MycoKey actions on maize: 2016-2019 survey on the incidence of total aflatoxins in Romanian maize samples

MycoKey

Project:

Integrated and innovative key actions for mycotoxin management in the food and feed chain

iBA București

HORIZON 2020

European Commission

General information

MycoKey project has been funded by European Commission under Horizon 2020 programme, Societal challenge 2 "Food security, sustainable agriculture and forestry, marine, maritime and inland water research and the bioeconomy challenge" – topic " Biological contamination of crops and the food chain".
It aims to deliver the first integrated ICT based solutions to address mycotoxin contamination along the food and feed chain, by using a holistic and sustainable approach.

Horizon 2020

Call: H2020-SFS-2015-2
Topic: SFS-13-2015 Type of action: RIA
Proposal number: 678781-2
Proposal acronym: MycoKey

Coordinator: National Research Council - Institute of Sciences of Food Production - Italy

23 *Scientific Partners*
5 *Industrial Partners*
1 *Producers Association*
3 *Small Medium Enterprises*

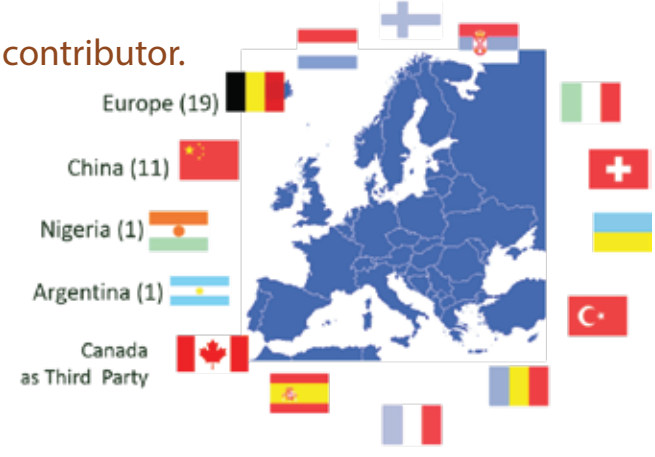
The MycoKey Consortium consists of 32 organizations from 14 countries of three continents (Europe, Asia and Africa).

It comprises

- 9 Universities
- 14 Research Centers and Institutions
- 8 companies
- 1 producer association
- and 1 Third party (external to the Consortium) as contributor.

EU-CHINA research cooperation

11 *Chinese Partners*



About MycoKey

Aims to generate innovative and integrated solutions that will support stakeholders in effective and sustainable mycotoxin management along food and feed chains

Will contribute to reduce mycotoxin contamination mainly in Europe and China
will address the major affected crops maize, wheat and barley, their associated toxigenic fungi and related mycotoxins (aflatoxins, deoxynivalenol, zearalenone, ochratoxin A, fumonisins)

Will integrate key information and practical solutions for mycotoxin management into a smart ICT

Tool (MycoKey App), providing answers to stakeholders, who require rapid, customized forecasting, descriptive information on contamination risk/levels, decision support and practical economically-sound suggestions for intervention

Tools and methodologies will be strategically targeted for cost-effective application in the field and during storage, processing and transportation

Alternative and safe ways to use contaminated batches will be also delivered

The focus of MycoKey

Innovating communications of mycotoxin management by applying ICT, providing input for legislation, enhancing knowledge and networks

Selecting and improving a range of tools for mycotoxin monitoring

Assessing the use of reliable solutions, sustainable compounds/green technologies in prevention, intervention and remediation

IBA BUCHAREST

Project responsible: Irina Smeu
Team members "Biocontrol of *Aspergillus flavus* to prevent aflatoxin production" task :
Elena Mirela Cucu, Alina Alexandra Dobre
Laboratory technicians: Constanta Pirvu, Veta Mertescu

WP 4 – Prevention in the field

Biocontrol of *Aspergillus flavus* to prevent aflatoxin production
Reduction of *Fusarium* inoculum and mycotoxins in barley, wheat and maize through agronomic measures
Biological control of *F. graminearum* with fungal and bacterial antagonists on preceding crop residues

WP 8 – Communication, Dissemination & Exploitation

Technological Workshops
Training for mycotoxin management

MycoKey actions on maize: 2016-2019 survey on the incidence of total aflatoxins in Romanian maize samples

Introduction

According to the Romanian Ministry of Agriculture and Rural Development, with regard to general agriculture data, from the 23.8 million hectares, which represents the Romanian territory, the agricultural exploitation is about 13.3 million ha (55.9%), of which about 8.3 million ha (55.9%) represents the arable land.

In Romania, cereals and oil plants represent approx. 80% of the total arable land. Maize (approx. 2512.8 thous. ha and a production of 11988.6 thous. tons) followed by wheat and rye (approx. 2123.1 thous. ha, with a production of 7609.2 thous. tons) are the main cereals cultivated in Romania.

Since the agriculture practices and also particular soil and climatic conditions have a major influence on the levels of mycotoxins in cereals such as maize, wheat and barley, a preventive system is required. Moreover, aflatoxins became a major concern for maize production in Europe, after various outbreaks were noted.

2016 2017 2018 2019

Aflatoxins are mycotoxins produced mainly by *Aspergillus flavus and Aspergillus parasiticus*. Aflatoxins can contaminate maize, cotton, pistachio nuts and peanuts during the growing season and after harvest. They are potent carcinogens and hepatotoxins and thus present serious food safety problems (IARC, 2012).

Considerable knowledge is available regarding maize – *A. flavus* – environment interaction components (e.g. Bhatnagar, 2012; Reverberi et al., 2013), but its translation to minimise pathogen impact on the safety of agricultural products is still a very complex task.

Aflatoxins became a main concern for maize production in Europe since 2003, when a severe outbreak was observed in Italy (Piva et al., 2006). Romania also faced contamination problems (Gagiu et al., 2018; Stanciu et al., 2018).

Therefore, a maize survey on the incidence of total aflatoxins was started by IBA Bucharest in 2016 and continued until 2019. Around 500 samples were collected from all Romanian counties.

Bhatnagar, D. 2012. Biotechnology Summit 2012, Merida, Yucatan, Mexico, pp. 192-196.

Gagiu, V., Mateescu, E., Armeanu, I., Dobre, A.A., Smeu, I., Cucu, M.E., Oprea, O.A., Iorga, E., Belc, N. 2018. Post-harvest contamination with mycotoxins in the context of the geographic and agroclimatic conditions in Romania. Toxins. 10, 533.

International Agency for Research on Cancer (IARC) (2002) vol. 82. IARC, Geneva, pp. 301-366.

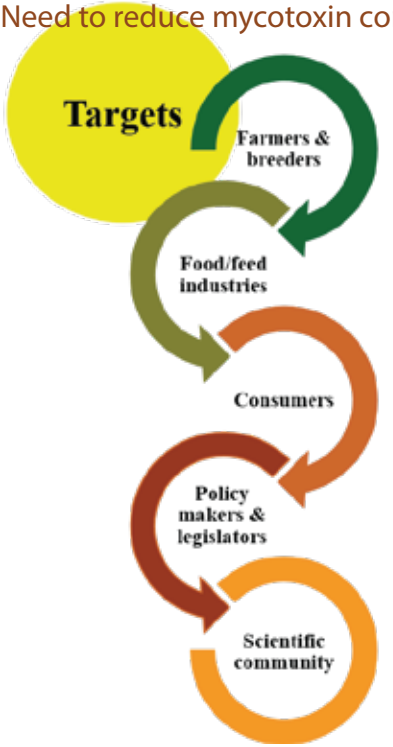
Piva, G., Battilani, P., Pietri, A. Emerging issues in Southern Europe: Aflatoxins in Italy. In: Barug, D., Bhatnagar, D., van Egmond, H.P., van der Kamp, J.W., van Osenbruggen, W.A., Visconti, A. (eds). The Mycotoxin Fact Book: Food and feed topics. Wageningen, the Netherlands: Academic Publishers. 2006. p. 139-153.

Reverberi, M., Punelli, M., Scala, V., Scarpari, M., Uva, P., et al. 2013. Genotypic and Phenotypic Versatility of *Aspergillus flavus* during Maize Exploitation. PLOS ONE 8(7): e68735.

Stanciu, O., Juan, C., Berrada, H., Miere, D., Loghin, F., Mañes, J. 2019. Study on Trichothecene and Zearalenone presence in Romanian wheat relative to wheather conditions. Toxins. 11(3): 163.

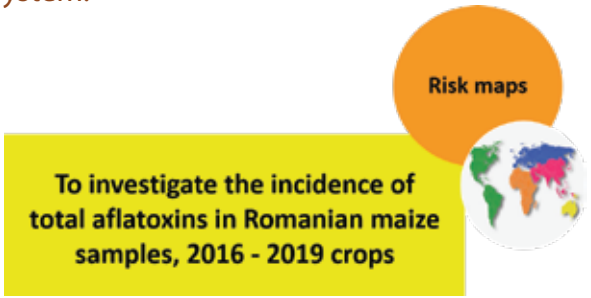
Crops & Mycotoxins Challenges

Climate impacts at different stages of the crop lifecycle
Emerging health risks linked to mycotoxins
Need for an effective and sustainable mycotoxin management along food and feed chain
Need to reduce mycotoxin contamination



Objective

A maize survey was conducted in Romania, to monitor the occurrence of total aflatoxins in maize samples collected during the 2016-2019 growing seasons from fields located in all counties. All samples were collected along with information regarding the applied agronomic practices and cropping system.

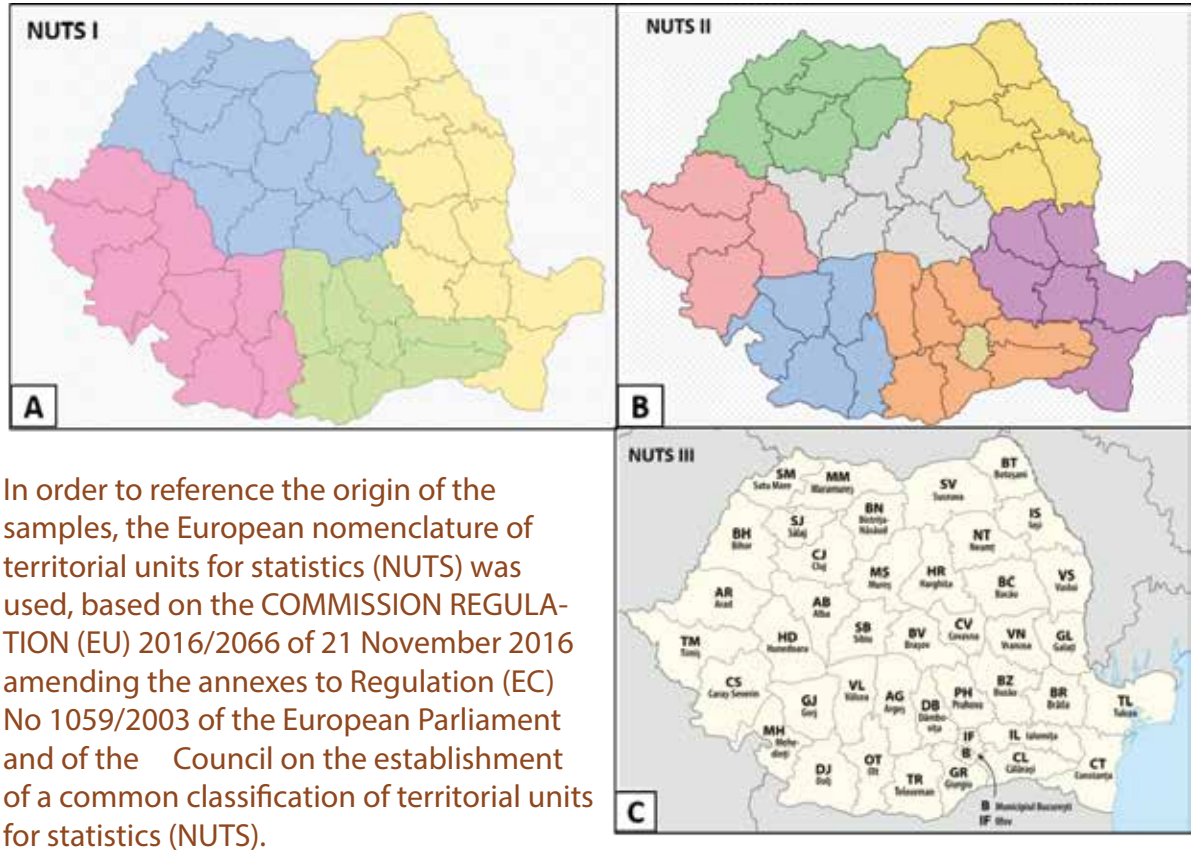


Sampling

Unprocessed maize samples were collected during the 2016-2019 harvest seasons from all around Romanian counties, along with information regarding specific location of maize fields, hybrid type, crop details and applied agronomic practices.
Sampling was performed according to the European Union (EU) guidelines[1]).

The sample collection was realised in collaboration with the Ministry of Agriculture and Rural Development and the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Agriculture.

1) Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs



In order to reference the origin of the samples, the European nomenclature of territorial units for statistics (NUTS) was used, based on the COMMISSION REGULATION (EU) 2016/2066 of 21 November 2016 amending the annexes to Regulation (EC) No 1059/2003 of the European Parliament and of the Council on the establishment of a common classification of territorial units for statistics (NUTS).

Distribution of the maize samples

NUTS III level

Maize monitoring questionnaire

Name and address incl. postal code Tel.: E-Mail:			Production type: Conventional ☐ Integrated ☐ Bio ☐
Variety:	Previous Crop, if **maize, please indicate if silage or grain maize:**		Pre-Previous Crop:
Die you observe Fusarium head blight in your field? Yes ☐ No ☐ comment:			
Combine with crop residue chopper	☐ yes	☐ no	☐ unknown
Additional chopping of crop residues	☐ yes	☐ no	
Plough	☐ yes	☐ no	
Incorporation of crop residues: chisel plough☐: spring tine harrow☐: disc harrow☐: rotary harrow☐: Rototiller ☐			Other: ☐ .....
No-till ☐ yes ☐ no			
Sowing date:	Begin of flowering (DC 61):		Harvesting date/yield:
N-fertilisation: kg N / ha	1	2	3
Fungicide, if used	Product	Date	Growth stage
Growth regulator, if used	Product	Date	Growth stage

Instructions for sampling

The grain sample can be taken directly after threshing form the combine, from trailers or from the silo. Please take ten individual subsamples and mix them carefully to one sample of approx. 1kg for the respective field.

- Field location
- Farmers' contacts
- Crop details
- Hybrid type
- Previous crop
- Crop residues

Screening of aflatoxin contamination

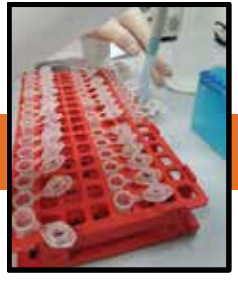
The incidence of total aflatoxins in maize samples was investigated using ELISA (Enzyme Linked Immunosorbent Assay) method (RIDASCREEN® Aflatoxin Total, R-Biopharm AG, Germany).

All samples were first finely ground using an ultra centrifugal laboratory mill (Retsch GmbH, Germany) and mixed thoroughly to achieve complete homogenization. Furthermore, 2 grams of grinded sample were homogenized in 10 mL 70% methanol (v/v). Samples were homogenized vigorously using an orbital shaker (GFL Gesellschaft für Labortechnik GmbH, Germany) and then filtered using Whatman No. 1 filter paper.

Further, samples were diluted according to the test kit manual, using distilled water.

50 µL standards and samples, in duplicate, were added into different wells. Enzyme conjugate and antibody solution were added to each well and further, the plate was incubated at room temperature for 30 minutes. After washing the plate (PBS tween buffer), 100 µL substrate/chromogen were added and after 15 minutes incubation time at room temperature, 100 µL stop solution were added.

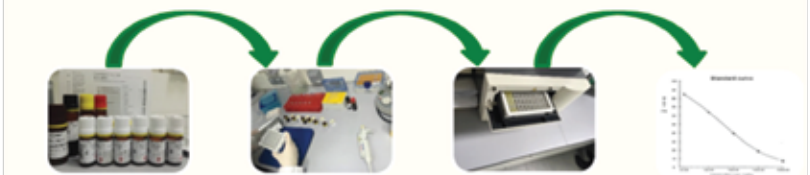
The plates were read on a Sunrise™ plate reader (Tecan Group Ltd., Switzerland). For each sample, two replicates have been used. The average of these samples has been employed in data analysis.
A reference material was used for each measurement (Trilogy Analitical Laboratory, US).

Method of analysis – RIDASCREEN® Aflatoxin Total kit
(R-Biopharm AG, Germany)

Sample preparation

ELISA method

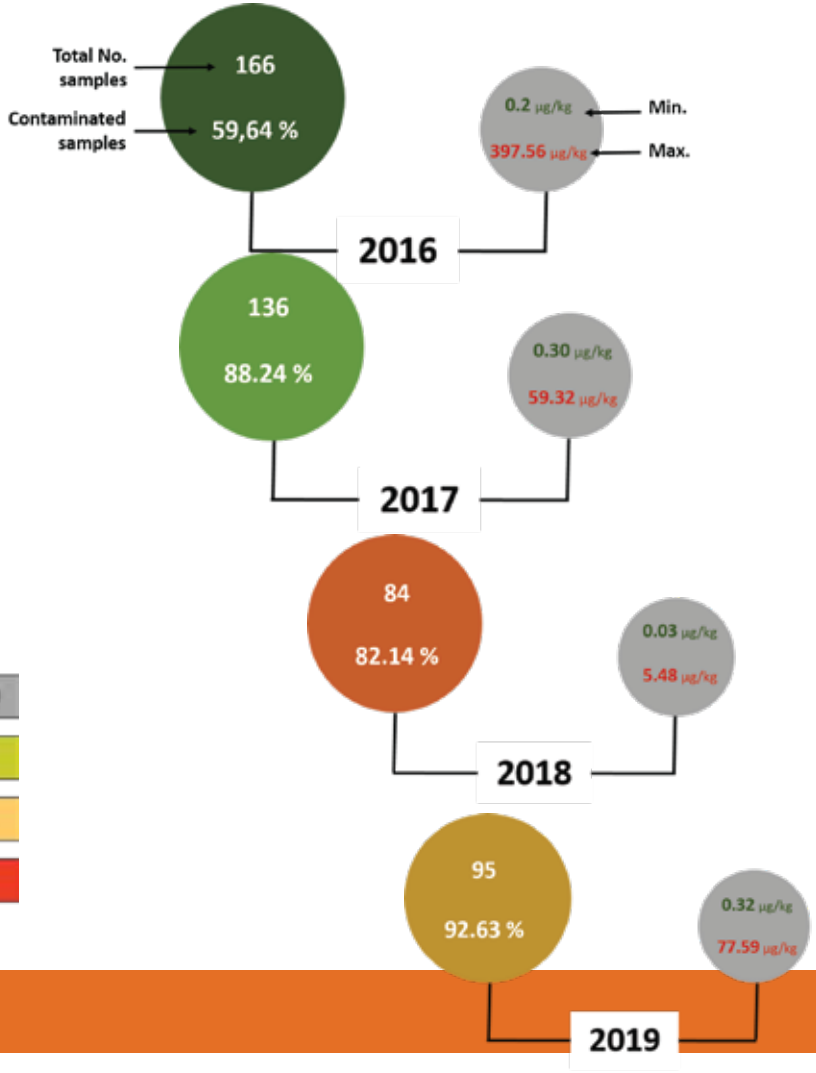


Results

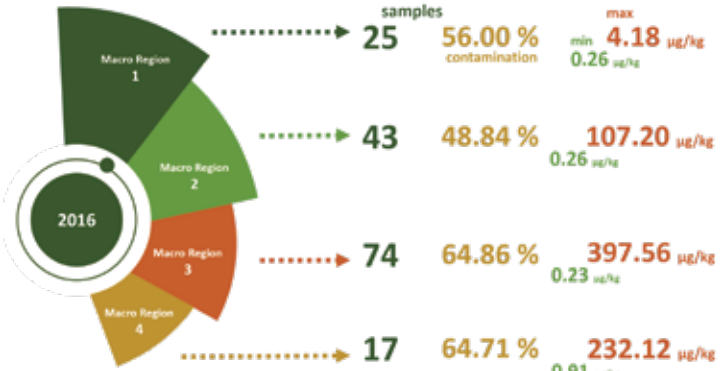
According to the European Union (EU) guidelines[2], the maximum levels of total aflatoxins for maize to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs are 10.00 µg/kg.

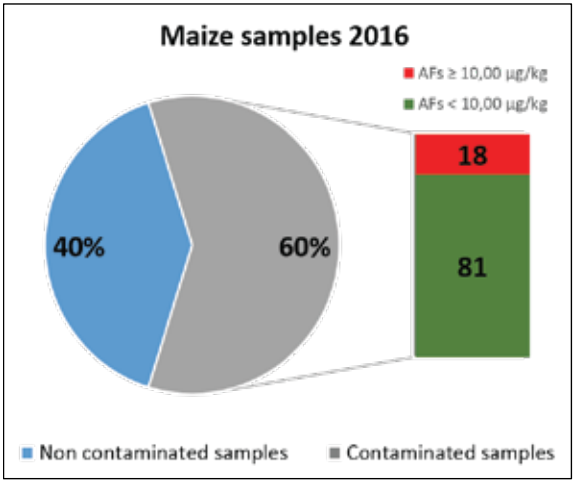
2) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

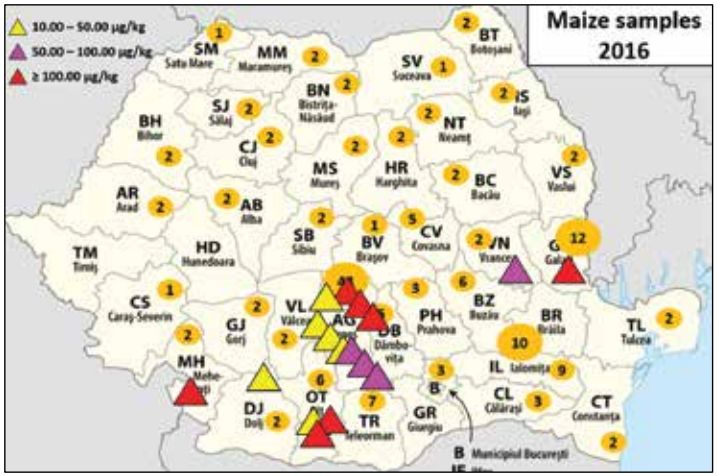
	2016	2017	2018	2019
Total maize samples	166	136	84	95
Contaminated samples	99	120	69	88
AFLA ≥ 10.00 µg/kg	18	4	0	1



Results - 2016







Out of the 166 maize samples assessed in 2016, 14 samples noted total aflatoxin levels higher than 10.00 µg/kg, the limit set by the Commission Regulation (EC) No 1881/2006 for maize (subject to soring or other physical treatment before human consumption or use in foodstuffs). When referring to the analysed maize samples, the southern regions of Romania were the most affected by the incidence of total aflatoxins:

9 samples from the southern region of Romania (Argeș county, South-Muntenia development region, Macro region 3);

3 samples from the southeastern region (Vrancea and Galați counties, South-Ea development region, Macro Region 2);

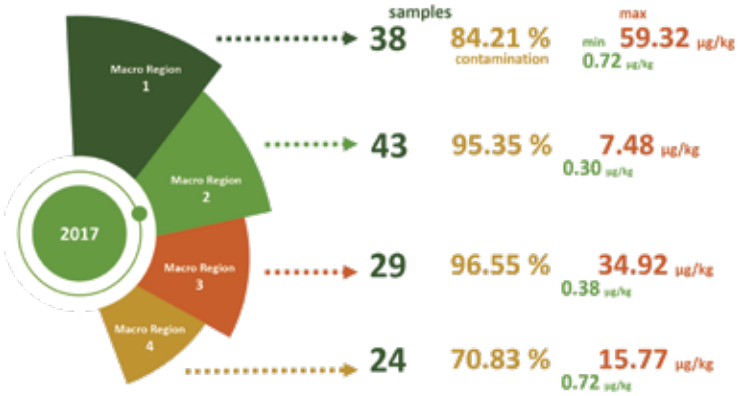
2 samples from the southwestern part of the country (Mehedinți and Olt counties, South-West Oltenia development region, Macro Region 4).

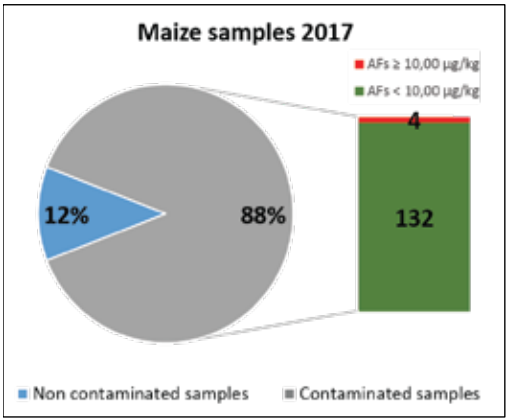
Argeș County noted the highest levels of aflatoxins (397,56 µg/kg). This county presents mainly moderate and strongly textured soils, with low permeability to water and low humus content, with slow nitrification and biologically poor biologically active soils.

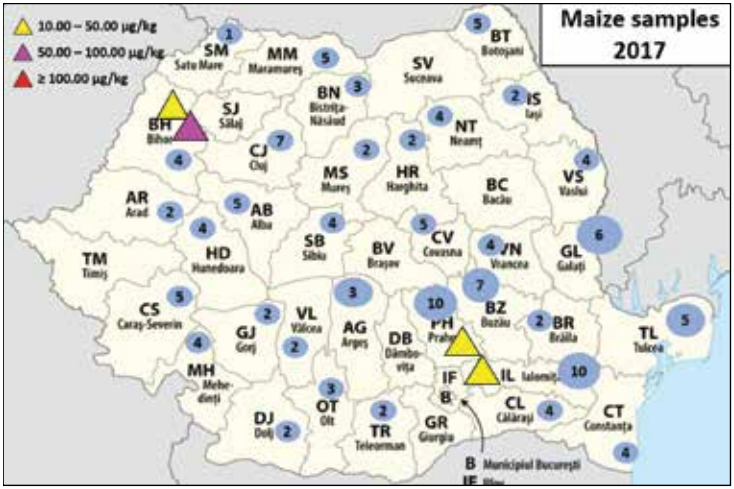
Taking into consideration the information provided by the monitoring questionnaires, the southern counties noted poor agricultural practices, also.

Romanian maize hybrids represented 13.5 % of the total number of recorded hybrids.

Results - 2017



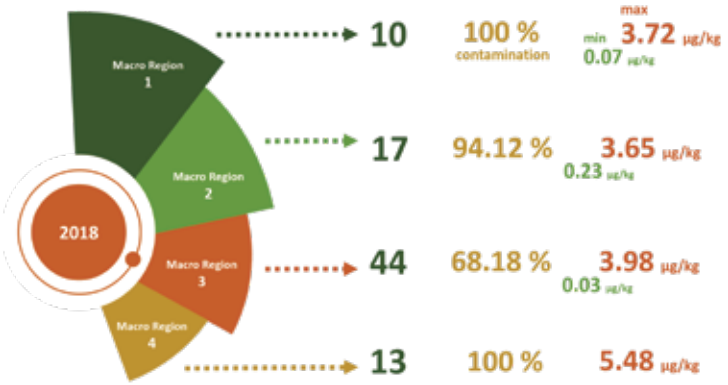


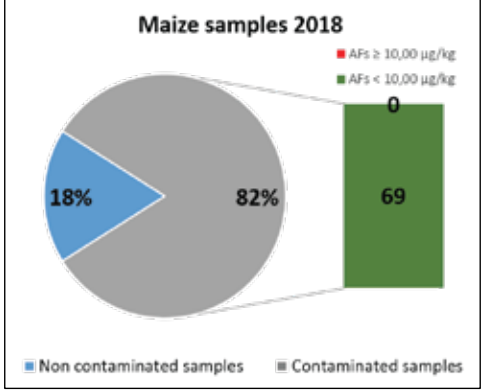


In 2017, the aflatoxin contamination was substantially lower than in 2016. Only 6 samples exceeded the concentration of 10.00 µg/kg. The highest concentration of total aflatoxins was 59.32 µg/kg, noted by a maize sample from Bihor county (North-West development region, Macro region 1).

The presence of total aflatoxins was independent of the type of maize hybrid and previous crop.

Results - 2018



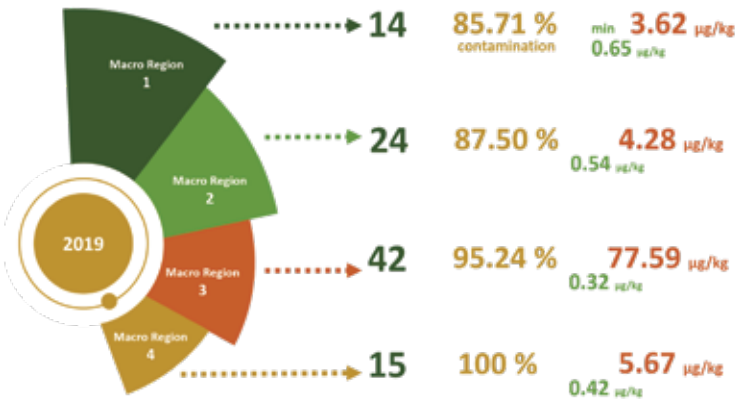


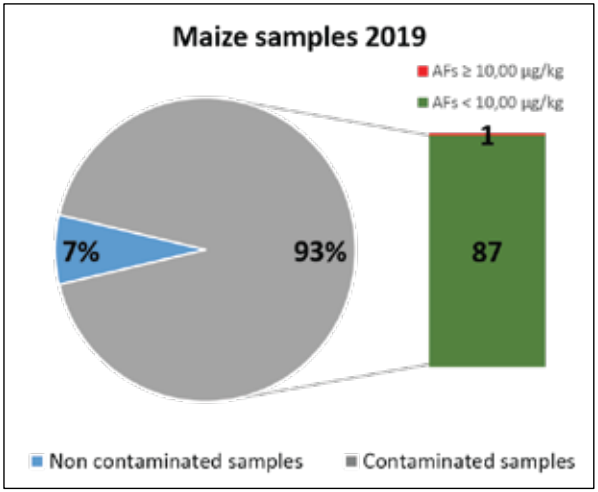
Out of the 84 maize samples which were assessed, only for 2 samples the origin of the fields was unknown. The majority of the samples were collected from the Macro Region 3 (52.38%), where the Sud-Muntenia development region registered the highest number of maize samples (43 samples). While there were received an average number of 2 maize samples from each county, Calarasi county was noted with 31 sent samples.

The highest concentration of total aflatoxins (5.48 µg/kg) was noted by a sample from Arad county, Vest development region (West), Macro Region 4. Out of the 84 analyzed maize samples, 69 samples noted an aflatoxin contamination. Regarding the contaminated samples, Macro Region 3 noted the highest number of samples with concentrations of total aflatoxins over 1.75 µg/kg (8 samples, which represented 18.18% of the total number of samples coming from this region). Macro Region 4 (the south-western and western areas of Romania) noted the smallest number of contaminated samples over 1.75 µg/kg (2 samples).

Regarding the type of hybrid, Pioneer (32 samples) and Maisadour Semences (20 samples) ranked as the first producers, followed by Dekalab (18 samples) and Syngenta (13 samples). The Romanian hybrids represented only a percent of 3.57% out of the total analyzed samples (3 samples). There was not observed a correlation between the hybrid type and the concentration of total aflatoxins

Results - 2019





When referring to the 2019 maize samples, Macro Region 4 of Romania was the most affected by the incidence of total aflatoxins (100% contaminated samples). Out of the total number of assessed samples (95 maize samples), 31 noted concentrations of total aflatoxins over 1.75 µg/kg (32.63%).

The highest concentration of total aflatoxins (77.59 µg/kg) was noted once again by a sample from Argeș county, South-Muntenia development region, Macro region 3. A number of 42 maize samples were collected from this Macro Region and 40 samples were found contaminated with total aflatoxins (95.24%), while 15 samples noted concentrations over 1.75 µg/kg.

Macro Region 1 noted only 2 samples with total aflatoxin concentrations over 1.75 µg/kg.

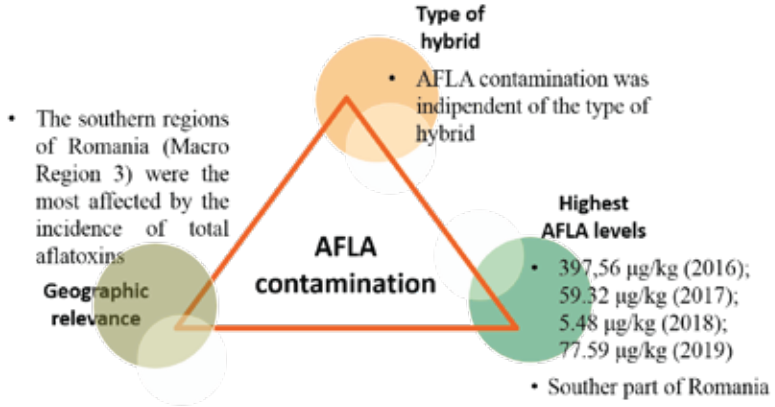
Conclusions

Aflatoxin contamination of maize samples (harvest 2016-2019) was independent of the type of hybrid. When referring to the analysed maize samples, the southern regions of Romania were the most affected by the incidence of total aflatoxins.

The highest concentration of total aflatoxins (397.56 µg/kg) was noted in 2016, by a maize sample from Argeș county (South-Muntenia development region, Macro region 3). Macro Region 3 noted to be the most affected region in regard to total aflatoxin contamination and Argeș county in special.

In 2016, there was noted the highest number of contaminated samples (18 samples), with concentrations over the maximum levels for total aflatoxins for maize to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs (10.00 µg/kg). However, the 2018 harvest did not noted any contaminated samples over this limit.

This comprehensive investigation of the Romanian maize samples was done for the first time in Romania for the occurrence of total aflatoxins. The present study can contribute to the effort to reduce fungi and mycotoxin contamination in maize and can represent an important step in the risk assessment strategies for mycotoxin mitigation.



Publications arising from the task

Publications

Smeu, I., Cucu, E.M., Dobre, A.A., Casian, H. (202X). Romanian maize cropping system: A three year survey on total aflatoxins. Toxins (in preparation).

Smeu, I., Cucu, E.M., Dobre, A.A., Casian, H. (202X). Romanian barley cropping system: A 2019 survey on deoxinivalenol and zearalenone. Annals. Food Science and Technology (in preparation).

Smeu, I., Cucu, E.M., Dobre, A.A., Casian, H. (202X). A 2018-2019 survey on deoxynivalenol (DON) in Romanian barley (Hordeum vulgare L.) samples. Journal of Plant Biology and Crop Research (submitted).

Smeu, I., Cucu, E.M., Dobre, A.A., Casian, H. (2020). A 2019 study on total aflatoxins in Romanian maize (Zea mays L.) samples. Romanian Journal of Plant Protection. Vol. XIII, pp. 1-8 (ISSN 2248 – 129X; ISSN-L 2248 – 129X).

Smeu, I., Casian, H., Cucu, E.M., Dobre, A.A. 2018. Recolta de porumb 2016: Studiu privind reducerea riscului de contaminare cu aflatoxine totale. Brutarul. XVIII(2), pp. 20.

Oral presentations

Smeu, I., Casian, H., Cucu, E.M., Dobre, A.A. – Importanța realizării hărților de risc privind contaminarea cu micotoxine. The XXVIII Conference of the National Plant Protection Society of Romania (SNPPR), 7-8 November 2019, Călimănești-Căciulata, Romania.

Smeu, I., Casian, H., Cucu, E.M., Dobre, A.A. - MycoKey actions on maize: 2016-2017 survey to decrease the risk of aflatoxin contamination in Romania. The 2nd MycoKey Technological Workshop, 7-8 May 2018, Bucharest, Romania.

Smeu, I., Casian, H., Cucu, E.M., Dobre, A.A. - Maize survey to decrease the risk of aflatoxin contamination in Romania. The International Conference of the University of Agronomic Sciences and Veterinary Medicine of Bucharest "Agriculture for Life, Life for Agriculture", 8-10 June 2017, Bucharest, Romania.

Smeu, I., Casian, H., Cucu, E.M., Dobre, A.A. - Recolta de porumb 2015-2016: Studiu privind reducerea riscului de contaminare cu aflatoxine totale. The 2017 Symposium of the Romanian Flour Milling and Bakery Specialists Association – ASMP, 30-31 August 2017, Bucharest, Romania.

Poster presentations

Smeu, I., Cucu, E.M., Dobre, A.A., Casian, H. – Elaborarea hărților de risc pentru contaminarea cu aflatoxine totale a culturii de porumb. Sesiunea anuală de comunicări științifice a Institutului de Cercetare-Dezvoltare pentru Protecția Plantelor – ICDPP București "Protecția plantelor – cercetare interdisciplinară în slujba dezvoltării durabile a agriculturii și a protecției mediului", 15 November 2019, Bucharest, Romania.

Dobre, A.A., Cucu, E.M., Casian, H., Smeu, I. – Incidența aflatoxinelor totale în probe de porumb din România, culturi agricole 2016-2018. The XXVIII Conference of the National Plant Protection Society of Romania (SNPPR), 7-8 November 2019, Călimănești-Căciulata, Romania.

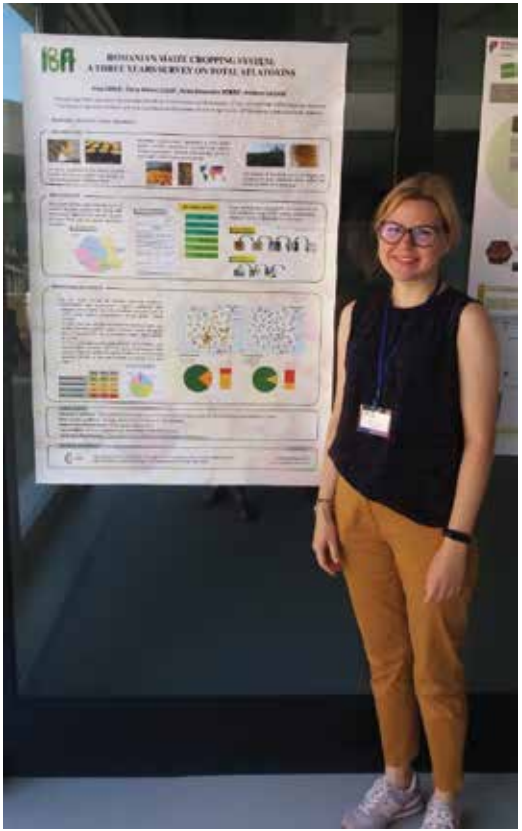
Smeu, I., Cucu, E.M., Dobre, A.A., Casian, H. - Romanian maize cropping system: A three year survey on total mycotoxins. 3rd International Conference on Food Contaminants. Challenges in Risk Assessment (ICFC 2019), 26-27 September 2019, Aveiro, Portugal.

Smeu, I., Cucu, E.M., Dobre, A.A. - Maize survey on aflatoxin contamination in Romania. 1st MycoKey International Conference, 11-14 September 2017, Ghent, Belgium.

Mitran, A., Belc, N., Cucu, E.M., Dobre, A.A., Smeu, I. - Biological control agents: tailored formulations for mycotoxin control. The International Conference of the University of Agronomic Sciences and Veterinary Medicine of Bucharest "Agriculture for Life, Life for Agriculture", 8-10 June 2017, Bucharest, Romania.

Smeu, I., Cucu, E.M., Dobre, A.A., Tivig, I.C., Adascalului, M., Niculae, O.M. - Preliminary 2000-2014 Romanian wheat survey to decrease the risk of mycotoxin contamination in cereals. The International Conference of the University of Agronomic Sciences and Veterinary Medicine of Bucharest "Agriculture for Life, Life for Agriculture", 8-10 June 2017, Bucharest, Romania.


ANF



Trainings, dissemination activities

IBA Bucharest hosted the meeting of the MycoKey's Working Group 1 – Maize (6 June 2018, Bucharest, Romania). The event reunited 14 participants from 6 countries (Italy, Romania, Nigeria, Argentina, Hungary, United States). The specific objective of WG1-maize is to prepare Good Agricultural Practices (GAP), Good Management Practices (GMP) and Good Storage Practices (GSP) for minimizing mycotoxins (aflatoxins, fumonisins and deoxynivalenol) along the maize chain, to reduce human and animal exposure, addressed to stakeholders. Based on WG1-maize discussions, short messages, suggestions for farmers, will be also discussed. They are crucial input for the MycoKey app, planned to keep farmers updated regarding the mycotoxin contamination risk during the growing season, based on predictive modelling.

IBA Bucharest organized the 2nd MycoKey technological workshop "MycoKey: a new approach for mycotoxin management in the maize chain in East Europe" (7-8 June 2018, Bucharest, Romania). The event hosted approx. 70 participants (scientific researchers, representatives of Romanian authorities and food industry) from 7 different countries. The workshop included 14 presentations on the mitigation of mycotoxin contamination in the maize chain, open discussions on smart management of mycotoxins, sustainable management of agricultural challenges, food security, sustainable agriculture, marine and maritime research and the bioeconomy, rural development programs, main challenges of Romanian maize processors, safe control: rapid detection of mycotoxins. More details: http://bioresurse.ro/the-2nd-mycokey-technological-workshop/

In the framework of the MycoKey fellowships, a short term mission took place (01.09.2017 – 31.10.2017), in collaboration with UCSC. The young researcher Ioan Cristian Tivig performed the research assignment "Selection of *Aspergillus flavus* strains as candidate biocontrol agents to prevent aflatoxin contamination in maize in Romania". This STM represents a support to the biocontrol of *A. flavus* actions within MycoKey project, the activities being part of WP 4.1, focused on the selection, isolation techniques and characterisation of Romanian atoxigenic strains of A. flavus, in order to reduce aflatoxin contamination in Romanian maize.

Bachelor students of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, A. Mitran (Faculty of Biotechnologies) and G. Bubu (Faculty of Agriculture) were trained in the ELISA laboratory of IBA Bucharest on biocontrol agents, artificial infection of wheat experimental fields and filamentous fungi assessment.

www.mycokey.eu

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