Atmospheric Cold Plasma (ACP) Treatment to Degrade Deoxynivalenol (DON) and its Impact on Grain Quality Parameters

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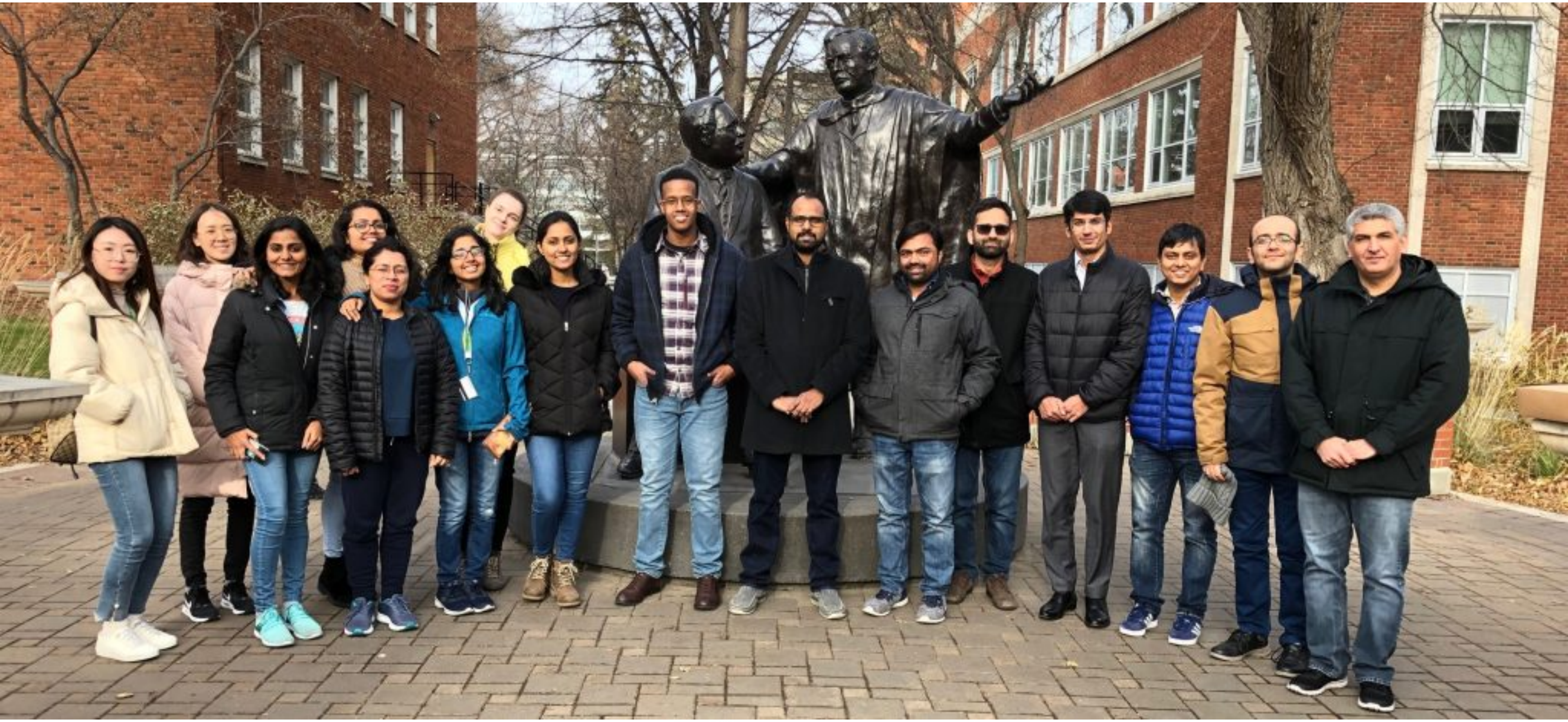
2020 National Fusarium Head Blight Forum
US wheat and barley scab initiative

Acknowledgements



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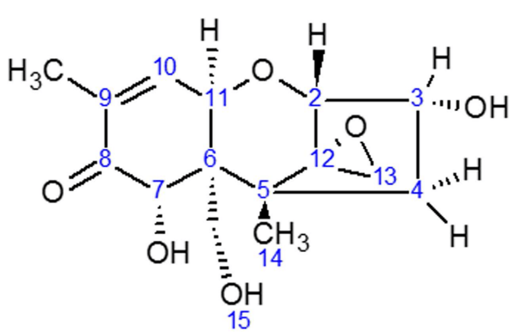
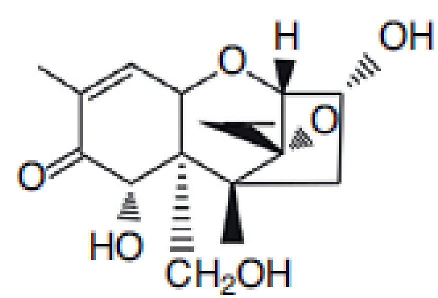
Introduction

- Mycotoxins are toxic substances that cause a significant annual economic loss to the agriculture and food industry
- Each year, approximately 25 % of agricultural commodities are contaminated by mycotoxins
- One of the major mycotoxins prevalently found in western Canada is deoxynivalenol (DON)
- Also known as vomitoxin, DON is a trichothecene mycotoxin produced by *Fusarium graminearum and F. culmorum*
- DON can cause vomiting, anorexia, growth retardation, immune suppression, inflammation and necrosis of various tissues, and diarrhea in animals
- Conventional approaches
- DON is resistant to high temperatures





Deoxynivalenol

FDA Advisory Levels for DON/ Vomitoxin

Intended Use	Grain or Grain By-Products	Vomitoxin Levels in Grains or Grain By-Products and Complete Diet ** [parts per million (p.p.m.)]
Human Consumption	Finished wheat products	1 p.p.m.
Swine	Grain and grain by-products not to exceed 20% of diet	5 p.p.m. (1 p.p.m.)**
Chickens	Grain and grain by-products not to exceed 50% of diet	10 p.p.m. (5 p.p.m.)**
Ruminating beef and feedlot cattle older than 4 months	Grain and grain by-products *	10 p.p.m. (10 p.p.m.)**
Ruminating dairy cattle older than 4 months	Grain and grain by-products not to exceed 50% of diet *	10 p.p.m. (5 p.p.m.)**
Ruminating beef and feedlot cattle older than 4 months, and ruminating dairy cattle older than 4 months	Distillers grains, brewers grains, gluten feeds, and gluten meals *	30 p.p.m. (10 p.p.m. beef/feedlot)** (5 p.p.m. dairy)**
All other animals	Grain and grain by-products not to exceed 40% of diet	5 p.p.m. (2 p.p.m.)**

* 88 percent dry matter basis ** Complete diet figures shown within parentheses

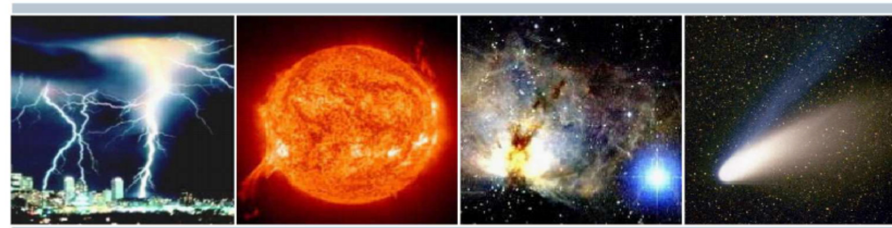
Commodity	Canada	Commodity	USA
Uncleaned soft wheat for human consumption	2 mg/kg	Finished wheat products	1 mg/kg
Diets for cattle & poultry	5 mg/kg	Grains and grain by-products destined for ruminating beef and feedlot cattle older than 4 months and chickens (not exceeding 50% of the cattle or chicken total diet)	10 mg/kg
Diets for swine, young calves, & lactating dairy animals	1 mg/kg	Grains and grain by-products (not exceeding 40% of the diet)	5 mg/kg

https://www.inspection.gc.ca/animal-health/livestock-feeds/regulatory-guidance/rg-8/eng/1347383943203/1347384015909?chap=1

Atmospheric Pressure Cold Plasma (ACP)

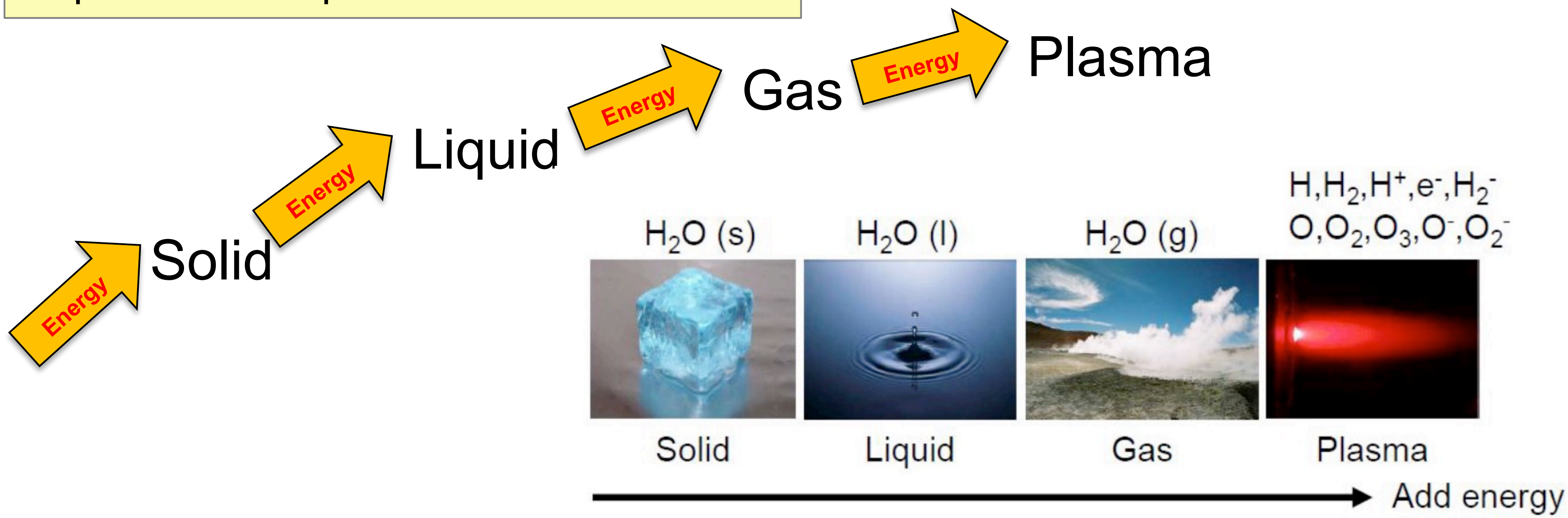
Plasma:

- Fourth state of matter
- Partially ionized gas with high energy
- Breaking down of gases to more basic components by applying energy
- A mixture (soup) of positive and negative charges as well as neutral particles and photons



More than 99% of all known visible matter is in plasma state!

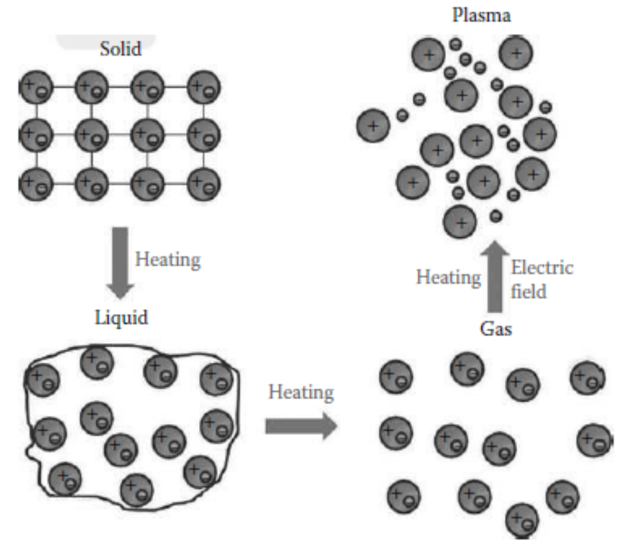
Examples: Northern lights, lightning
Applications: Plasma TV, fluorescent lamp etc.



Reference: Liu and Lu; NN Misra et al , Non-thermal Plasma Inactivation of Food-Borne Pathogens, Food Engineering Reviews (2011), 3, Issue 3-4, 159

Atmospheric Pressure Cold Plasma (ACP)

- Thermal plasma (e.g., sun's surface)
- Non-thermal atmospheric pressure plasma: Used in food processing
- Electrical discharge plasma: The plasma generated by electric or electromagnetic fields
- Several reactive species with short lifetimes



During plasma process

Ionization	e	+	M	$\rightarrow$	M^+	+	2e		
Dissociative ionization	e	+	AB	$\rightarrow$	A^+	+	B	+	2e
Electron attachment	e	+	M	$\rightarrow$	M^-				
Dissociative electron attachment	e	+	AB	$\rightarrow$	A^-	+	B		
Excitation	e	+	M	$\rightarrow$	M^*	+	e		

- Degradation of mycotoxins: chemical reactions with reactive species generated in the plasma volume such as positive and negative ions, O, O_3, OH, H_2O_2, and NOx
- Collision with electrons and ions leading to cleavage of molecular bonds

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Application of ACP to reduce mycotoxins in animal and poultry feed products (grains)

Current projects:

- Rapid detection and degradation of mycotoxins in animal and poultry feed materials (Supported by Alberta canola producers commission and Alberta agriculture and forestry)
- Development and understanding the efficacy of advanced technologies for mycotoxin detection and degradation in feed materials (Supported by Natural Sciences and Engineering Research Council, Canada)

In collaboration with McGill university (detection of mycotoxins) and industry partners

PhD student: Ehsan Feizollahi
Postdoctoral Fellow: Dr. Basheer Iqdiam

Alberta Agriculture and Forestry
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McGill UNIVERSITY
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Objectives

Overall objective

- Develop ACP based mycotoxin decontamination method

Specific objectives

- Understand the efficacy of ACP to degrade DON, zearalenone, T2- and HT-2 toxins, and ergot alkaloids on grains (e.g., barley, wheat, oat, canola)
- Identify the important product and process parameters, influencing the ACP efficacy to develop an effective mycotoxin decontamination technology
- Identify the degradation mechanisms

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Comprehensive REVIEWS in Food Science and Food Safety

Cold Plasma for Effective Fungal and Mycotoxin Control in Foods: Mechanisms, Inactivation Effects, and Applications

N.N. Misra, Barun Yadav, M.S. Roopesh, and Cheorun Jo

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2020.1743967
Taylor & Francis
Taylor & Francis Group

REVIEW

Check for updates

Factors influencing the antimicrobial efficacy of Dielectric Barrier Discharge (DBD) Atmospheric Cold Plasma (ACP) in food processing applications

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applied sciences
MDPI

Article

Effects of Atmospheric-Pressure Cold Plasma Treatment on Deoxynivalenol Degradation, Quality Parameters, and Germination of Barley Grains

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Received: 18 April 2020; Accepted: 17 May 2020; Published: 20 May 2020

check for updates

Food Engineering Reviews
https://doi.org/10.1007/s12393-020-09241-0

Check for updates

Degradation of Deoxynivalenol by Atmospheric-Pressure Cold Plasma and Sequential Treatments with Heat and UV Light

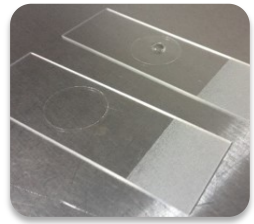
Ehsan Feizollahi[1] · Muhammad Arshad[1] · Barun Yadav[1] · Aman Ullah[1] · M. S. Roopesh[1]

Received: 16 March 2020 / Accepted: 9 July 2020

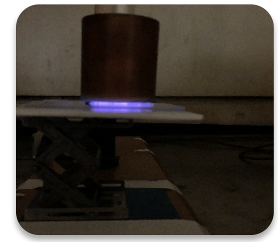
Reduction of pure DON by ACP

Methodology

20 µg DON/ml ACN: water

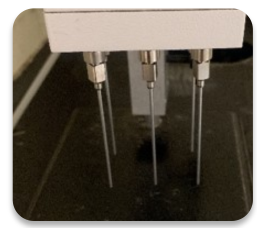


Air drying
(75 min)



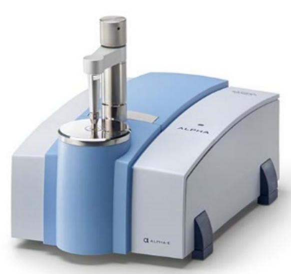
ACP treatment
Voltage: 30 kV,
Current: 1 A
Frequency: 3500 Hz
Pulse width: 10µs

Dissolving DON:
1 ml methanol, vortex for
1 min



Nitrogen evaporation

HPLC: acetonitrile/water (15/85, v/v) as mobile phase



FTIR spectrophotometer

Feizollahi, E., Arshad, M., Yadav, B., Ullah, A., Roopesh. M. S. (2020). Degradation of deoxynivalenol by atmospheric-pressure cold plasma and sequential treatments with heat and UV light. Food Engineering Reviews. https://doi.org/10.1007/s12393-020-09241-0.

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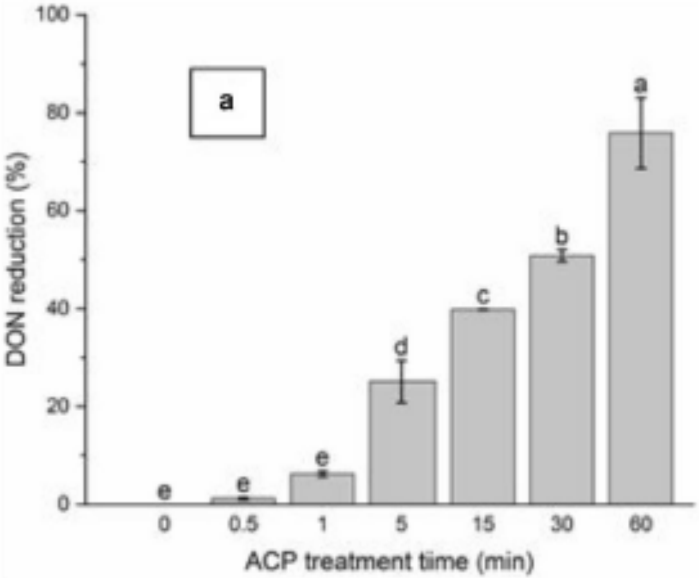
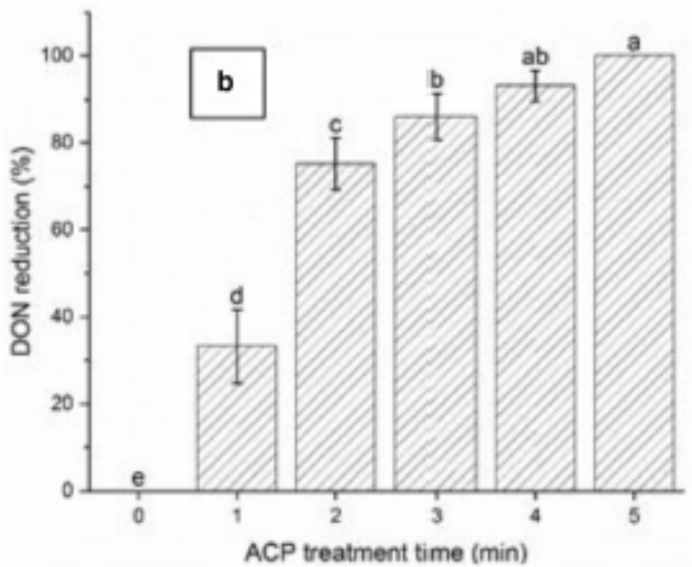
Results and Discussion



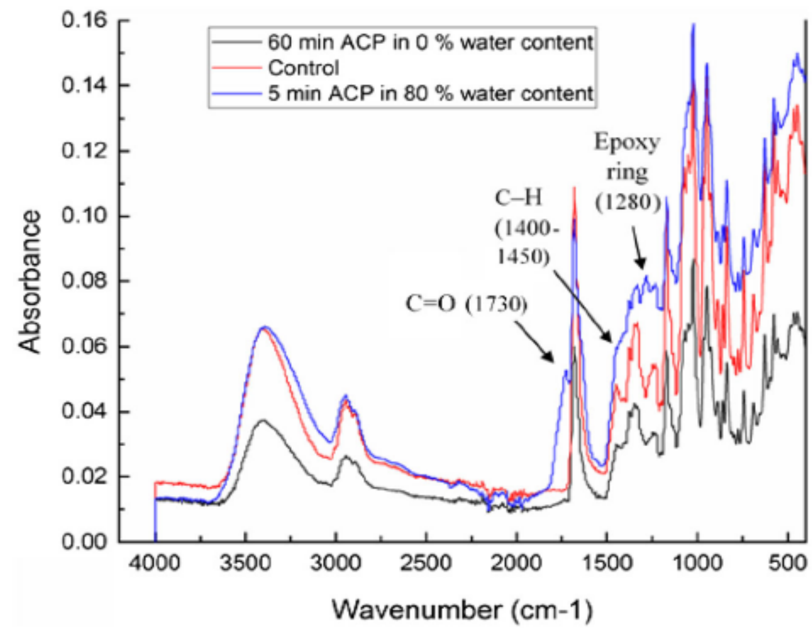


Effect of ACP treatment times on DON degradation (%)
a: 0% water, 0% ACN
b: DON (20 µg/ml) in ACN/water (20/80, v/v).

Treatment method	Average concentration (µg/ml)	Average reduction (%)
Control sample 0% ACN + water	20.2 ± 0.1^{a}	0^{d}
Control sample 100% ACN + water	20.1 ± 0.2^{a}	0^{d}
sample with 0% ACN + water, 2 min ACP	17.3 ± 1.0^{b}	14.1 ± 5.1^{c}
sample with 5% ACN + water, 2 min ACP	0^{d}	100^{a}
sample with 20% ACN + water, 2 min ACP	0^{d}	100^{a}
sample with 80% ACN + water, 2 min ACP	3.0 ± 1.8^{c}	84.8 ± 9.1^{b}

Values with different letters in the same column are significantly different ($p < 0.05$, $n = 3$)

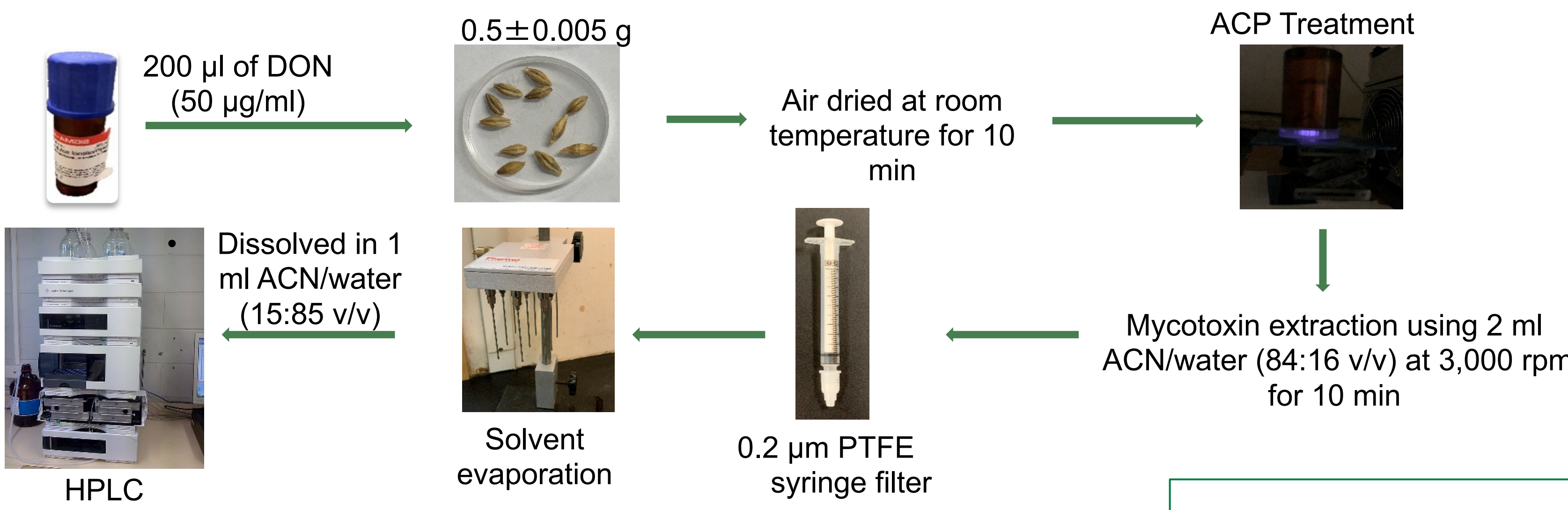
Effect of the initial ACN/ water content on the reduction of DON concentration by ACP

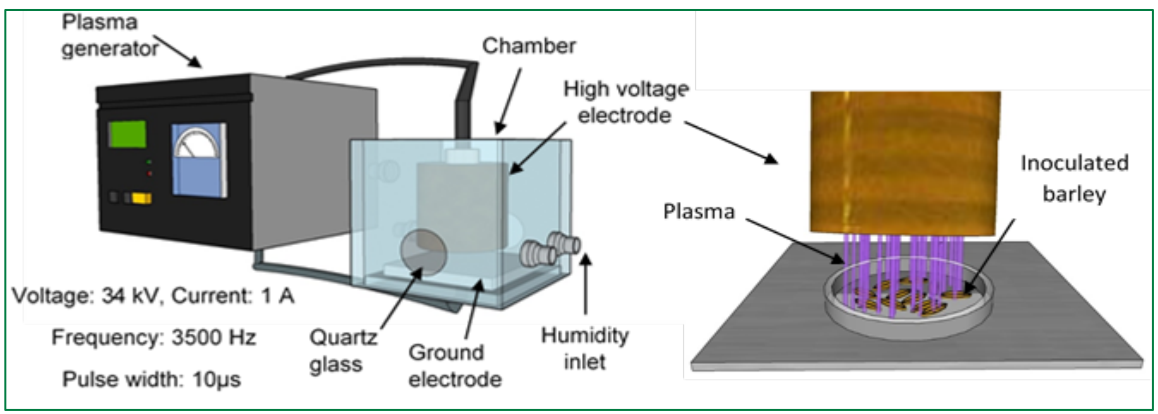


ATR-Fourier transform infrared spectrum of DON in solution (0.8 mg DON/ml ACN/water (20/80, v/v)) and dry mode (0% water, 0% ACN) after ACP treatment

Reduction of DON on barley by ACP

Reduction of DON on barley





ACP treatment system

Black comet C-25, StellarNet Inc., Tampa, USA

Dräger detector tubes

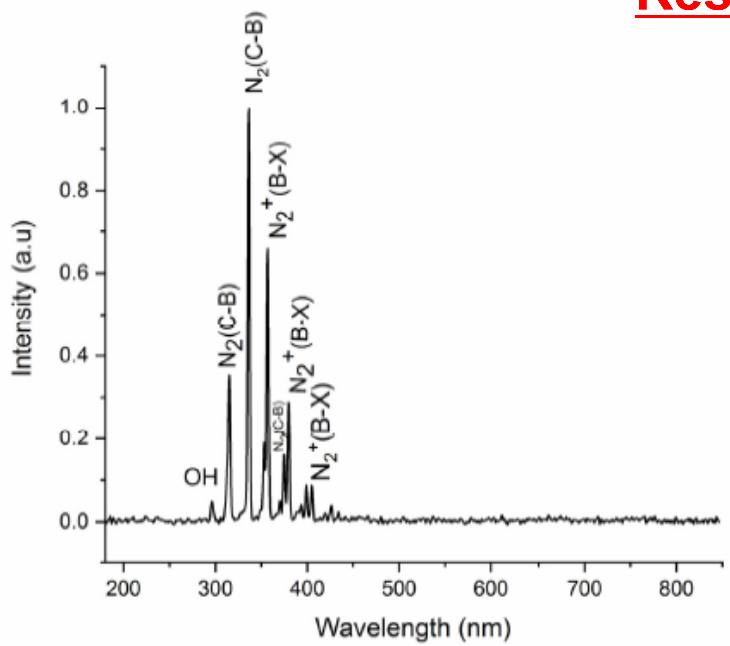
ACP characterization

Process/ product parameters

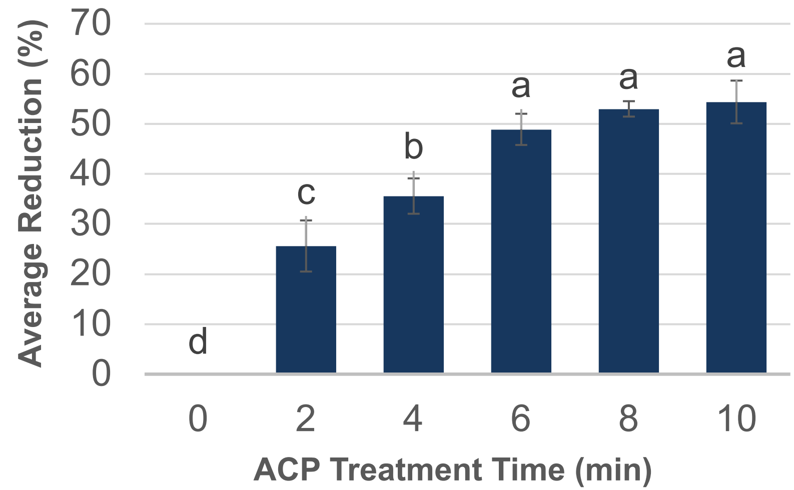
- Relative humidity
- Post treatment storage
- Moisture content

Quality and germination analysis

Results and Discussion



Optical emission spectra of air ACP



Efficacy of ACP Treatment on DON degradation on barley

Ozone, nitrous oxides and hydrogen peroxide concentration during ACP treatment

ACP Treatment Time(s)	O_3 (ppm)	H_2O_2 (ppm)	NO_x (ppm)
60–80	600	100	400
360–380	675	150	470
600–620	675	200	480

$$O + O_2 + M \rightarrow O_3 + M$$

Ozone generation

$$e + H_2O \rightarrow e + {}^{\cdot}OH + {}^{\cdot}H$$

$${}^{\cdot}OH + {}^{\cdot}OH + \rightarrow H_2O_2$$

$$HO_2^{\cdot} + HO_2^{\cdot} \rightarrow H_2O_2 + O_2$$

H_2O_2 generation

$$O_3 + HO_2^{\cdot} \rightarrow 2O_2 + {}^{\cdot}OH$$

$${}^{\cdot}OH + O_3 \rightarrow HO_2^{\cdot} + O_2$$

$${}^{\cdot}H + O_3 \rightarrow {}^{\cdot}OH + O_2$$

$${}^{\cdot}H + O_3 \rightarrow HO_2^{\cdot} + {}^{\cdot}O$$

$$2O_3 \rightarrow 3O_2$$

$${}^{\cdot}O + O_3 \rightarrow O_2 + O_2$$

$${}^{\cdot}N + O_3 \rightarrow NO + O_2$$

$$NO^{\cdot} + O_3 \rightarrow NO_2 + O_2$$

$$NO_2^{\cdot} + O_3 \rightarrow NO_3 + O_2$$

Nitrous molecules generation and ozone depletion

Results and Discussion

Quality Parameters of Barley Grains after ACP Treatment

Germination pouch

Treatment	N_2 (%)	Protein (%)	Carbon (%)	β-Glucan (%)	MC (g Water/100 g Sample)
Control	1.71 ± 0.02 [a]	10.68 ± 0.15 [a]	44.1 ± 0.2 [a]	3.96 ± 0.14 [a]	9.7 ± 0.1 [a]
6 min ACP	1.62 ± 0.05 [a]	10.39 ± 0.33 [a]	44.07 ± 0.21 [a]	3.98 ± 0.08 [a]	9.6 ± 0.0 [a]
10 min ACP	1.64 ± 0.05 [a]	10.26 ± 0.29 [a]	43.93 ± 0.55 [a]	4.23 ± 0.25 [a]	9.4 ± 0.2 [a]

Germination analysis of Barley Grains by by WinRHIZO software after ACP Treatment

Treatment	Average Root Length (cm)	Average Root Surface Area (cm^2)	Average Root Diameter (mm)	Root Volume (cm^3)	Shoot Length (cm)	Number of Roots	Germination Percentage (%)
Control	44.2 ± 17.8 [a]	6.4 ± 2.5 [a]	0.46 ± 0.04 [a]	0.07 ± 0.03 [ab]	6.8 ± 1.7 [a]	5.7 ± 0.6 [a]	80
1 min ACP	33.7 ± 19.4 [ab]	4.9 ± 2.5 [b]	0.49 ± 0.11 [a]	0.06 ± 0.03 [b]	6.4 ± 1.9 [a]	5.2 ± 1.5 [a]	83.3
6 min ACP	42.2 ± 15.7 [ab]	6.3 ± 2.2 [a]	0.49 ± 0.06 [a]	0.08 ± 0.03 [a]	7.4 ± 1.6 [a]	5.5 ± 0.8 [a]	93.3
10 min ACP	32.6 ± 20.0 [b]	4.8 ± 2.5 [b]	0.50 ± 0.07 [a]	0.06 ± 0.03 [b]	6.3 ± 2.1 [a]	5.1 ± 1.3 [a]	90

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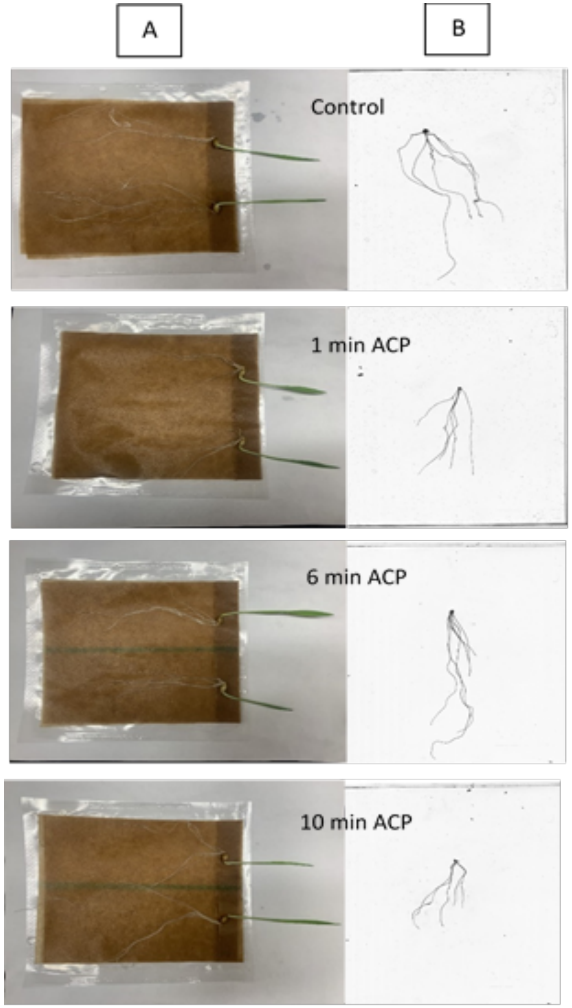
Results and Discussion



A: photo images. B: scanned root images using WinRHIZO software

Germination improvement by plasma. (Work done in our lab)

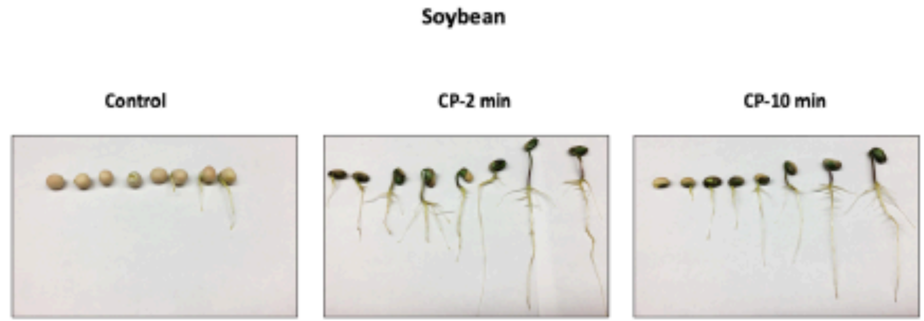


Figure 4. Influence of cold plasma (CP) treatment for 2 min and 10 min on soybean seed germination after one week

Figure 5. Influence of cold plasma (CP) treatment for 2 min and 10 min on lentil seed germination after one week

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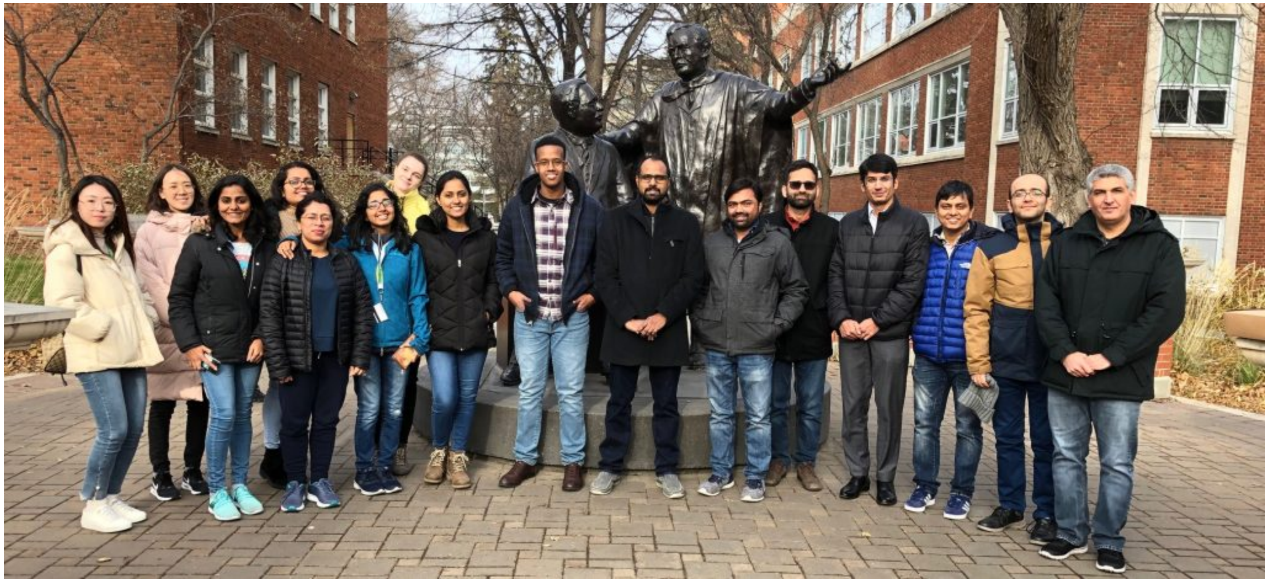
Concluding Remarks

- ACP has the potential to reduce DON on barley
- DON reduction by ACP is affected by product and process parameters
- Quality of barley was not affected by ACP and the germination % was increased

On-going and Future Research

- Testing of ACP to reduce other mycotoxins (e.g., ZEA, T2-, HT-2 toxins and ergot alkaloids in grains (e.g., wheat, oats, canola)
- Decontamination of natually contaminated grains by ACP
- DON reduction mechanisms & pathways
- Explore scale-up opportunities

Thank you!



Questions??

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