



After three years of research and extensive programming and modelling, **MyToolBox – the smart way to tackle mycotoxins along the food- and feed chain** (www.mytoolbox.eu/) was presented to a wider audience at its first stakeholder meeting in China in April 2019. It served not only as a demonstration of its scientific results, but also to understand the end-users' needs and requirements to tackle mycotoxin contamination in their industry field. Above all, the stakeholders' feedback was vital to understand the usability of mycotoxin research – and thus the MyToolBox e-platform.

MyToolBox' scientific results promise high-impact and applicability all along the food and feed production chain: The use of resistant maize cultivars resulted in a reduction of aflatoxin B₁ of up to 98% by also ensuring similar yields compared to other cultivars. Using an atoxigenic *Aspergillus* strain isolated for application in Serbia reduced mycotoxin contamination up to 86%. To complement these mycotoxin reduction strategies in fields, forecasting models were developed and validated to estimate the mycotoxin contamination risks in cereal fields based on weather data recorded closest to the fields. If harvests are nevertheless deemed unfit for food or feed, using recombinant enzymes in bioethanol production to treat ZEN and FUM contamination offers a promising alternative, by reducing FB₁ and ZEN by 99% and 89% respectively (detoxification was verified by determining their non-toxic hydrolyzed metabolites, HFB₁ and hydrolyzed ZEN).

After harvest, effectively monitoring storage conditions of cereals in silos is key for further processing: The MyToolBox sensors include a novel early-warning parameter of CO₂ which allows a more sensitive indication of risk from mycotoxin contamination – ZEN in wheat, aflatoxin in maize – between 3-5 days earlier than detected by temperature changes. The last year of the project's duration will serve to validate the developed sensors in our Chinese partners' silos. In addition, sorting of dried figs ensures Further processing of wheat combines multiple technologies to produce flour, including sorting out of unfit kernels, milling, fractionation and micronization. The most effective combination has been tested in industrial scale at the MyToolBox partner BARILLA by which up to 15% more branched material can be used for pasta production – and thus could be redirected to human consumption instead of feed for livestock. This fibre-rich material has been included in pasta formulation, increasing dietary fibre content by 3% in the final wholegrain pasta product and thus providing for a significant health benefit to the consumer – the novel MyToolBox pasta. In addition, untargeted LC-MS based ¹³C labelling provided a full mass balance of deoxynivalenol and its degradation products formed during baking of crackers, biscuits and bread, indicating DON to degrade largely to its less toxic metabolite isoDON (Stadler, D., et al. (2018): <https://doi.org/10.1016/j.foodchem.2018.11.150>).

These scientific results, complemented by readily available information, have been integrated into the MyToolBox e-platform. Extensive feedback from stakeholders located at various points of the food and feed production chain ensured usability, appeal and understandability of the information and tools presented in the e-platform – which will be demonstrated soon at the Smart Farm Conference in Venlo, the Netherlands, on June 27, 2019 (<https://smartfarmingconference.com/>) and at the World Mycotoxin Forum in Belfast, UK, from October 14-16, 2019 (<https://www.worldmycotoxinforum.org>).

Impacts:

Biofumigation studies (WP1) showed allyl isothiocyanates exhibit the highest inhibitory effect with near complete inhibition of *F. graminearum* mycelial growth at 100 mg/L. Studies of biocontrol with crop debris treatments are continuing.

Using aflatoxin resistant plant cultivars resulted in up to 98% reduction in aflatoxin B₁.

Studies of biocontrol with biopesticides are continuing. So far biopesticides show reductions of up to 17% and a novel non-triazole fungicide has shown high efficacy (91% reduction).

The use of atoxigenic *Aspergillus* strain in maize fields in Serbia reduced aflatoxin B₁ up to 86%.

In modelling different sampling and analysis techniques, on-site detection with lateral flow devices are more cost-effective than aggregate sampling when analysing wheat for fumonisins. Testing maize for aflatoxins requires an aggregate sample before analysis, thus on-site detection is less suitable.

The MyToolBox sensors include a novel early-warning parameter of CO₂ which allows a more sensitive indication of risk from mycotoxin contamination which is between 3-5 days earlier than that detected by temperature changes for) before ZEN and aflatoxin contamination which exceeds legislative limits of wheat and maize respectively.

Samples of dried figs were analysed for aflatoxins and metabolites from *Aspergillus* and other fungal genera using an LC-MS/MS based multi-mycotoxin method developed and validated at IFA/BOKU. Determining the most accurate wavelength bands to accurately classify aflatoxin B₁ contaminated dried figs required testing the camera and algorithms at BOKU in Tulln. Fig slices were inoculated with O-methylsterigmatocystin (Afla precursor), tenuazonic acid (an *Alternaria* metabolite that was found in large concentrations in all sample classes), kojic acid and ochratoxin A that have similar reflective characteristics like AFB₁ or its less-toxic precursor (as used for the experiments), increasing the risk to sort out figs that are not contaminated with AFB₁. These tests provided valuable results to fit the algorithms to AFB₁ contamination. The developed prototype will be demonstrated in Oct. 2019.

NIR-VIS optical sorter and standard technology optical sorter reduce mycotoxin load in an uncleaned wheat batch (contamination levels below maximum limits) by 50%.

Debranning results in declining DON contamination from the outermost to the innermost layer of the wheat kernel, but bioactive compounds are also declining. The best ratio for further processing is the second bran fraction powder.

Hammer milling to large particles (300–700 µm) produced a higher level of micronization and a more homogeneous granulation distribution than the micronizer, also providing for a better DON/fibre ratio in the final product. In addition, large particles represent a better DON/fibre ratio than fine particles (< 250 µm). Further processing of large hammer milled particles is thus preferred.

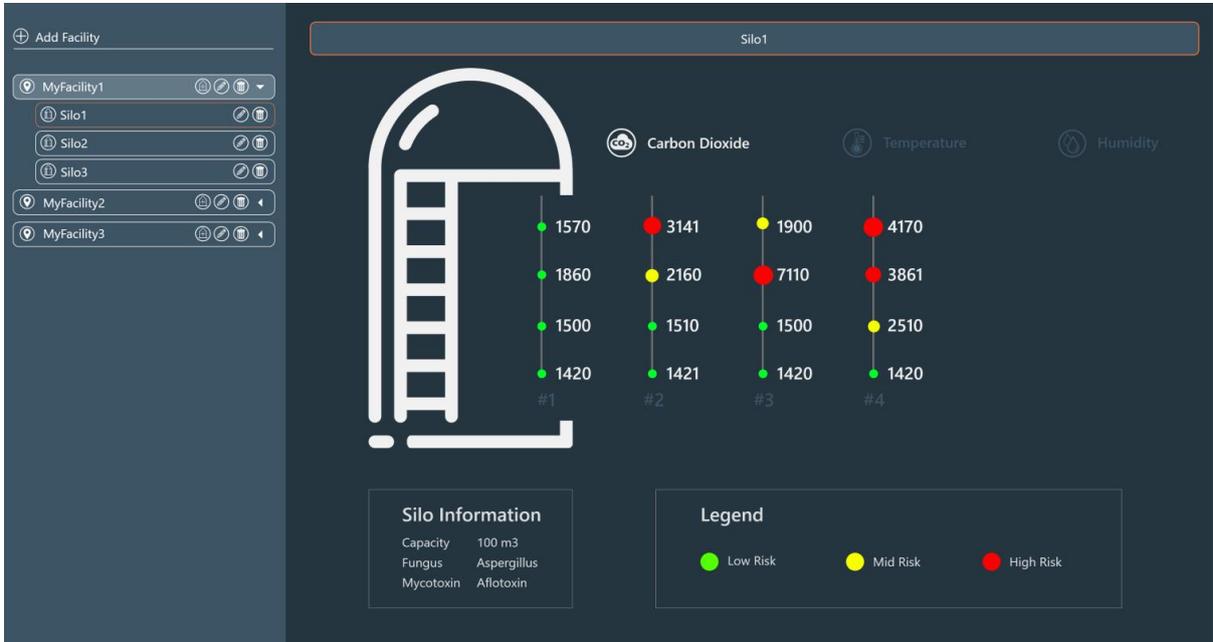
Lower DON/fibre ratio of the large hammer milled particles was achieved by a sieving sifter rather than turbo separator.

Optimizing grain milling procedures allows to use up to 15% more branned material for pasta – i.e. human consumption – than for feed. Additionally, fibre content was increased by 3% in the final wholegrain pasta product, providing for a significant health benefit to the consumer.

ZEN and FUM can be reduced significantly in bioethanol production by using recombinant enzymes: After treatment FB₁ and ZEN levels were reduced by 99% and 89%, respectively. Detoxification was also verified by determining non-toxic HFB1 and hydrolyzed ZEN as a result of successful mycotoxin degradation by using novel enzymes.

Biogas production is not inhibited by mycotoxin contaminated grains as feeding substrate. Methane yield and quality showed no significant difference between uncontaminated and contaminated grains fed to the continuous digesters. Selected microorganisms were also not affected by mycotoxin contamination in fed substrate.

Furthermore, much effort was put into adapting scientific outcomes into a language and format attractive and easy to read to lay persons. As such, MyToolBox is on track in achieving the intended impact: Providing a toolkit for effective monitoring and reduction of mycotoxin contamination all along the food and feed chain, thus enabling increasing productivity and competitiveness of European agriculture and food sectors, and increasing consumer confidence in agro-food products by contributing to standard setting and legislation. Safe use options of contaminated batches such as biofuel production and novel milling procedures ensure valuable use of contaminated batches. The vital cooperation for the development of silo sensors, as well as the scientific exchange with regards to the use of atoxigenic *Aspergillus* strains and feed additives ensure a long-term collaboration with China.



Integrated sensor platform visualising the mycotoxin contamination risk in reference to EU legal limits for food or feed integrated into the sensors, linked to the MyToolBox e-platform.



MyToolBox e-platform landing page (status: March 2019).

