydrogen peroxide in PAW was determined colorimetrically by using UV-VIS spectrometry.

Results are indicating enhancement of growth of plant fresh matter weight in all variants treated by plasma activated water compared to control group (TW). In comparation of variants treated by PAW, it was found that PAW F variant had more fresh matter weight than the other variants (PAW DW and PAW TW). Determination of dry matter weight has shown that PAW F had more content of dry matter compared to other variants. PAW has high nitrate, nitrite and hydrogen peroxide content which can affect overall vitality of plant. Vitality of plant was determined by chlorophyll fluorescence. The principle of the method consists in measuring the activity of photosystem II (PSII). Fluorescence measurement results are showing inhibition of photosynthesis activity by treating plants with PAW compared to control group (TW), which means treating plants with PAW lowers overall vitality. Elemental analysis was used to determine content of nitrogen in leaves and roots of plants. Using PAW TW to water plants accumulated higher content of nitrogen in both aboveground and underground parts of plant compared to other variants. Overall, results are showing promising alternative source of nitrogen in PAW which is prepared more environmentally friendly way than inorganic fertilizers.

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31. Plasma treatment of freshly pressed apple juice in a flow system

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The consumption of fruit juices worldwide shows a constant upward trend, especially for juices without preservatives and other additives. This is due to increased consumer awareness, who pay attention to flavor qualities (lack of unpleasant aroma and artificial colors) and the positive impact on health.

Apple juice is one of the most liked and frequently chosen beverages by Poles. It is characterized by the presence of many vitamins and minerals. It also constitutes a rich source of dietary fiber (especially pectin). Additionally, it is considered a good source of antioxidants (mainly polyphenols).

True juice (not from concentrate, NFC) is defined as a product made from fruits or vegetables, devoid of any additives (water, preservatives, or antioxidants). Therefore, to maintain the quality of the product, preservation is necessary before the distribution and storage process. The most popular method so far has been pasteurization, which requires the use of high temperatures. Unfortunately, many substances that beneficially affect human body function belong to the group of thermolabile compounds, which is why low-temperature methods for extending the shelf life of such beverages are currently being sought.

The aim of the study was to develop a flow system for cold atmospheric plasma treatment to improve the microbiological safety of juices while preserving their basic physicochemical values.

32. Effect of Chrome-Nickel Doping on the Structure and Properties of Diamond-like Carbon Films

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The diamond-like carbon (DLC) films production process has undergone extensive approaches to improve their properties and functions, so it can meet the needs of several applications in broad fields such as biological implants, automotive industry, micro/nano-electromechanical systems (MEMS/NEMS), magnetic storage devices, solid-state batteries and more. Thus, various metal dopants such as Ag, Ti, Ni, Cr, Ag, Mo, etc. are used to deposit metal doped diamond-like carbon films. The usage of two metals doping of chrome and nickel was reported in several literature research.

The Chrome-Nickel doped amorphous diamond-like carbon thin films (Cr/Ni-DLC) were deposited on Si (100) substrates by magnetron sputtering. The graphite and the Cr-Ni cathode currents were fixed at 1.5 A and 0.25 A, respectively. The Cr-Ni target consists of 80% Ni and 20% Cr. The deposition duration was 10 min. The Cr and the Ni contents in the coatings were regulated by adjusting a slit wide in a shield mounted above the Cr-Ni target. It was obtained that the concentration of Cr and Ni in DLC films varies from ~2 to ~5 at.% and from ~4.5 to ~22 at.% for Cr and Ni, respectively. The increase of the Cr-Ni content led to a slight increase in the oxygen amount up to 19 at.% in DLC films. The Raman spectroscopy revealed that the sp3 C-C bond fraction decreased with increase of the Cr-Ni content due to the metal catalyst effect as obtained, which effectively promoted the graphitization and slight oxidation. The microhardness measurement indicated that the addition of low amount of Cr-Ni slightly reduced the hardness of DLC films. The atomic force microscopy (AFM) was used to reveal the surface morphology and nano-tribological properties. The AFM results demonstrated that the surface roughness was reduced with the addition of low amount of Cr-Ni. The lowest friction coefficient was obtained when the Cr-Ni content in doped DLC film was about 2.0 and 4.5 at.%. Further increasing of Cr Ni doping content led to high friction coefficient.

Keywords: Cr/Ni-doped diamond-like carbon, microstructure, friction coefficient, nano hardness.