MICOTOSSINE ED ENDOTOSSINE NELLA BOVINA DA LATTE: CONOSCERLE E PREVENIRLE

UNIVERSITÀ CATTOLICA DEL SACRO CUORE - PIACENZA

UNIVERSITÀ CATTOLICA del Sacro Cuore

CONTROLLO DELLE MICOTOSSINE NEGLI ALIMENTI E STRATEGIE DI CONTENIMENTO

Giuseppina Avantaggiato

Consiglio Nazionale delle Ricerche

ISPA

ISTITUTO DI SCIENZE DELLE PRODUZIONI ALIMENTARI

Toxic chemical compounds produced by moulds

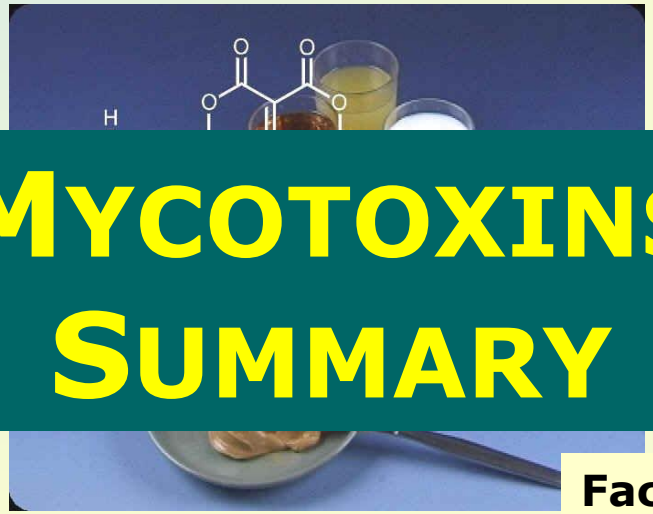
	Known	Postulated
Mold species	1,100	1,500,000
Secondary metabolites	3,200	3,000,000
Mycotoxins	>300	30,000

Occur in a wide variety of commodities:
- raw agricultural products
- processed foods
- imported products
- animal products (milk, meat, eggs)

A truly mycotoxin-free food supply cannot be guaranteed

Up to 25% of the world's foods are significantly contaminated

MYCOTOXINS SUMMARY



Contamination is an **additive process**, beginning in the **field** and increasing during **harvest**, **drying**, and **storage**

"Mycotoxin contamination is **unvoidable** and **unpredictable**, which makes it a unique challenge to food supply"

Contamination and the severity of the problem **vary from year to year** and also **from one geographic region to another**

Factors affecting occurrence in the food chain:
- availability of water
- temperature
- inoculum concentrations
- microbial interactions
- mechanical damage
- insect infestation

Managing Mycotoxins Pre- and Post-Harvest

1. Pre-harvest Control

... during crop growth

2.

3.

Pre-Harvest Control

Pre-harvest control of fungal infection and toxin formation is the **best way to manage post-harvest contamination**

- **Reduction of plant stress**
  (crop rotation, plant-spacing, irrigation, ...)
- **Avoidance of critical environmental conditions**
  (timing of planting)
- **Minimization of crop residues** and other **sources of inoculum** (weeds) by soil preparation
- Use of **crop protection chemicals** that are antifungal agents
- **Breeding for cultivars resistant** to fungal infection
- Development of **transgenic plants**
- Use of **biocompetitive agents**

Managing Mycotoxins Pre- and Post-Harvest

1. Pre-harvest Control

... during crop growth

2. Decontamination

biological/physical/chemical treatments

... During storage, food/feed processing

3.

DECONTAMINATION

PHYSICAL TREATMENTS

- **Cold processing methods:**

  cleaning (screening), **dehulling**, **pearling**, density segregation and fractionation, washing/soaking, **dry/wet milling**

- **Hot processing methods:**

  roasting/baking, extrusion, steam rolling/flaking

- **Irradiation**

  (including microwaves), ultrasound

- **Solvent extraction**

Screening/Cleaning

- **High levels of mycotoxins** in dust, debris and damaged kernels
- **Screening out fine materials** reduces mycotoxin content
- The process is **noninvasive**, and does not alter the product
- ***It is simple but incomplete !!!***

Effect of the Process on Mycotoxin Levels

Aflatoxins Fumonisins	Significant reduction (30-40%)
Deoxynivalenol Zearalenone	Small reduction

Physical Treatments

Case study on aflatoxin reduction in maize

AIM

- **Reduction of aflatoxins in maize by combining mechanical and optical sorting technologies**

Pascale et al., 2014

Separator – Aspirator – Sortex

Results - Trial #1*

Lot	Fraction	Aflatoxin B_1 (µg/kg) Mean ± SD *(n=3)*	Total AFs (µg/kg) Mean ± SD *(n=3)*	AFB_1 reduction (%)	Total AFs reduction (%)
A	Incoming product	24.2 ± 0.3	25.4 ± 0.3	65	65
	End product	8.4 ± 0.3	8.8 ± 0.3		
B	Incoming product	62.0 ± 1.5	64.3 ± 1.6	78	78
	End product	13.5 ± 0.2	14.3 ± 0.2		

Cleaning Line (#1)*:

- primary size classification + removal of light particles through an aspiration channel (**SEPARATOR** + **ASPIRATOR**)
- optical sorting of spatial and spectral defects (**SORTEX**)

Separator – Aspirator – Concentrator – Sortex

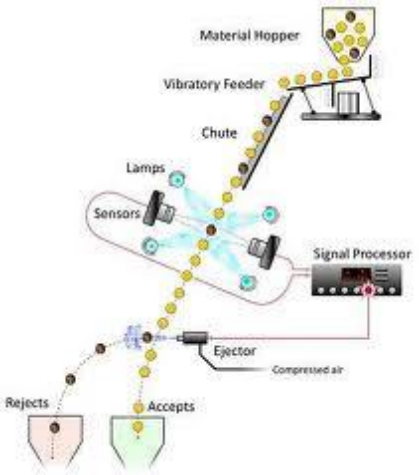
Results - Trial #2*

Lot	Fraction	Aflatoxin B_1 (µg/kg) Mean ± SD *(n=3)*	Total AFs (µg/kg) Mean ± SD *(n=3)*	AFB_1 reduction (%)	Total AFs reduction (%)
A	Incoming product	28.8 ± 2.1	30.2 ± 2.2	84	84
	End product	4.5 ± 0.2	4.7 ± 0.2		
B	Incoming product	23.5 ± 0.5	24.5 ± 0.5	76	76
	End product	5.7 ± 0.3	6.0 ± 0.3		

Cleaning Line (#2)*:

- primary size classification + removal of light particles through an aspiration channel (**SEPARATOR** + **ASPIRATOR**)
- additional kernel gravity separation (**CONCENTRATOR**)
- optical sorting of spatial and spectral defects (**SORTEX**)

EFFICIENCY OF SORTING



Optical Sorter *(Pearson et al. 2004)*

Kansas AF and **FB co-contaminated maize** (pre-cleaned)

- **AFLATOXINS** at 51 ppb reduced by **83%** by removing 5%
- **FUMONISINS** at 18 ppm reduced by **82%** by removing 5%

Manual Sorting *(Van der Westhuizen et al. 2010)*

- **FUMONISINS** at 1.67 ppm reduced by **84%** by removing **3.9%**

Procedure	Mycotoxin	Product	Efficiency
removal of damaged kernels	AFLA	peanuts pistachio	+++ +++
fluorescence sorting	AFLA	corn, cottonseed	+++
sieving	FUM	corn	++
flotation	AFLA	corn, peanuts	+++
rinsing	DON, ZEA, FUM	wheat, corn	++
wet–milling	AFLA, ZEA	corn	+/–
roasting	AFLA	corn, peanuts	+ or –
heat processing	OTA TRIC FB_1	flour cereal products corn products	++ – –
γ–radiation	T–2, ZEA, DON AFLA	wheat wheat	++ ++*
sunlight	AFLA	wheat	+++

+++ 90–100% reduction; ++ 50–90% reduc; + 10–50% reduc; +/– mycotoxin is distributed into the fractions; + or – mycotoxin reduction depends on the procedure; * procedure leaves behind residual metabolites

DECONTAMINATION

CHEMICAL TREATMENTS

- **Acids** (formic and propionic acids)
- **Alkali** (ammonium, sodium hydroxide)
- **Oxidizing reagents** (hydrogen peroxide, ozone)
- **Reducing agents** (bisulphite, sugars)
- **Chlorinating agents** (chlorine)

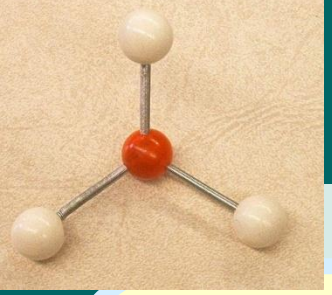
Detoxification ability depends on:

parameters related to the contaminated products

parameters associated with the process (such as T and P)

incubation time

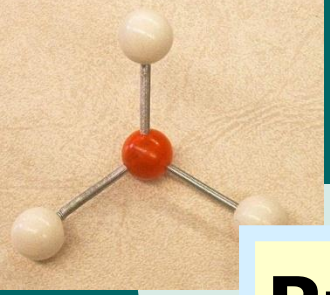
level of the mycotoxin in the product



AMMONIATION

- **Ammonium hydroxide** or **gaseous ammonia (7%)**
- **AFLATOXIN reduction up to 99%**
  corn, peanut meal-cakes, cottonseed
- **Irreversible** (***if the reaction proceeds to completion***)
- Identification of several **decomposition compounds**
- **No toxic effects** related to the process in animals
- **Reduction/Elimination** of aflatoxicosis signs in animals

- **Some changes in the nutritional quality** of the feed (decrease in lysine and sulphur containing amino acids)
- **Adequate aeration** after ammoniation for acceptance of the feed by animals



AMMONIATION

PROCEDURES

1. **High Temperature/High Pressure**
   (80-120°C/35-50 psi/20-60 min) used at feed mills
2. **Ambient Temperature/Atmospheric Pressure**
   (14-42 days) used on the farm

PERMITTED USES OF AMMONIATION PROCEDURES

- The **FDA** does not permit interstate shipment of ammoniated feeds
- Some **States of the USA**, **Mexico**, **South Africa**, **Senegal**, **Brazil** approve the process
- Some **European Countries** import ammonia-treated feeds

Chemicals	Mycotoxin	Product	Efficiency
calcium hydroxide	FB_1	Corn	++
	ZEA	Corn	++
	DON	Corn	++
hydrogen peroxide	AFLA	Peanuts	+++
hydrogen peroxide / sodium bicarbonate	FB_1	Corn	+++
sodium bisulphite	DON, AFB_1	Feed	+++
sodium chloride	AFLA	Peanuts	+++
ammonia	AFLA	Corn	+++
	AFLA	Peanut meal	+++
	FUM	Corn	+
ammonia with calcium hydroxide (at 96 °C)	OTA	Swine feed	+++

+++ 90–100% reduction; ++ 50–90% reduction; + 10–50% reduction;

DRAWBACKS

PHYSICAL TREATMENT

- Expensive
- Uncertain results, often connected with high feed/food losses

⇒ **Limited practical application**

CHEMICAL TREATMENT

- Expensive and time consuming
- Change in palatability and nutritive value
- Decreased feed quality
- Toxic by-products possible

⇒ **No practical application**

Managing Mycotoxins Pre- and Post-Harvest

1. Pre-harvest Control

... during crop growth

2. Decontamination

... during food/feed processing

3. Mycotoxin-Detoxifying Agents

... additives for mycotoxin decontamination of feeds

Mycotoxin-Detoxifying Agents

COMMISSION REGULATION (EC) No 386/2009

of 12 May 2009

amending Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the establishment of a new functional group of feed additives

"Substances for Reduction of Feed Contamination by Mycotoxins"

- **suppress or reduce absorption**
- **promote excretion**
- **modify mode of action of mycotoxins**

No increase of the existing max/guidance levels

Improvement of feed quality (lawfully on the market)

MYCOTOXIN-DETOXIFYING AGENTS

EFSA S/T Report, 2009 (www.efsa.europa.eu)

REVIEW of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and safety

AFSSA, INRA (*France*), CODA-CERVA (*Belgium*), IRTA (*Spain*), **ISPA (*Italy*)**

SCIENTIFIC OPINION
EFSA Journal 2010; 8(7):1693

Statement on the establishment of GUIDELINES for the assessment of mycotoxin-detoxifying agents

Positive Opinions of EFSA and EU Authorisations for Mycotoxin-Detoxifying Agents

efsa
European Food Safety Authority

1. **EFSA Journal 2011 → EU Reg. 1060/2013**

   Authorisation of the Bentonite as additive (Binder) for All Animal Species and for the reduction of AFB1 contamination of feed

   Studies in dairy cows showing significant reduction in AFB_1 excretion via milk (AFM_1)

2. **EFSA Journal 2013 → EU Reg. 1016/2013**

   Authorisation of the micro-organism DSM 11798 as additive (Biotransforming Agent) for Pigs and for the reduction of DON contamination of feed

   Short-term studies showing the transformation of DON to the less toxic metabolite (DOM-1)

3. **EFSA Journal 2014 → EU Reg. 1115/2014**

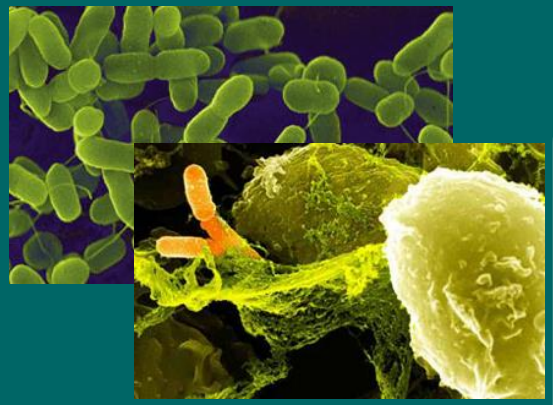
   Authorisation of fumonisin esterase EC 3.1.1.87 as additive (Biotransforming Agent) for Pigs and for the reduction of Fumonisins contamination of feed

   Short-term/long-term studies showing the hydrolysis of FBs into less toxic metabolites

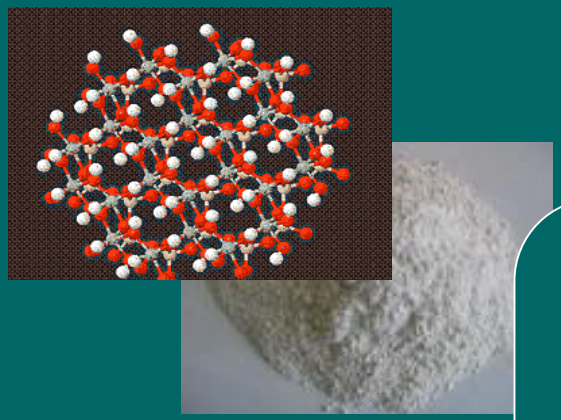
Mycotoxin-Detoxifying Agents

Mode of Action

Biodegradation



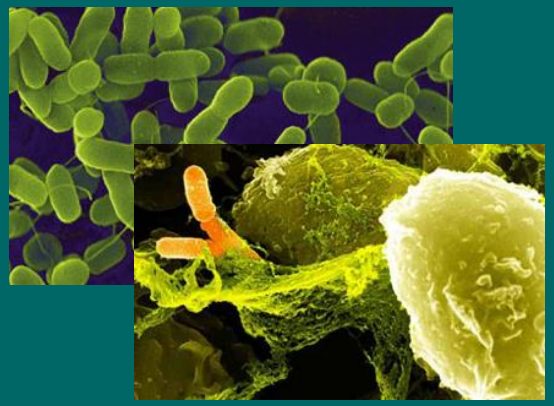
Adsorption



Bioprotection

Mycotoxin-Detoxifying Agents

Biodegradation



Biotransformation/biodegradation

of mycotoxins by

microorganisms and/or **enzymes**

- + **specific**
- + **irreversible**
- + **no toxic by-products/residues**

Microorganisms

Bacteria *Yeast*

Fungi

Mycotoxins → **Non-Toxic Metabolites**

Enzymes

Biotransformation/Biodegradation

- Although mycotoxins transformation by microorganisms has been evidenced since 1960's
- Studies in this topic have been limited
- The majority of studies used **mixed cultures of microorganisms** from various sources (animal guts, soils, plants)
- **Few active microorganisms have been identified**
- **Few commercial products are available on the market for microbial degradation**

Biotransformation / Biodegradation

Mycotoxin-biotransforming agents		Source	Toxin	Reference
Bacteria	*Nocardia asteroides* *Mycobacterium fluoranthenivorans* *Rhodococcus erythropolis*		**AFB_1**	Wu *et al.*, 2009
	Mixed culture (*Alcaligenes, Bacillus, Achromobacter, Flavobacterium, Pseudomonas*)		**ZEA**	Megharaj *et al.*, 1997
	Curtobacterium sp. 114-2		**T2**	Ueno *et al.*, 1983
	***Eubacterium* sp. BBSH 797** (anaerobic bacteria isolated from GI of ruminants)	Biomin®	**DON**	Fuchs *et al.*, 2002
Yeast	*Trichosporon mycotoxinivorans*	Biomin®	**OTA + ZEA**	Schatzmayr *et al.*, 2003
Enzyme	**FUMzym** (identified in a *Sphingopyxis* sp., isolated from soil, and the gene used to transform the yeast *Komagataella pastoris*)	Biomin®	**FB_1**	Heinl *et al.*, 2010

Commercial products available on the market

Aerobic and anaerobic *in vitro* testing of feed additives claiming to detoxify DON and ZEA

Hahna et al., *Food Additives and Contaminants: Part A* (2015)

- **Twenty** (**20**) **commercially available products** claiming DON and/or ZEA detoxification by microbial or enzymatic processes
- *In vitro* testing under **aerobic** and **anaerobic conditions**
- Selection of **only one promising product** containing bentonite, inactivated yeast, BBSH 797, etc.

TOXIN REDUCTION (%) AFTER DIFFERENT INCUBATION TIMES

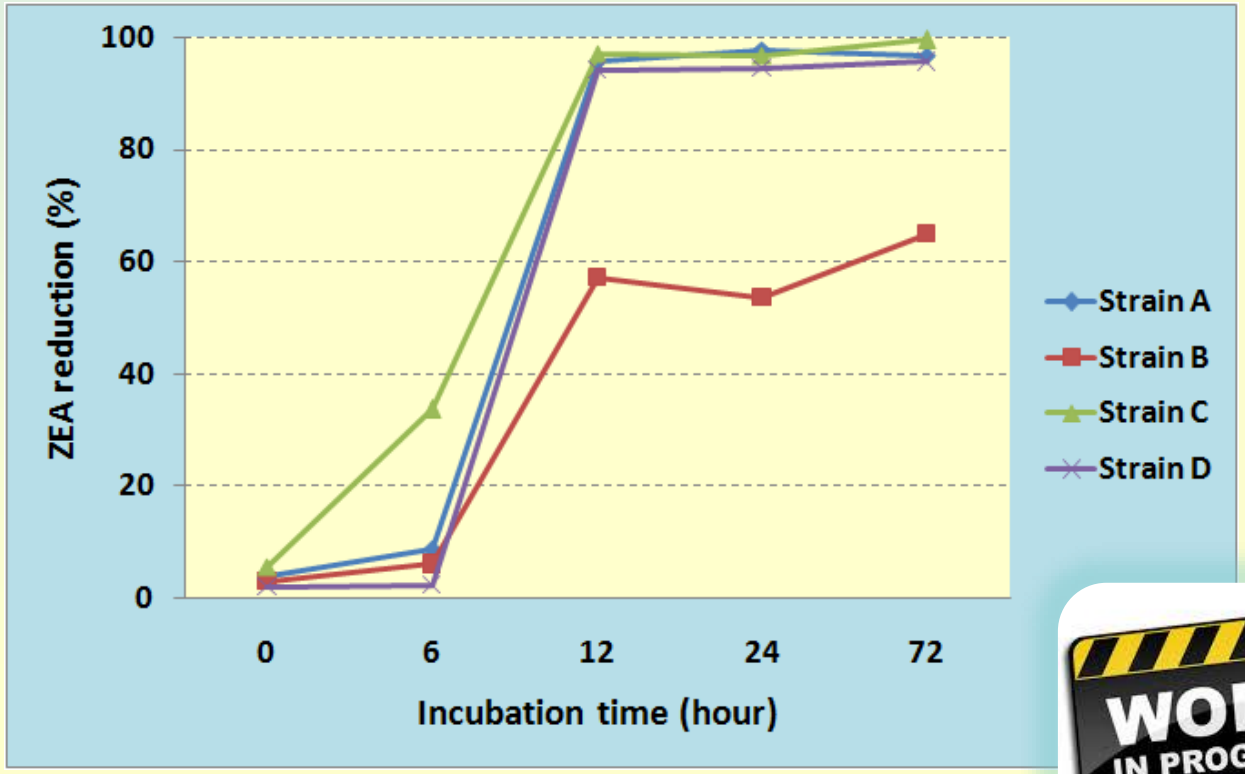
Condition	DON	ZEA		
	24 h	***1 h***	***3 h***	***24 h***
Aerobic	3	75	97	100
Anaerobic	97	28	68	100

Time requested to detoxify DON into DOM-1

Aerobic and anaerobic *in vitro* testing of *Bacillus* strains to detoxify DON and ZEA

Reduction of ZEA by *Bacillus* strains grown in tryptic soy broth containing 1 µg/mL of toxin and incubated at 30°C in aerobic and anaerobic conditions

Avantaggiato et al., 2015

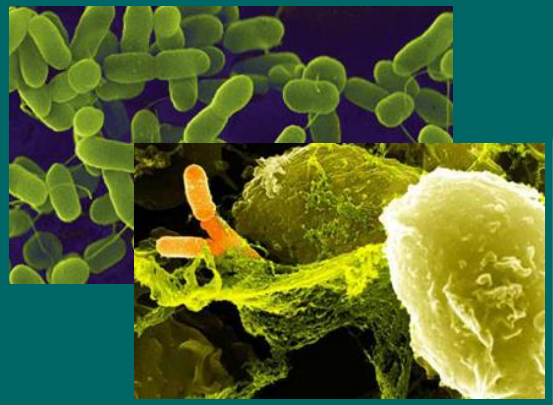


All strains were ineffective in reducing DON (1 µg/mL)

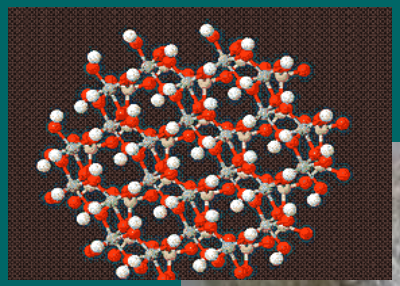
Mycotoxin-Detoxifying Agents

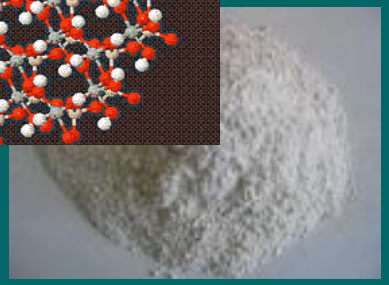
Mode of Action

Biodegradation



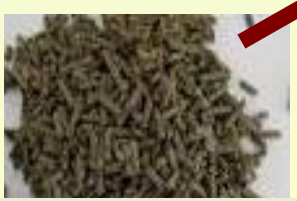
Adsorption



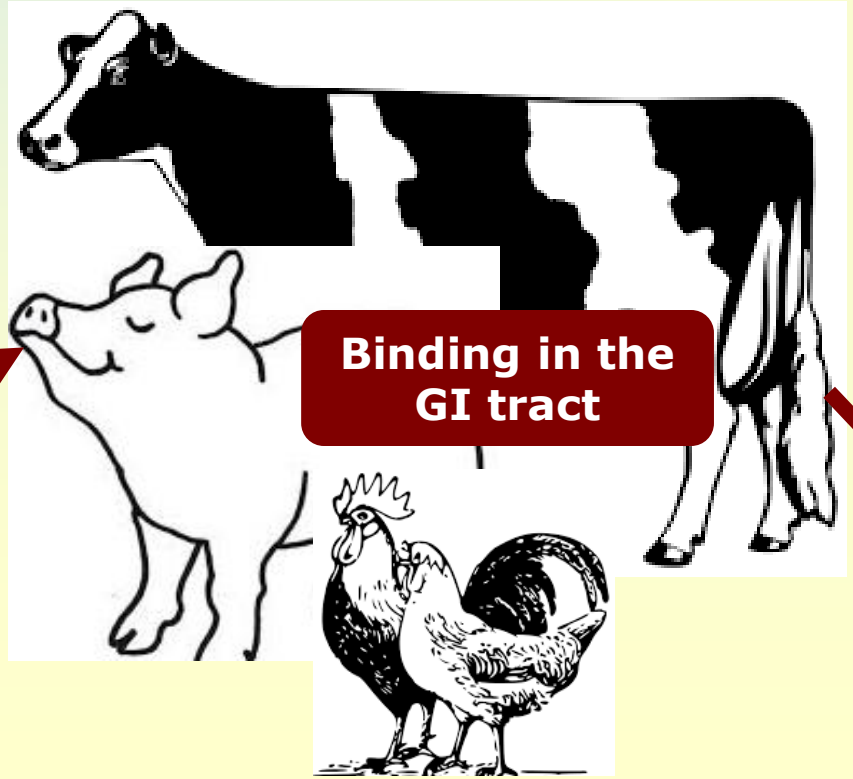


The Concept of Mycotoxin Adsorbents

- Largely used to **decontaminate animal feeds**
- **Bind mycotoxins** in the gastro-intestinal tract and form stable complexes which are excreted through the faeces
- **Decrease mycotoxins absorption and promote their excretion**



Mycotoxins



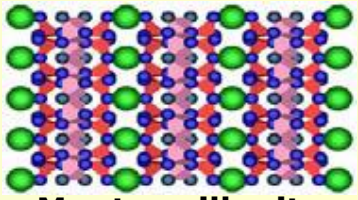
Increased excretion

Mycotoxin Adsorbents

- **Silicate Products**

  Phyllosilicates (*silicate sheets*) - montmorillonite, sepiolite

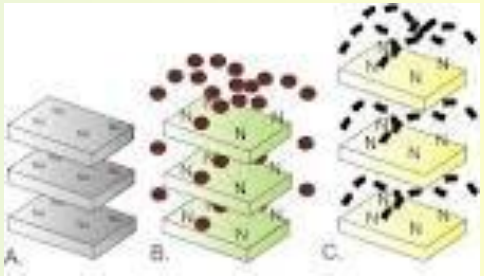
  Tectosilicates (*silicate frameworks*) - zeolites, clinoptilolite



Montmorillonite

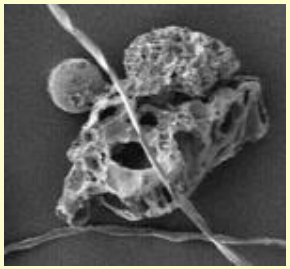
- **Chemically treated silicates**

  Organoclay: interlayer cations are replaced by quaternary alkylammonium ions - **TOXIC**



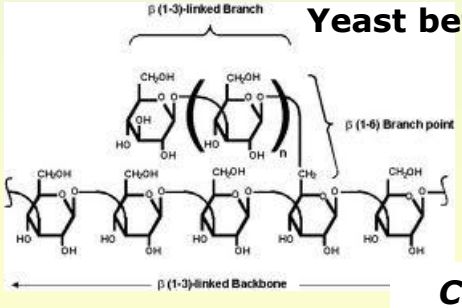
- **Carbon based Products**

  Activated or **superactivated carbon**



Carbon

- **Glucan based Products**



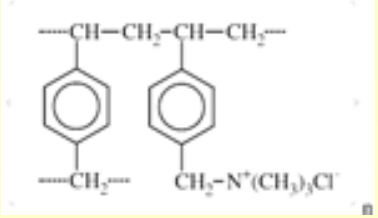
Yeast beta-glucan

- **Inorganic polymers**

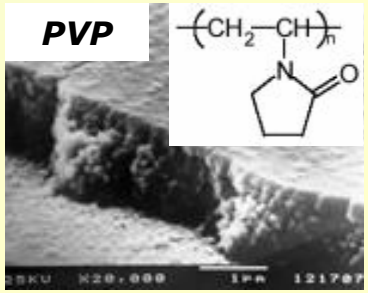
  Cholestyramine

  Polyvinylpyrrolidone (PVP)

Cholestyramine

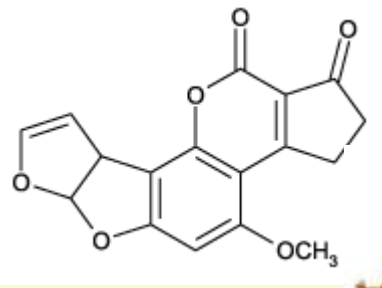


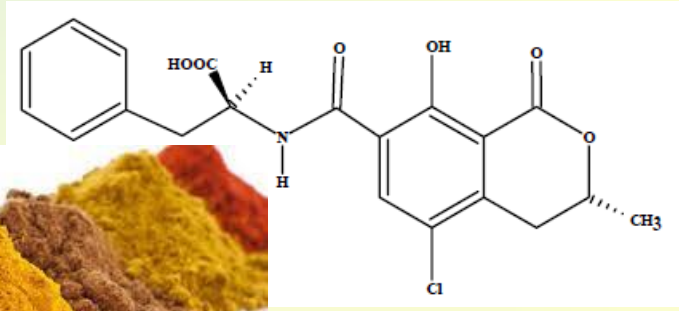
PVP

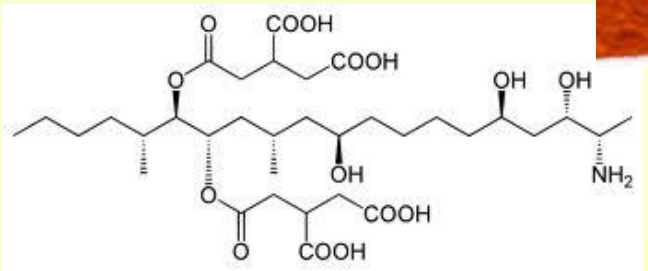


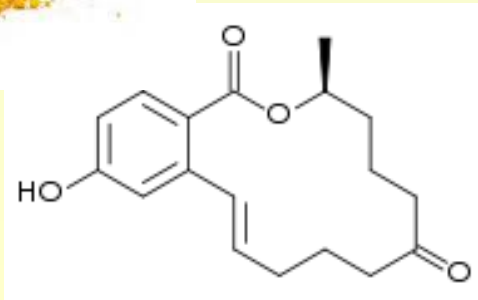
Multi-Mycotoxin Adsorbents

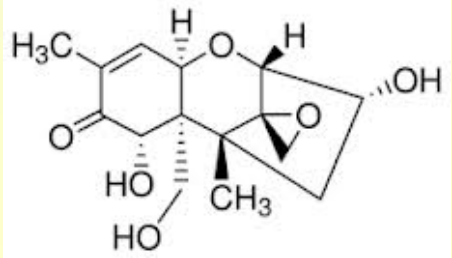
- **Three products** are registered in the **EU** as detoxifying agents for **AFB_1** in ruminants (**bentonite**), **DON** in pigs (bacterium **DSM 11798**), and **FB_1** in pigs (**fumonisin esterase** produced by Komagataella pastoris DSM 26643)
- More than **100 commercial products** are available on the market
- Most commercial products claims the ability to **adsorb several toxins**
- **Simultaneous multi-toxin adsorption has been poorly addressed**











In Vitro Selection of Multi-Toxin Adsorbing Agents

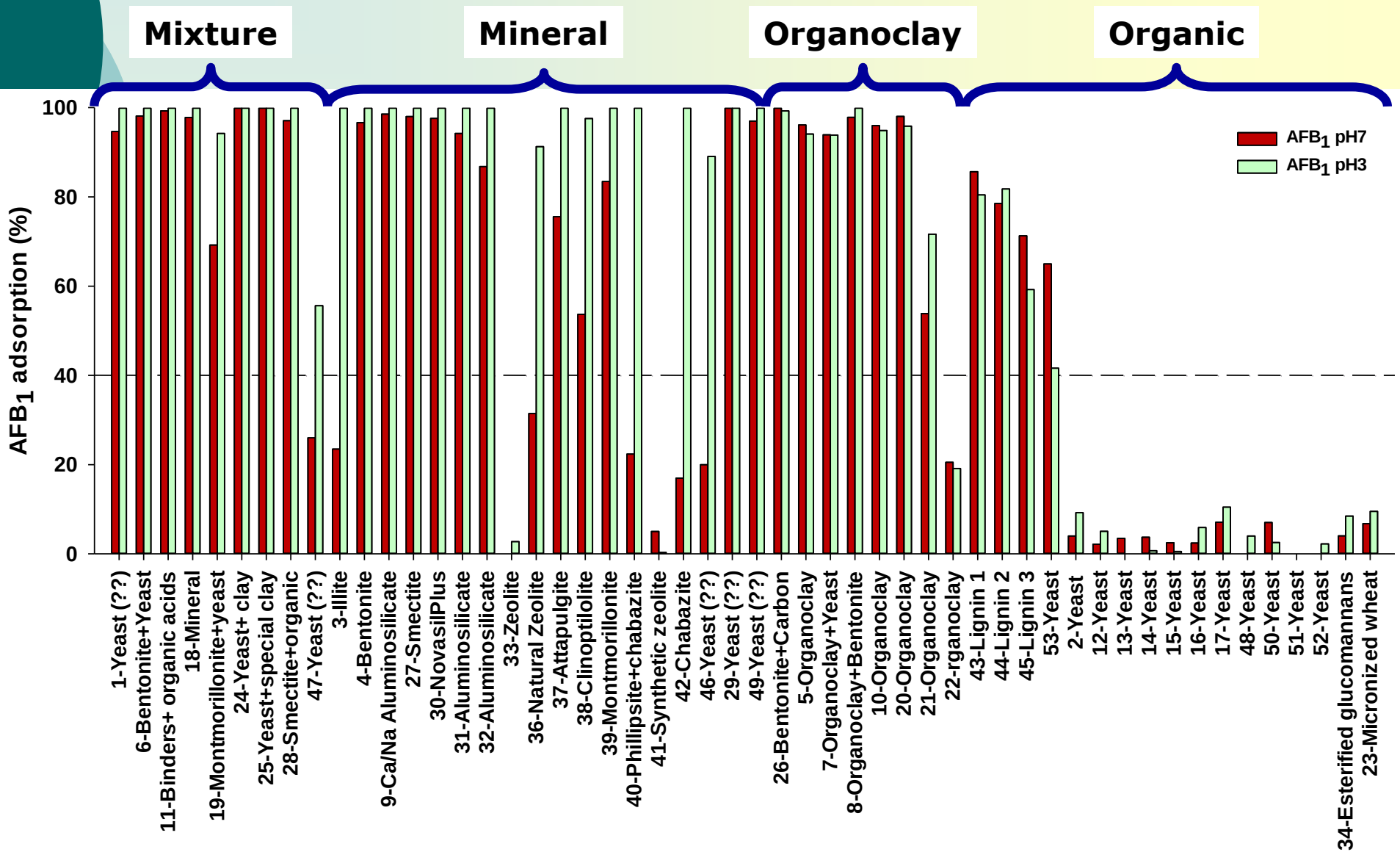
Companies: 26

Products: 52

Organics (17) **ash content <15%**	**Mixtures (17)** **ash content 40-90%**	**Minerals (18)** ***ash content* >90%**
Yeasts (13) Vegetables (4)	Mineral + Organic Organoclays	Phyllosilicates (9) Tectosilicates (6)

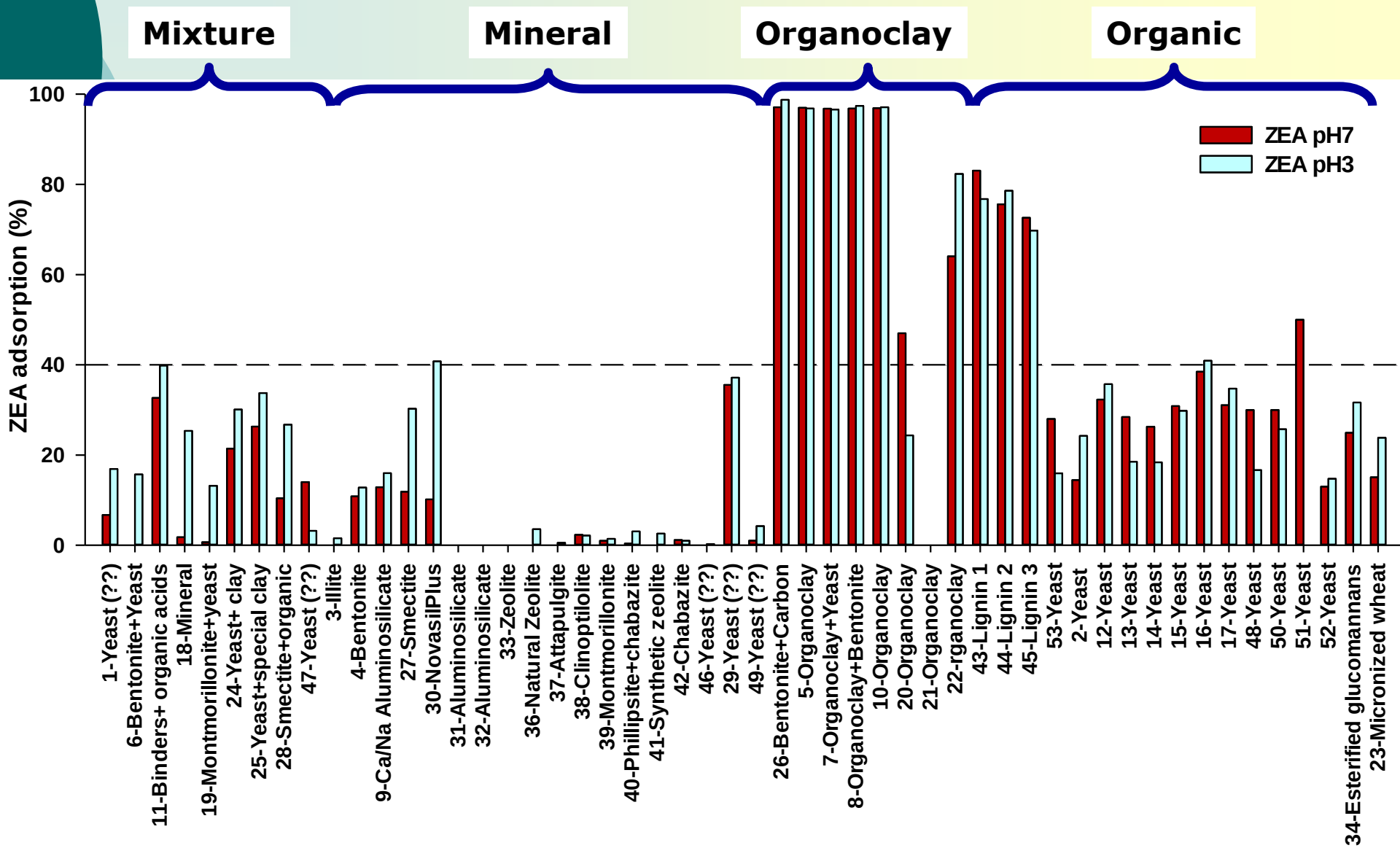
AFB_1 Adsorption by Organic and Inorganic Materials

Adsorbent dosage: 0.1% (w/v) - AFB_1 concentration: 1 µg/mL – Temp.: 37°C



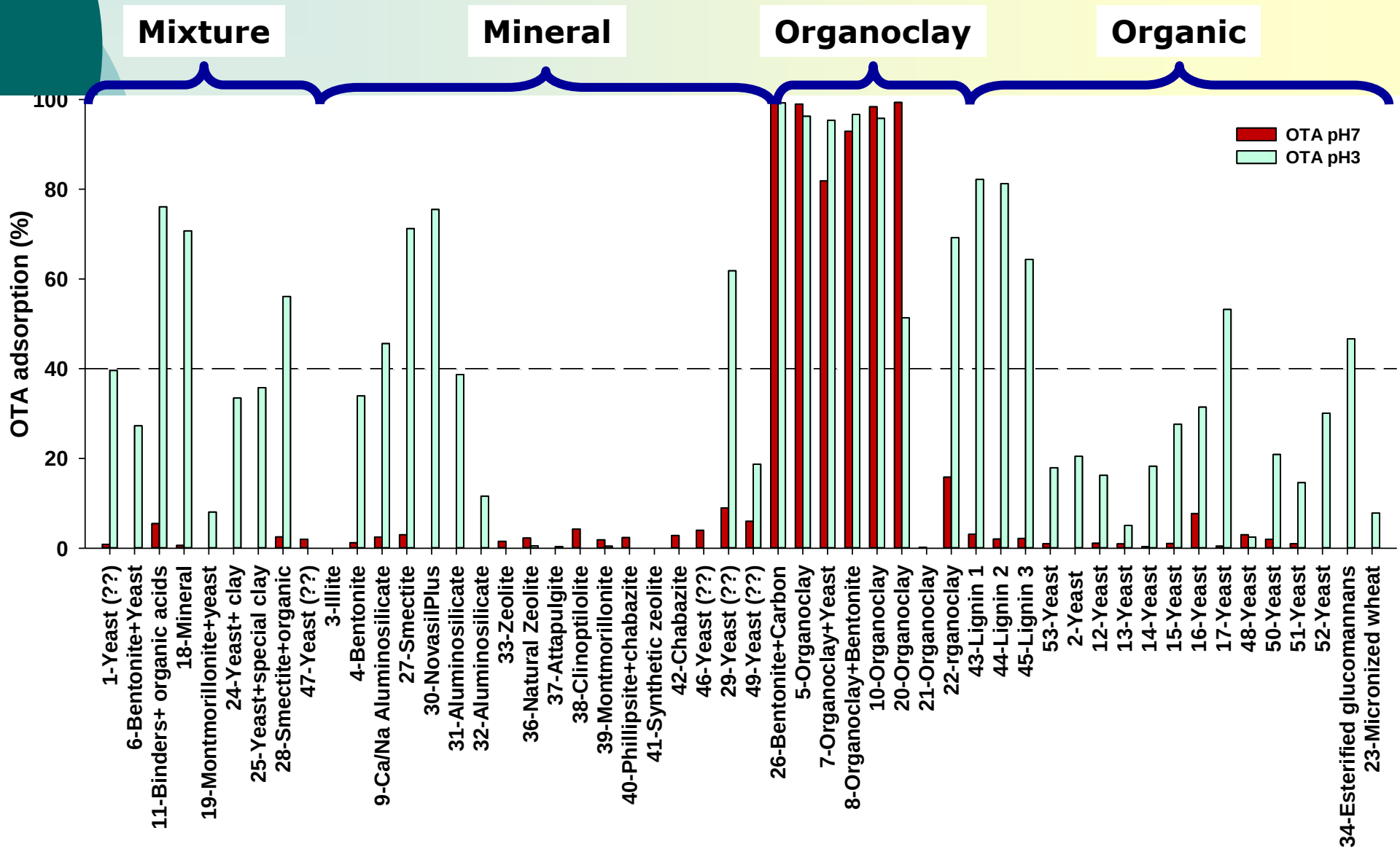
ZEA Adsorption by Organic and Inorganic Materials

Adsorbent dosage: 0.1% (w/v) – ZEA concentration: 1 µg/mL – Temp.: 37°C



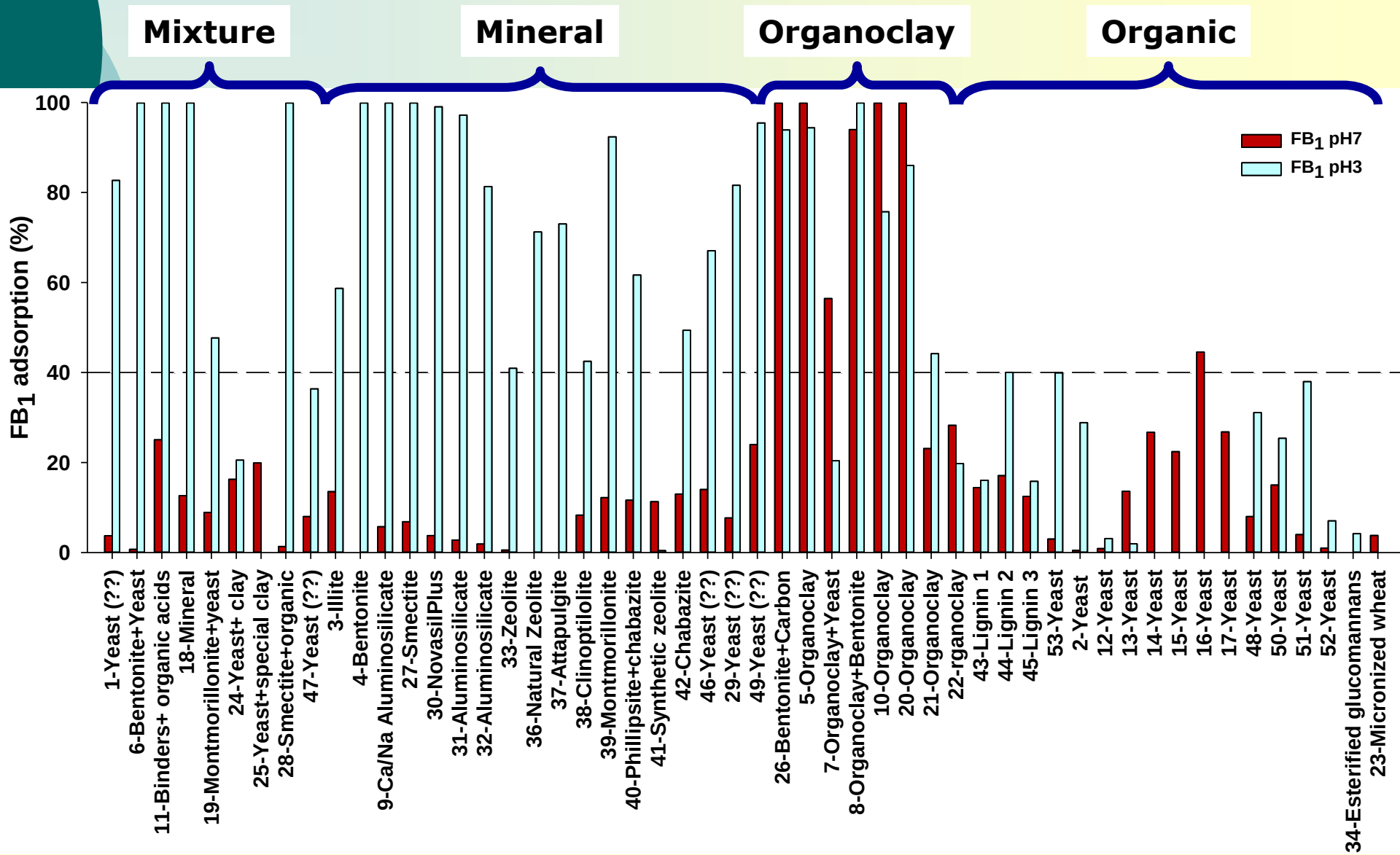
OTA Adsorption by Organic and Inorganic Materials

Adsorbent dosage: 0.1% (w/v) – OTA concentration: 1 µg/mL – Temp.: 37°C



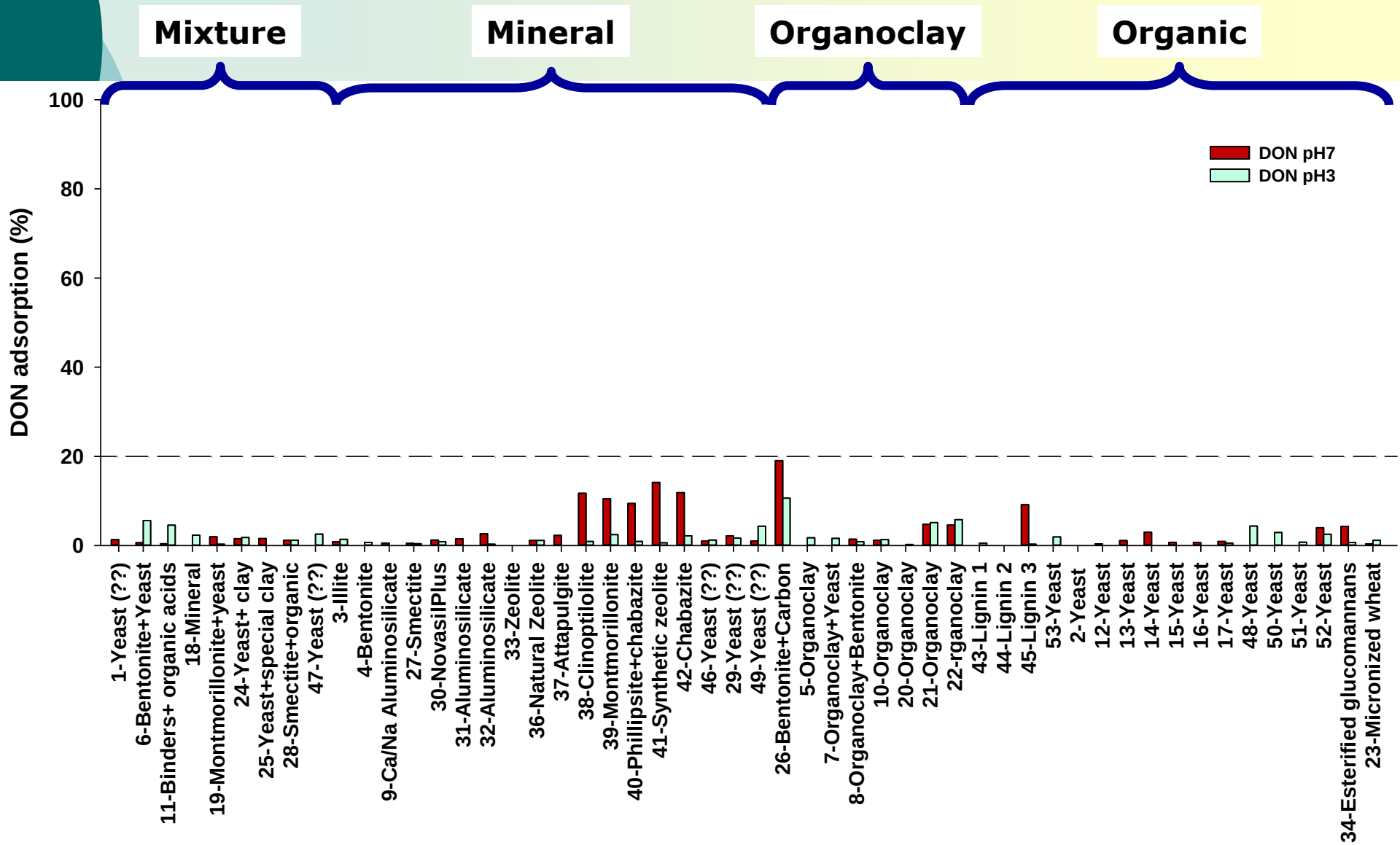
FB_1 Adsorption by Organic and Inorganic Materials

Adsorbent dosage: 0.1% (w/v) – FB_1 concentration: 1 µg/mL – Temp.: 37°C



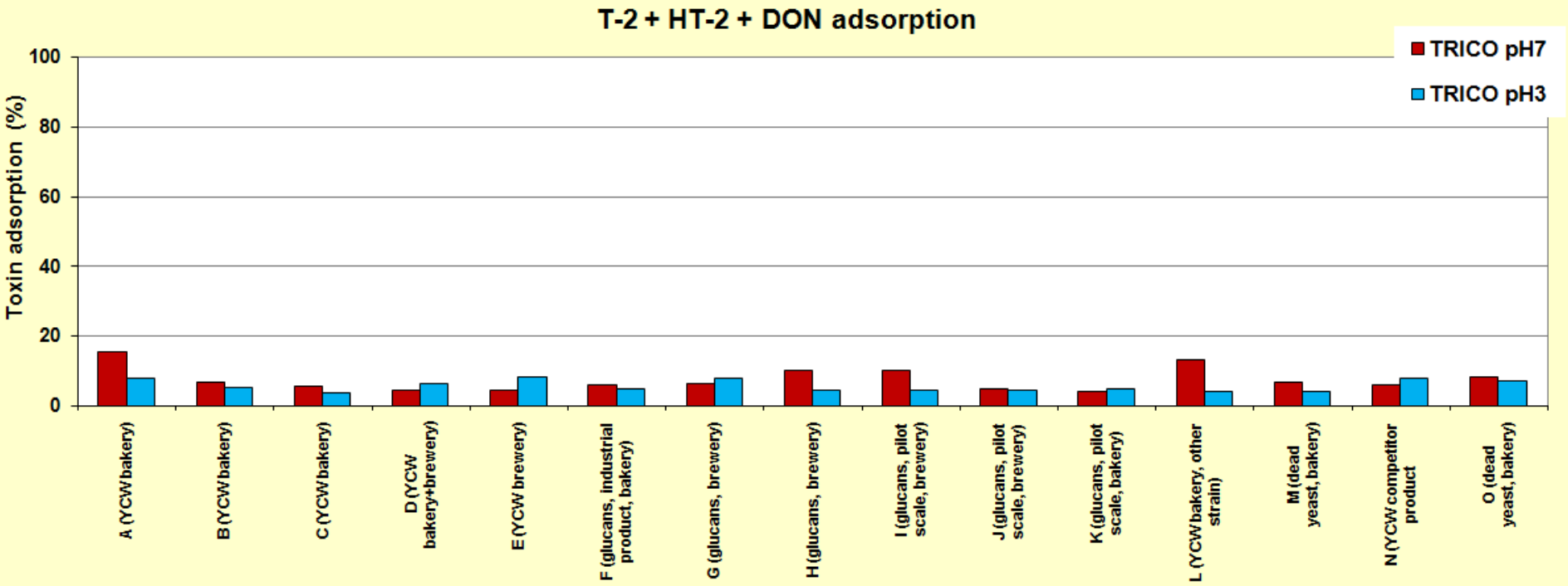
DON Adsorption by Organic and Inorganic Materials

Adsorbent dosage: 0.1% (w/v) – DON concentration: 1 µg/mL – Temp.: 37°C



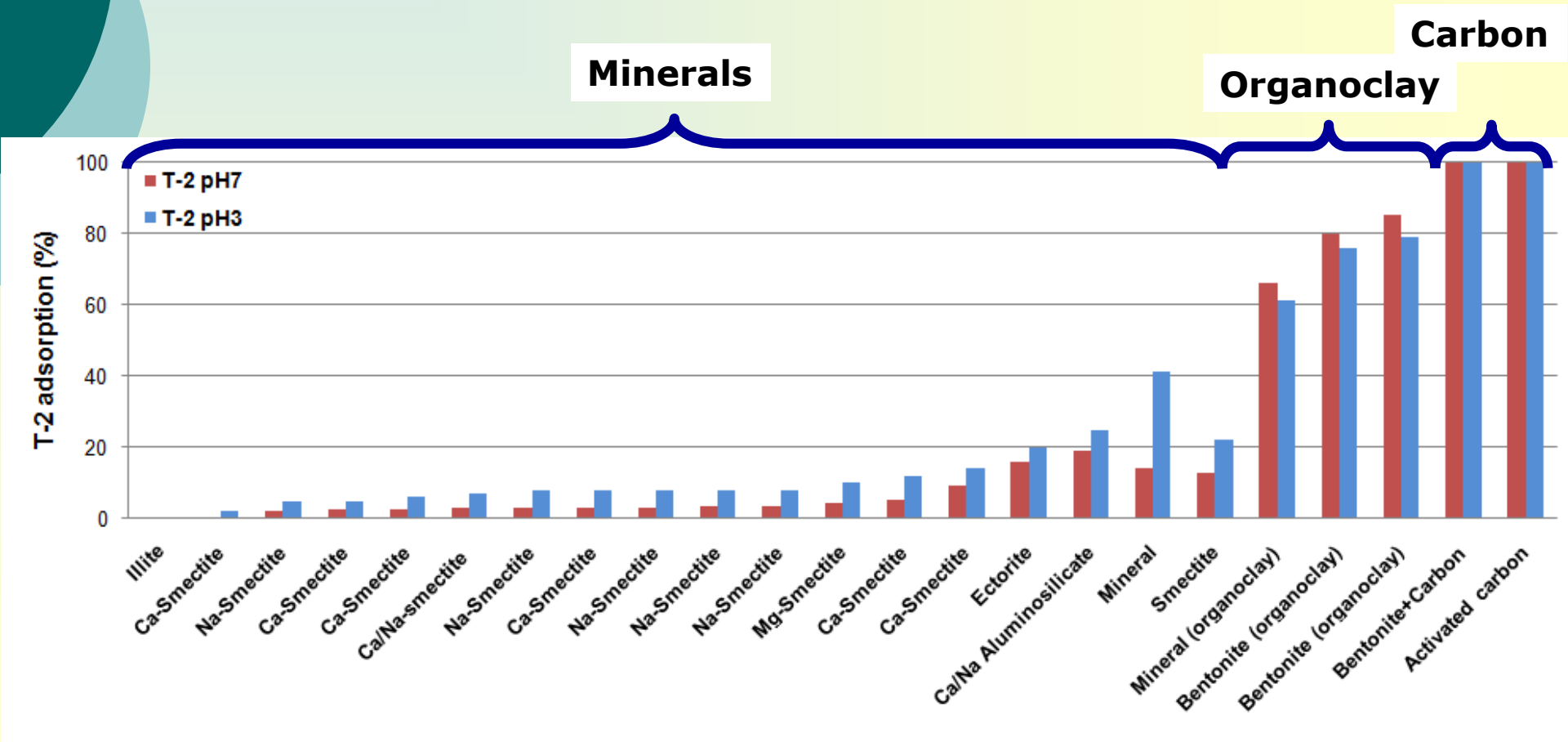
TRICO Adsorption by YCW products

Adsorbent dosage: 0.1% (w/v) - Toxins concentration: 1 µg/mL - Temp.: 37°C



T-2 Toxin Adsorption by Inorganic Materials

Adsorbent dosage: 0.1% (w/v) – T-2 concentration: 1 µg/mL – Temp.: 37°C



Selection of Multi-Mycotoxin Adsorbents

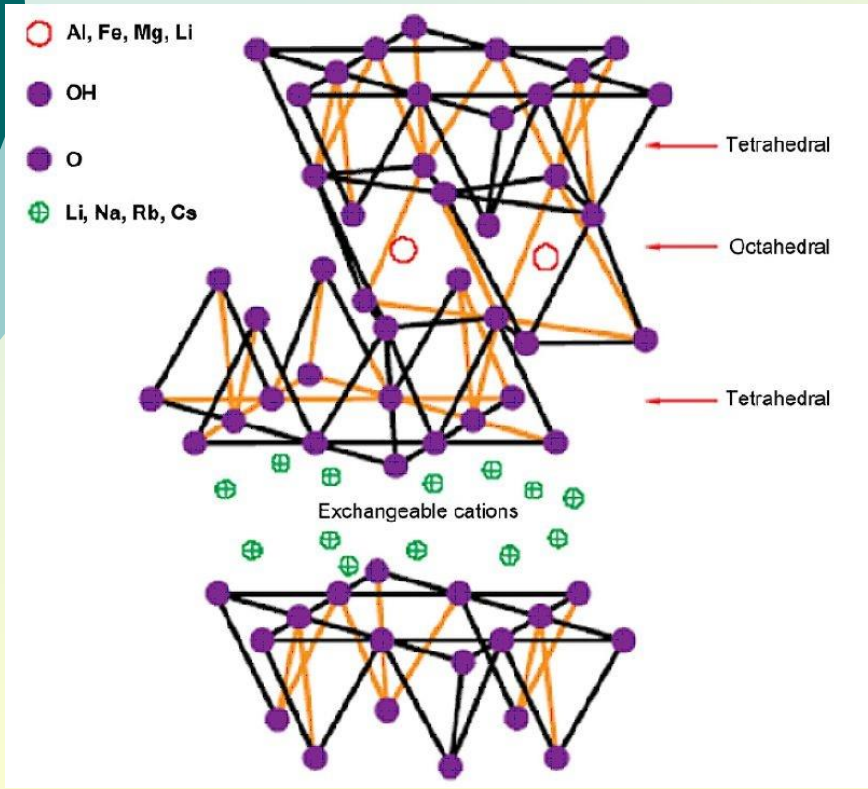
	Mycotoxin adsorption (%)											
	AFB_1		ZEA		OTA		FB_1		T-2		DON	
	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3
Organoclay 1*	96	94	97	97	99	96	100	94	85	79	0	2
Organoclay 2*	96	95	97	97	98	96	100	76	78	76	1	1
Organoclay 3*	98	96	47	24	99	51	100	86	66	61	0	0
Bentonite + Carbon	100	99	97	99	100	99	100	94	100	100	19	11
Activated Carbon	100	100	100	100	100	100	100	100	100	100	100	100

* The identity of the adsorbents provided by some Companies did not match with ash content and XRD analysis. They were stated as **minerals** but were identified as **organoclays**.

Organoclays are not suitable for feed ingredients due to toxicity of the interlayer quaternary alkylammonium ions

Mineralogical Analysis - XRD

- **X-Ray Diffraction analysis:** information on the mineralogical composition of a mineral



- **Basal spacing (d_{001}): distance** between two parallel plane layers of the crystal
- It provides information on the features of the **geometry of stacking of layers**, as well as any **material present between layers**

Basal spacing (d_{001}):

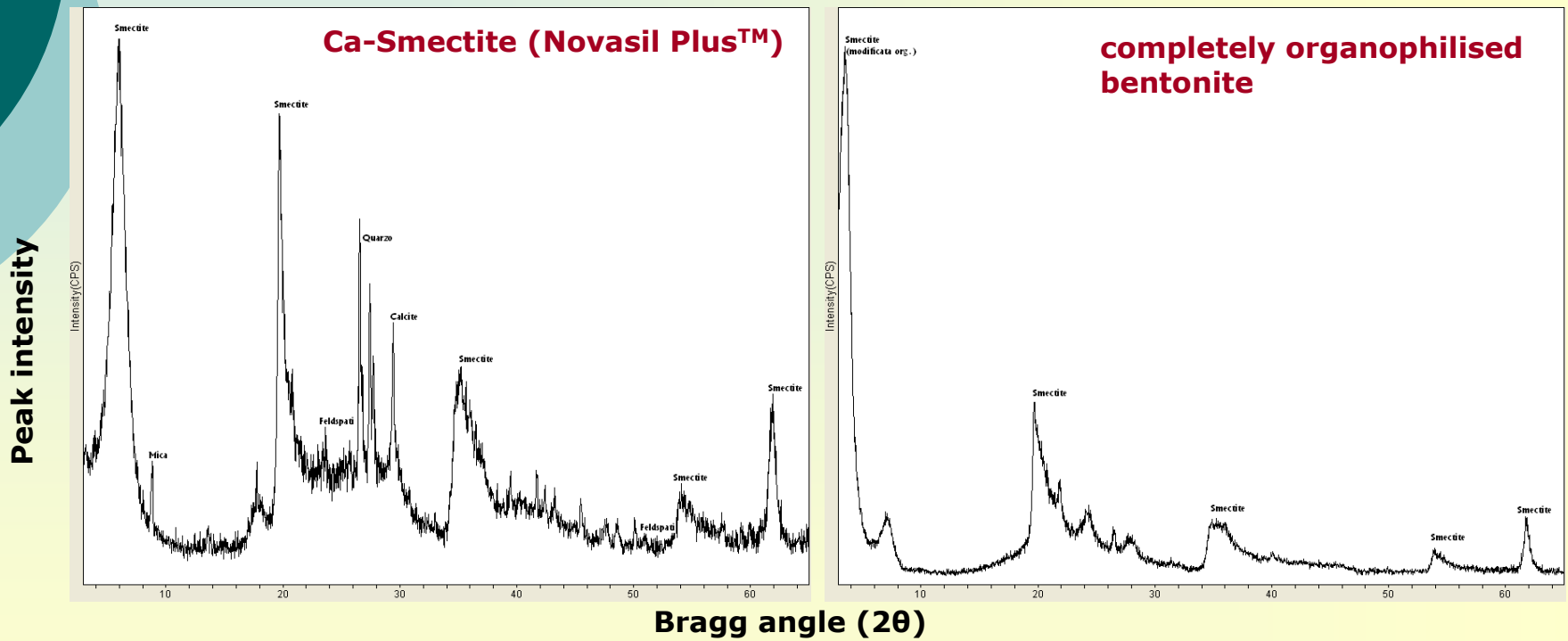
- **monovalent cation-saturated bentonite ~12.6 Å**
- **divalent cation-saturated bentonite ~15.4 Å**
- **organo-bentonite > 17 Å, depending on the surfactant**

Mineralogical Analysis - XRD

- **Basal spacing** (d_{001}): monovalent cation-saturated bentonite **~12.6 Å**

  divalent cation-saturated bentonite **~15.4 Å**

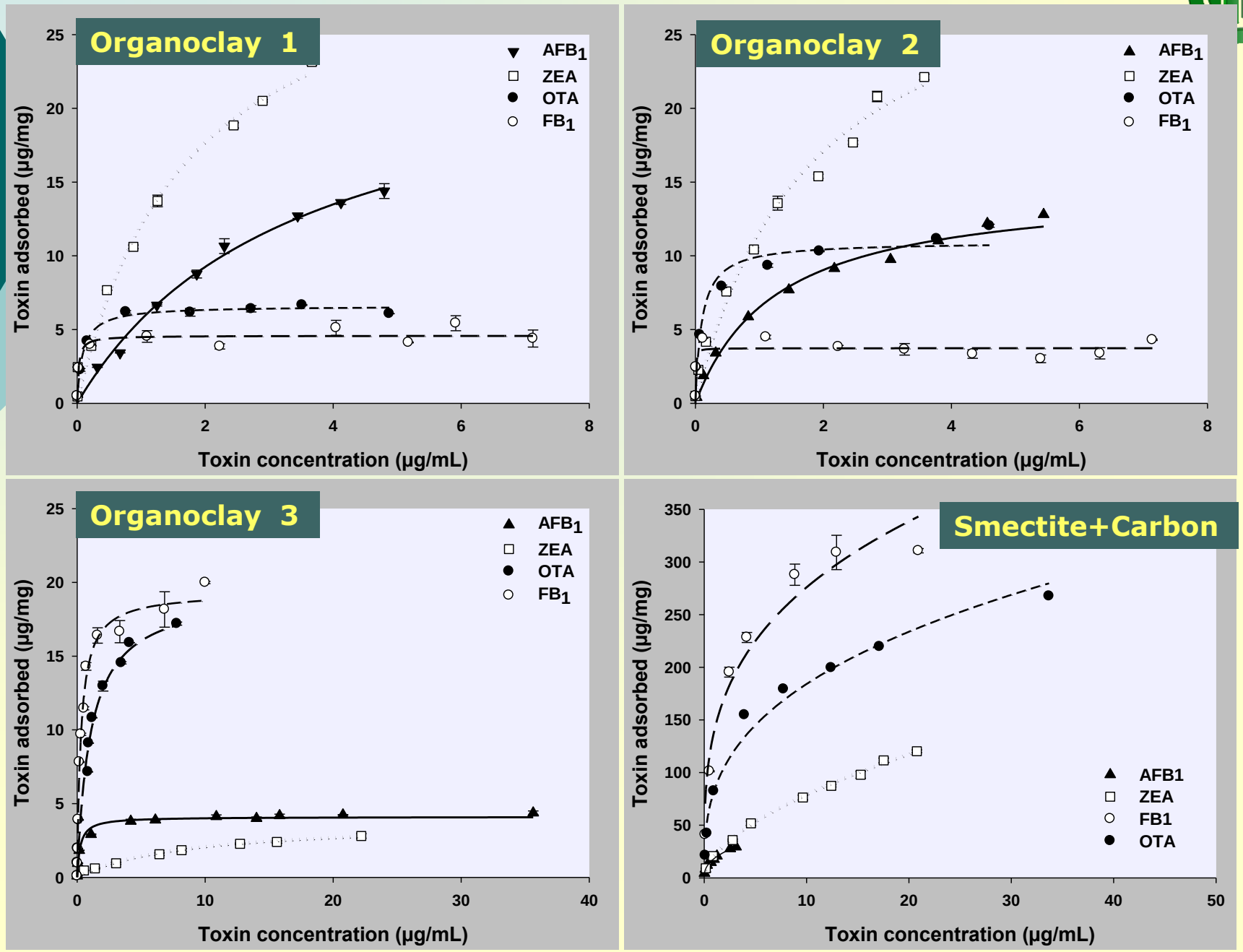
  organo-bentonite **> 17 Å**, depending on the surfactant



Product	d_{001}
Na-Smectite (Sigma)	12.20
Ca-Smectite (Novasil)	14.67
No.26 (Blend)	14.24

Product	d_{001}
No. 1 (organoclay)	24.53
No. 2 (organoclay)	29.43
No. 3 (organoclay)	28.98

EQUILIBRIUM ADSORPTION ISOTHERMS for AFB_1, ZEA, OTA, FB_1



EQUILIBRIUM ADSORPTION ISOTHERMS for AFB_1, ZEA, OTA, FB_1

Organoclay 1

	pH3		pH7	
	B_{max}	K_L	B_{max}	K_L
AFB_1	26	0.44	25	0.30
ZEA	31	0.51	33	0.58
OTA	35	0.45	7	11.51
FB_1	-	-	5	-

Organoclay 2

	pH3		pH7	
	B_{max}	K_L	B_{max}	K_L
AFB_1	15	0.97	15	0.79
ZEA	30	0.52	32	0.56
OTA	21	1.02	11	10.00
FB_1	-	-	4	-

Organoclay 3

	pH3		pH7	
	B_{max}	K_L	B_{max}	K_L
AFB_1	4	4.22	4	4.17
ZEA	2	0.17	4	0.12
OTA	6	0.15	19	0.95
FB_1	3	1.14	19	3.25

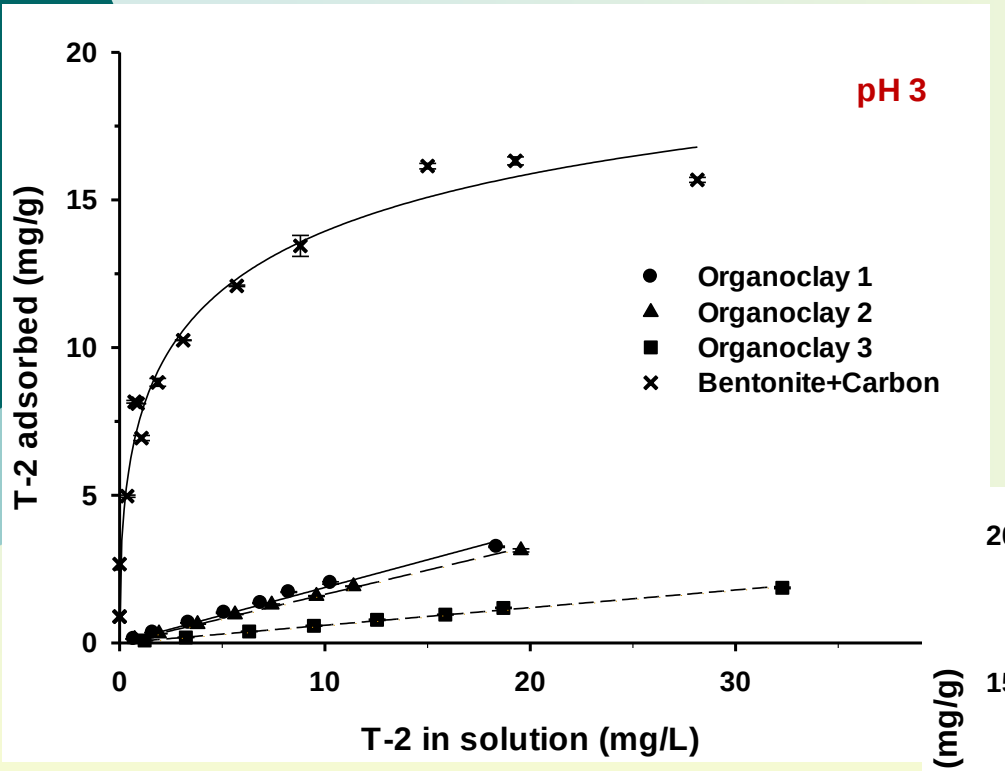
Toxin concentration (µg/mL)

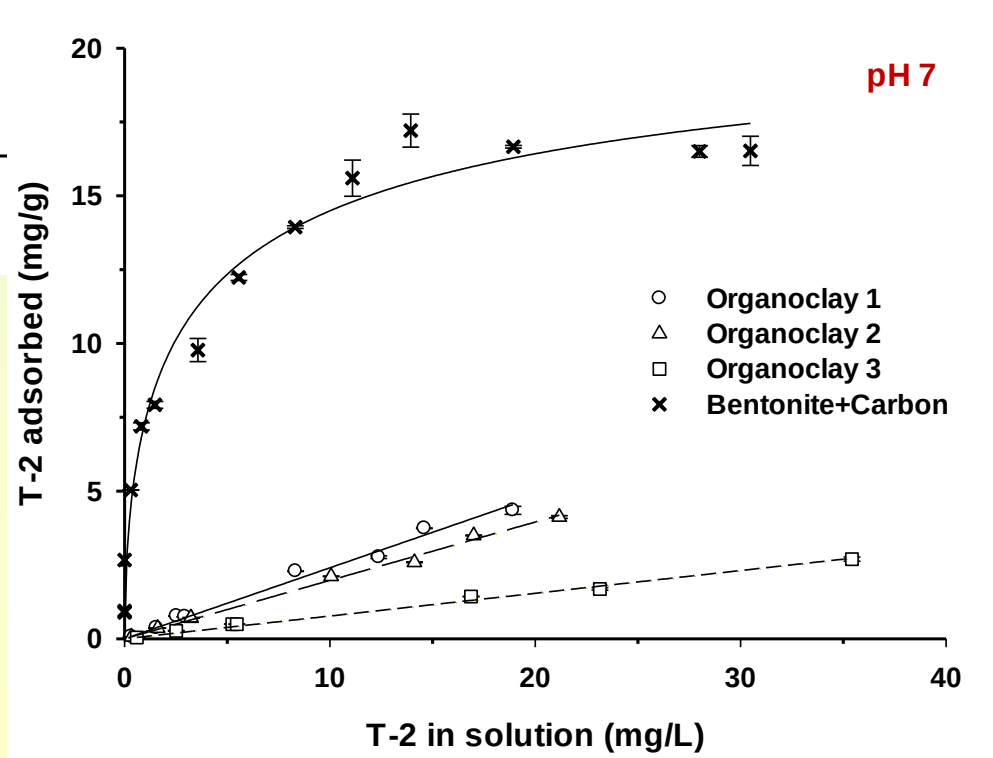
Bentonite+carbon

	pH3		pH7	
	B_{max}	K_L	B_{max}	K_L
AFB_1	29	2.99	34	1.40
ZEA	85	0.09	184	0.08
OTA	154	0.04	256	0.41
FB_1	50	-	332	0.67

Toxin concentration (µg/mL)

EQUILIBRIUM ADSORPTION ISOTHERMS for T-2 TOXIN





Chemisorption Index

- Determination of the **strength of toxin binding** of "selected adsorbents"

Product	Toxin	Adsorption (%) pH3	Desorption (%) pH7	MeOH 1	MeOH 2	MeOH 3	Total	Chemisorption Index
Bentonite +carbon	AFB_1	100	0.1	19	0	0	19	0.8
	ZEA	99	0.4	63	20	5	89	0.1
	OTA	100	0.0	0	0	0	0	1.0
	FB_1	100	0.0	0	0	0	0	1.0
Organo-clay 1	AFB_1	96	0.2	33	33	12	78	0.2
	ZEA	98	0.0	71	24	1	97	0.0
	OTA	99	0.0	66	24	1	91	0.1
	FB_1	99	0.0	28	7	1	37	0.6
Organo-clay 2	AFB_1	94	0.2	38	33	14	86	0.1
	ZEA	97	0.0	77	22	0	99	0.0
	OTA	98	0.0	60	25	1	86	0.1
	FB_1	98	0.0	15	4	1	20	0.8
Organo-clay 3	AFB_1	94	0.2	49	24	10	83	0.2
	ZEA	89	2.1	95	2	0	98	0.0
	OTA	99	0.1	62	1	0	63	0.4
	FB_1	98	0.0	83	4	2	89	0.2

Multi-Mycotoxin Adsorption Efficacy

- **Mycotoxin adsorptions** decrease in the order: **AFB_1>FB_1>ZEA>OTA>T-2**
- **All products** (but carbon based products and activated carbons) are **ineffective** towards **DON**
- **Minerals** adsorb preferably **AFB_1** (pHs 3 and 7), **OTA** and **FB_1** (pH 3)
- **Tectosilicates** (zeolites) are less effective than **phyllosilicates** (bentonites)
- **Yeast cell wall based products** adsorb preferably **ZEA**
- **Organoclays** (minerals with the cations of the interlayer replaced by quaternary alkylammonium ions) can act as **multi-toxin adsorbents**

However …

Organoclays are weak mycotoxin adsorbents, showing very low values for maximum adsorption capacity and affinity, and for chemisorption index

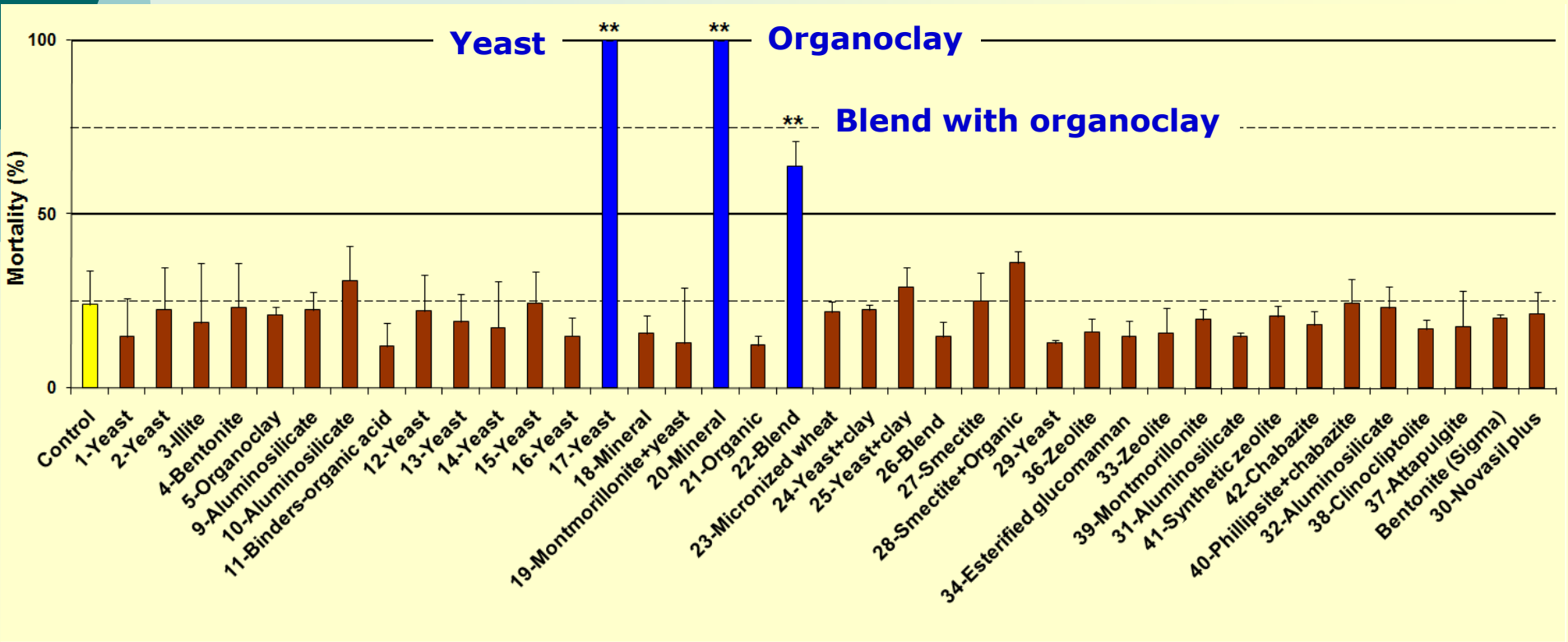
Organoclay can be toxic…

Brine Shrimp Lethality Assay

- Potential **toxicity** of mycotoxin adsorbents towards ***Artemia salina***

48-hr mortality

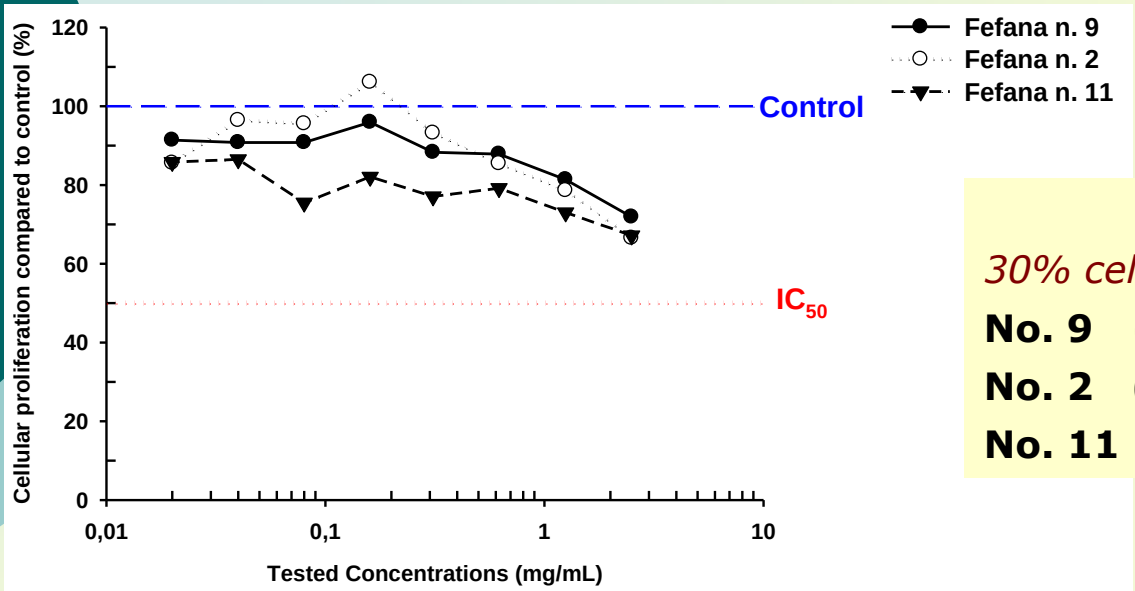
Amount of product extract tested: 0.25% w/v (2.5 mg/mL)



** statistical significance (Sheffe test: $P<0.01$) compared to the control ($n=4$)

Cytotoxicity Assay - Intestinal Cell Line

- **Human colon carcinoma cell line HT-29**



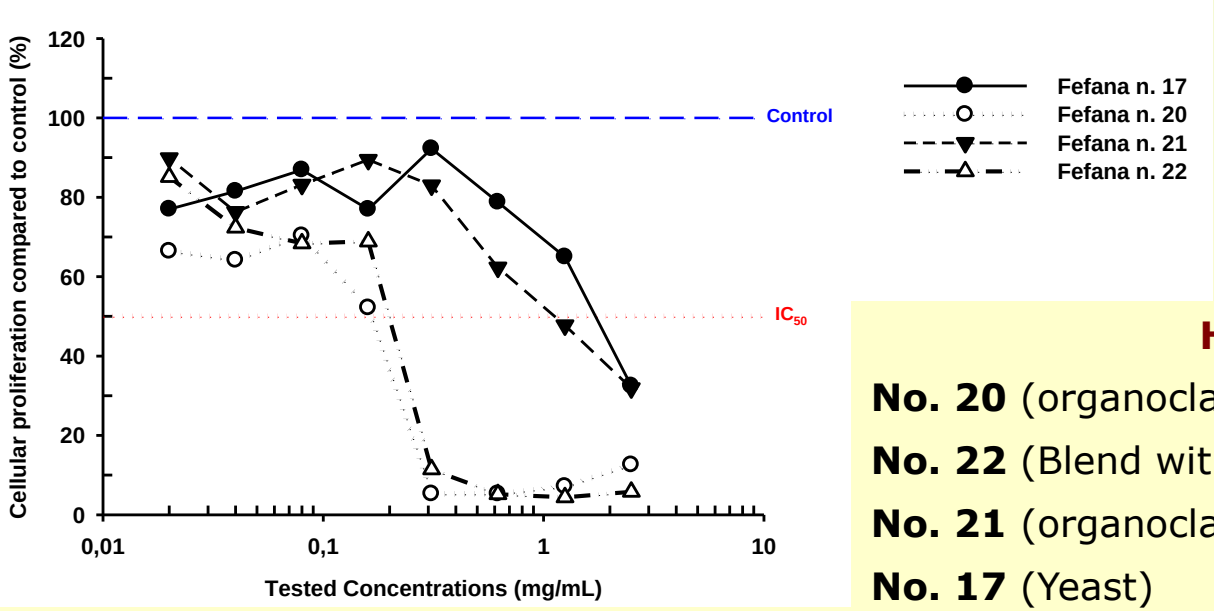
MINOR TOXICITY

*30% cellular inhibition at **2.5 mg/mL** level*

No. 9 (Ca/Na Aluminosilicate)

No. 2 (Yeast)

No. 11 (Binders+organic acids)



HIGH TOXICITY

No. 20 (organoclay) IC_{50}= **0.16 mg/mL**

No. 22 (Blend with organoclay) IC_{50}= **0.22 mg/mL**

No. 21 (organoclay) IC_{50}= **0.80 mg/mL**

No. 17 (Yeast) IC_{50}= **1.6 mg/mL**

Selection of Multi-Mycotoxin Adsorbents

Adsorbent dosage: 0.1% (w/v) – Toxin conc: 1 µg/mL – Temp.: 37°C

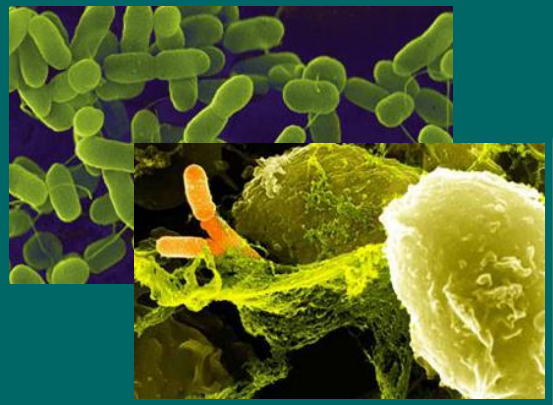
F.I.S. FEED INDUSTRY SERVICE

	Mycotoxin Adsorption (%)							
	AFB_1		ZEA		OTA		FB_1	
	pH3	*pH7*	*pH3*	*pH7*	*pH3*	*pH7*	*pH3*	*pH7*
Na-Smectite	99	83	2	3	10	0	88	6
Na-Smectite	99	97	4	7	29	2	92	9
Na-Smectite	87	84	15	6	1	1	27	1
Na-Smectite	100	100	18	4	55	3	100	8
Na-Smectite	100	98	28	8	74	3	96	8
Na-Smectite	100	100	34	14	77	4	100	4
Ca-Smectite	100	100	36	20	71	2	100	20
Na-Smectite	100	99	38	24	75	1	99	8
Humalite	73	58	47	31	39	0	47	1
Humate	79	29	60	20	63	0	79	21
Leonardite 1	80	43	67	22	64	2	64	3
Biopolymer	**85**	**81**	**73**	**74**	**82**	**3**	**38**	**2**
Na-Smectite	**98**	**98**	**77**	**81**	**90**	**14**	**100**	**26**
Na-Smectite	**99**	**99**	**79**	**77**	**88**	**6**	**100**	**27**
Na-Smectite	**100**	**99**	**84**	**76**	**77**	**4**	**100**	**13**
Leonardite 2	**91**	**95**	**86**	**88**	**87**	**5**	**92**	**10**
Na-Smectite	**100**	**99**	**89**	**82**	**80**	**25**	**99**	**25**

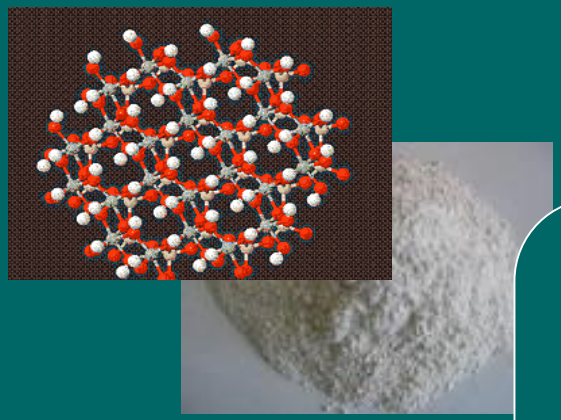
Mycotoxin-Detoxifying Agents

Mode of Action

Biodegradation



Adsorption



Bioprotection

BioProtection

Natural Ingredients

- **Provide immune support**
- **Counter the toxic side effects caused by mycotoxins** (oxidative stress, immunosuppression, inflammatory effects)
- **Adsorb mycotoxins** thus reducing their absorption in the GI tract (BIOSORPTION)

Grape pomaces

Artichoke by-products

Biopolymer from agricultural by-products

Bioprotection

BioSorption

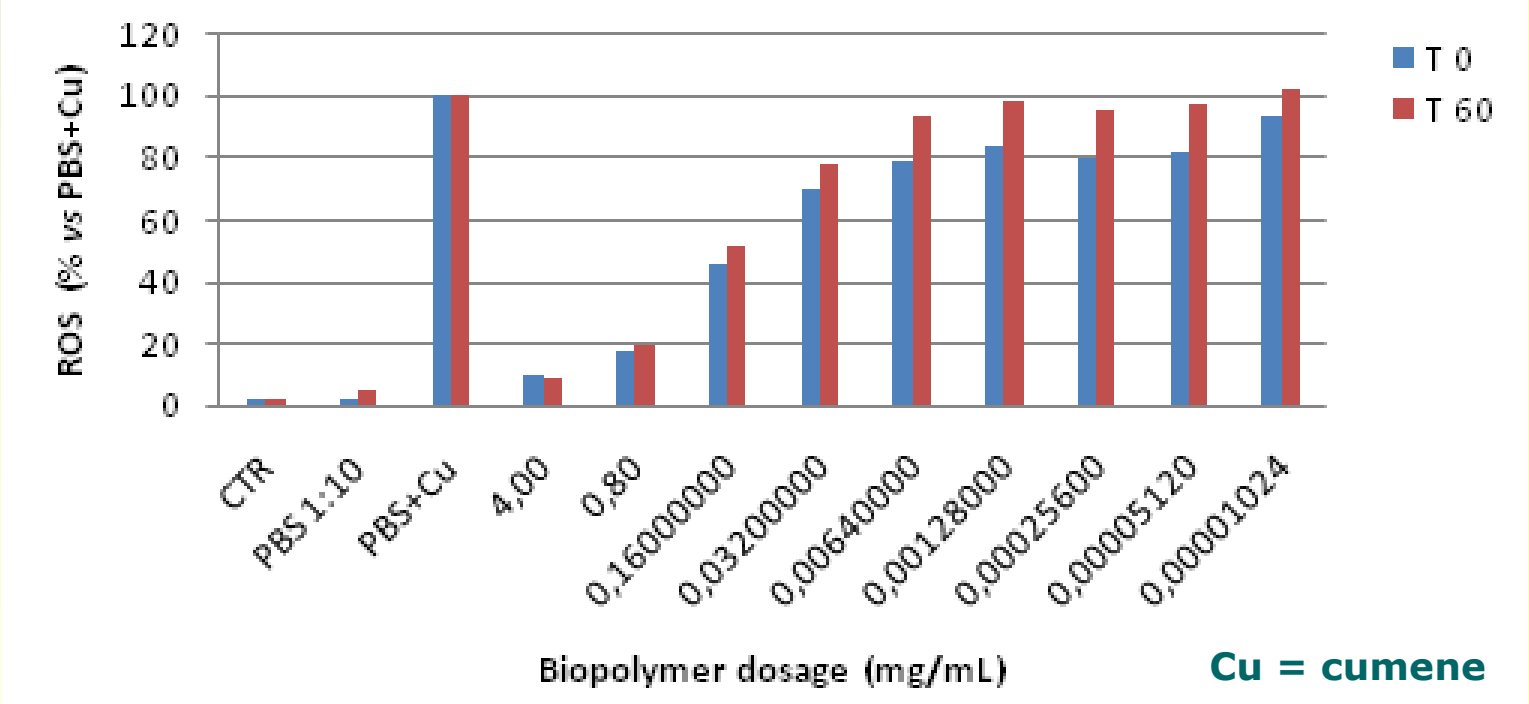
	Mycotoxin adsorption (%)							
	AFB_1		ZEA		OTA		FB_1	
	pH7	pH3	pH7	pH3	pH7	pH3	pH7	pH3
Biopolymer	100	100	97	97	87	87	41	0
Grape pomace	97	97	92	92	91	91	46	54
Artichoke by-product	67	69	91	90	13	30	15	0

Adsorbent dosage: 2% (w/v)
Multi-mycotoxin solution: 1 µg/mL
37°C; pH 7 – 3

BioProtection

	Mycotoxin adsorption (%)							
	AFB_1		ZEA		OTA		FB_1	
	pH7	pH3	pH7	pH3	pH7	pH3	pH7	pH3
Biopolymer	100	100	97	97	87	87	41	0

Antioxidant activity of PBS extracts of a biopolymer on HT-29 human colon cell line using DCFH-DA probe

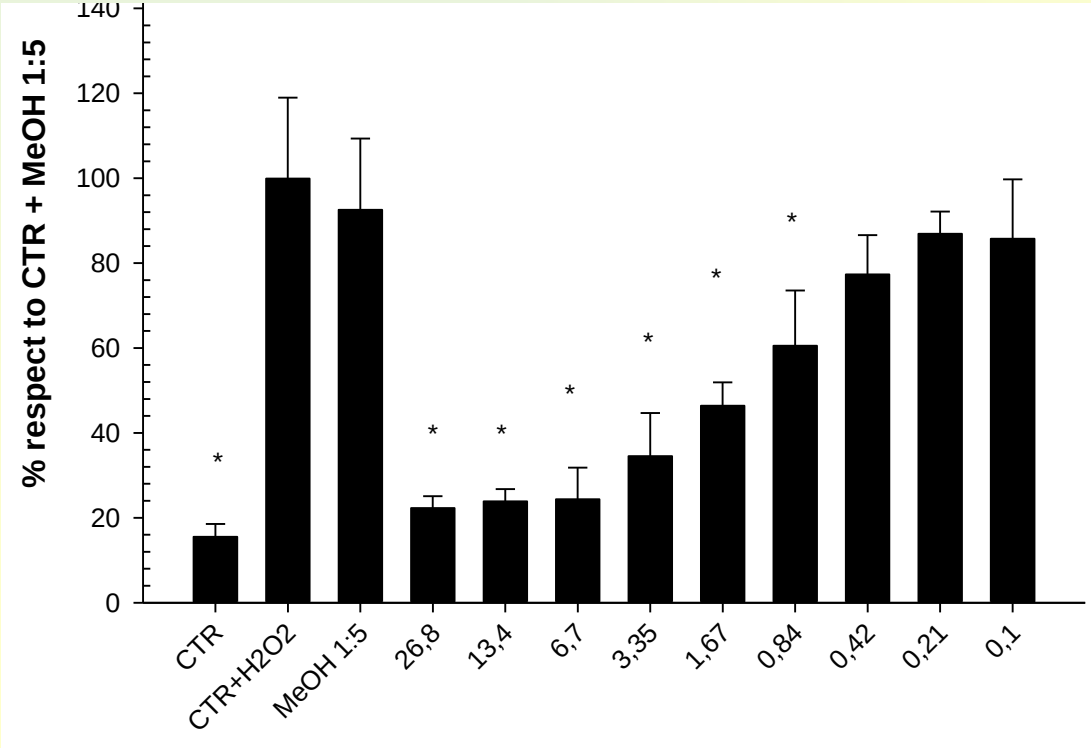


Cu = cumene

BioProtection

	Mycotoxin adsorption (%)							
	AFB_1		ZEA		OTA		FB_1	
	pH7	pH3	pH7	pH3	pH7	pH3	pH7	pH3
Artichoke by-product	67	69	91	90	13	30	15	0

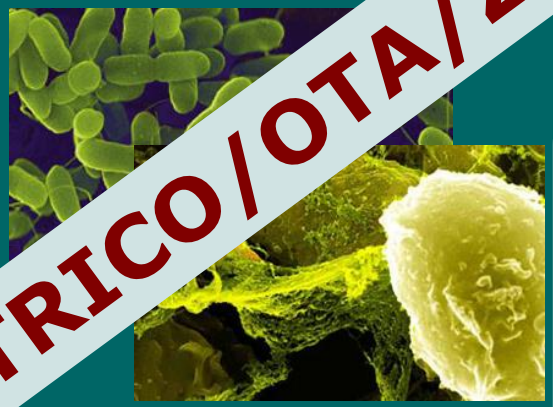
Antioxidant activity of MeOH extracts of artichoke on HT-29 human colon cell line using DCFH-DA probe



Mycotoxin-Detoxifying Agents

Efficacy

Biodegradati



TRICO/OTA/ZEA

Adsorption

AFLA/FUM/ZEA

Bioprotec

All mycotoxins

ACKNOWLEDGEMENTS

Donato Greco

Vito D'Ascanio

Francesco Grieco

QUESTIONS ???

giuseppina.avantaggiato@ispa.cnr.it

www.ispa.cnr.it