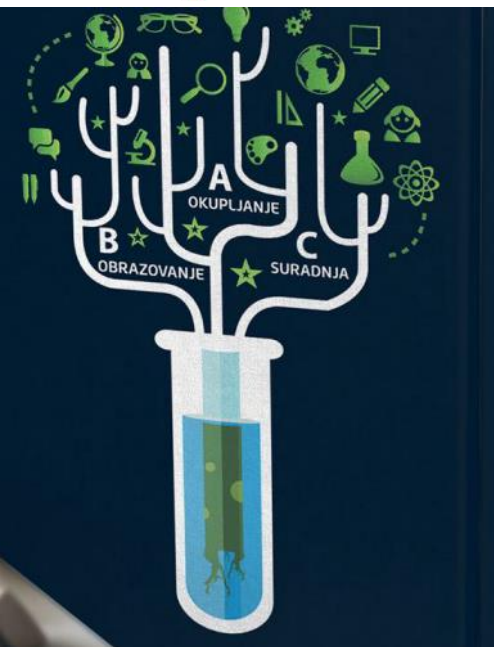




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Prisutnost nereguliranih mikotoksina u Hrvatskoj

Bojan Šarkanj

Sveučilište Sjever, Odjel za prehrambenu tehnologiju

bsarkanj@unin.hr

9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



mykes (greek) = gljive
toxicum (latin) = otrov } = **Mikotoksin**

Toksični sekundarni metaboliti plijesni, relativno male molekulske mase (do 700 Da)

Najznačajniji producenti: *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp. *Claviceps* spp.

Širok raspon štetnog djelovanja: karcinogeni, mutageni, teratogeni, estrogeni, imunotoksini...

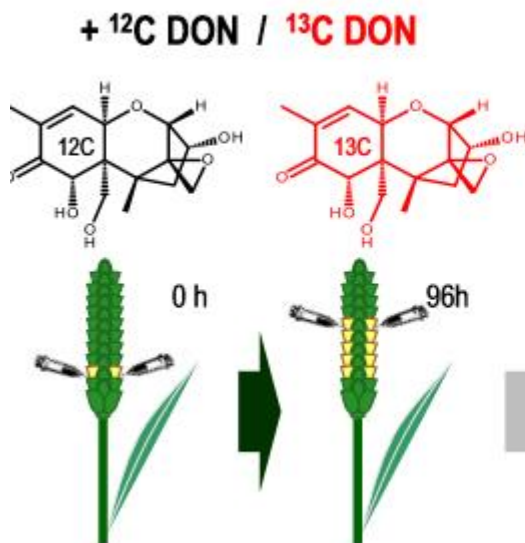


9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



Maskirani **Mikotoksini** = Biljni metaboliti mikotoksina



JSM Mycotoxins **67** (1), 11 - 16 (2017)
<http://doi.org/10.2520/myco.67-1-8>

JSM
Mycotoxins



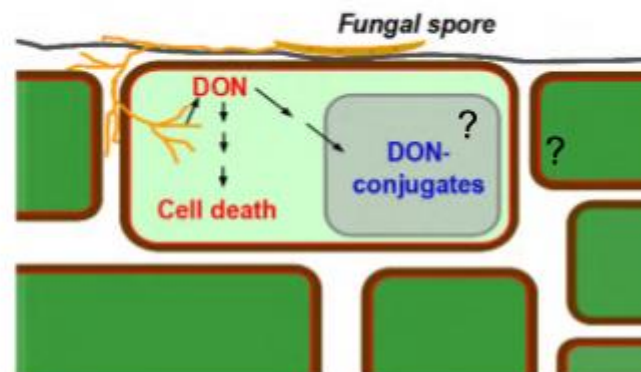
Mini Review

www.jstage.jst.go.jp/browse/myco

Mycotoxin testing: From Multi-toxin analysis to metabolomics

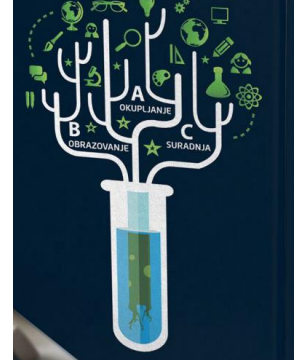
Rudolf Krška¹, Michael Sulyok¹, Franz Berthiller¹, Rainer Schuhmacher¹

¹University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology (IFA-Tulln),
 Center for Analytical Chemistry, Konrad-Lorenz-Straße 20, 3430 Tulln, Austria

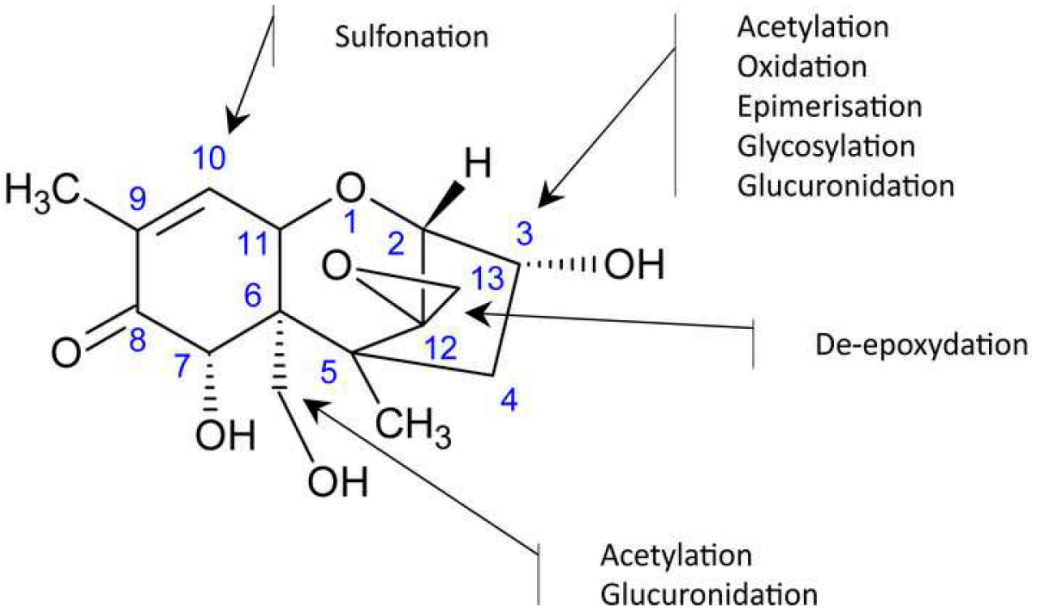


MYCOTOXINS

MASKED
MYCOTOXINS



Modificirani
Mikotoksini



Review

DOI: 10.2478/aiht-2018-69-3108

Yesterday *masked*, today *modified*; what do mycotoxins bring next?

Marija Kovač¹, Drago Šubarić², Mateja Bulaić¹, Tihomir Kovač², and Bojan Šarkanj^{2,3}

Table 1 Systematic definition of “modified mycotoxins”

1st level	2nd level	3rd level	4th level	Example
Free mycotoxins				DON, Aflatoxin B ₁ , 3-acetyl-DON, 15-acetyl-DON
Matrix-associated mycotoxins	Complexes, physically dissolved or trapped Covalently bound			Fumonisin bound to starch, OTA- and DON-oligosaccharides Aflatoxin B ₁ -epoxide
Modified mycotoxins	Biologically modified	Functionalised (phase 1-metabolites) Conjugated (phase 2 – metabolites)	Conjugated by plants (= masked according to ILSI) Conjugated by animals Conjugated by fungi	DON-3-glucoside DON-3/8/15-glucuronide, HT2-3/4-glucuronide ZEN-14-sulfate Deepoxy-DON (=DOM-1) norDON A-C, N-carboxy-methyl-FB ₁ , 14-(R)-OTA DON-sulfonate, norDON A-C (under alkaline conditions)
	Chemically modified	Differently modified Thermally formed Non-thermally formed		

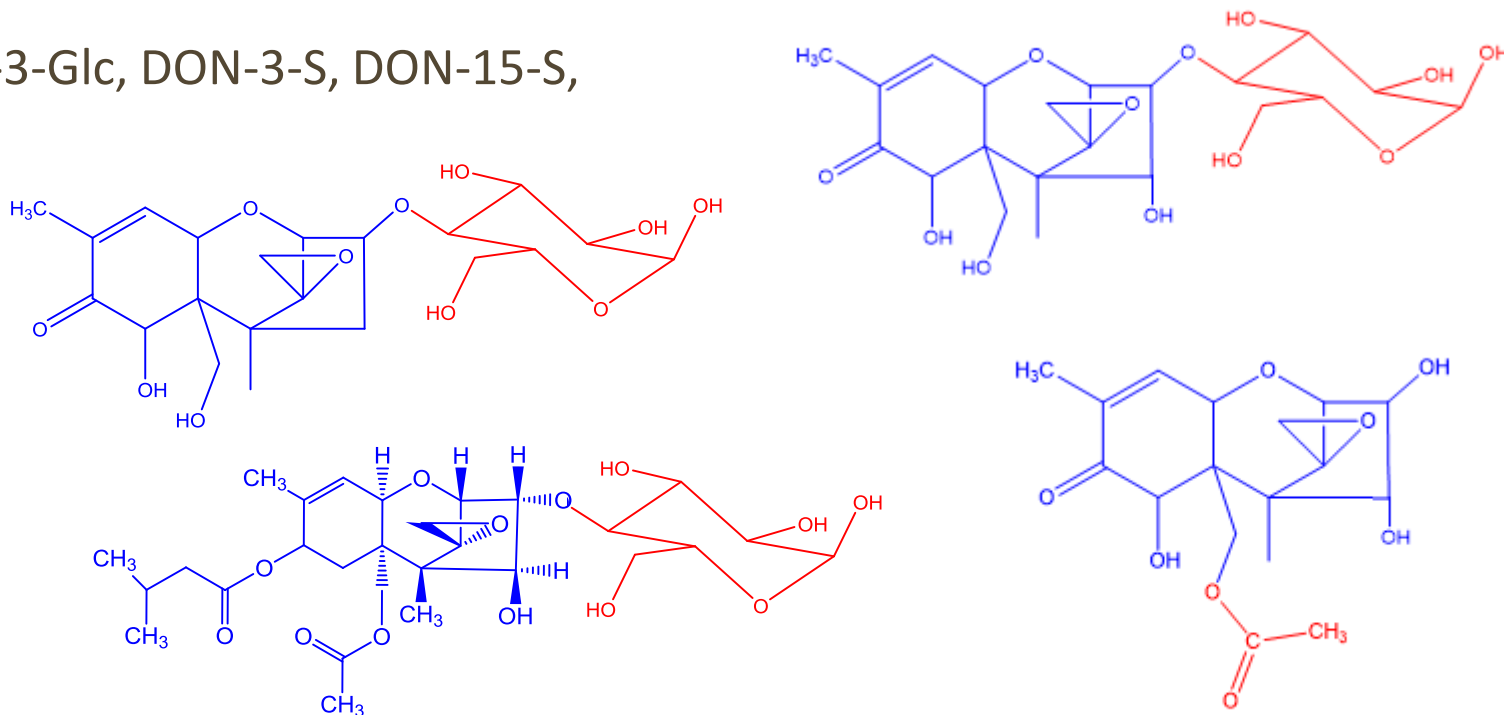
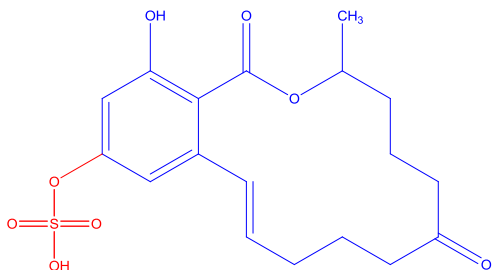
9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



Modificirani mikotoksini:

- DONs: 3-AcDON, 15-AcDON, DON-3-Glc, DON-3-S, DON-15-S,
- NIV glucoside,
- HT-2 glucoside,
- ZON-4-sulfate,

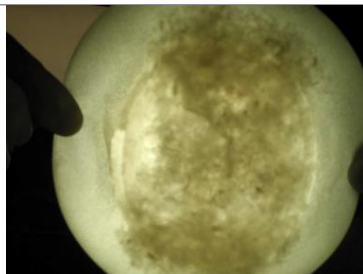


9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

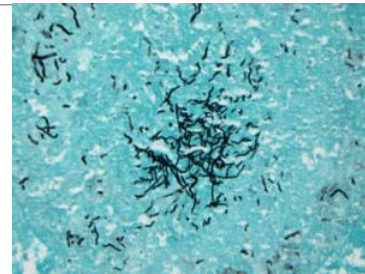
Pojavnost nereguliranih mikotoksina u Hrvatskoj



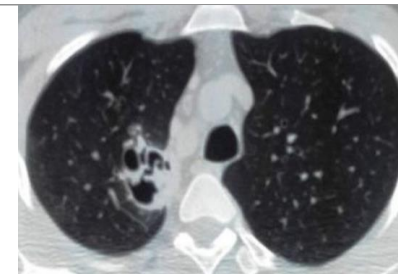
Wright et al., 2006



Wright et al., 2006



Wright et al., 2006



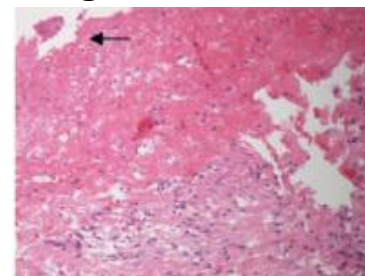
Tiwari et al., 2014



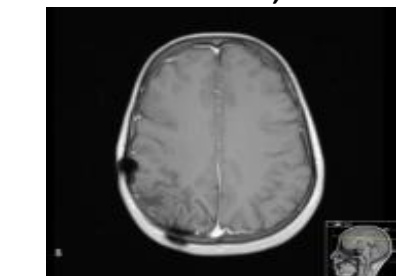
Li et al., 2009



Li et al., 2009



Li et al., 2009



Bangash, 2017



El-Tawil & El-Tawil, 2003



El-Tawil & El-Tawil, 2003



Cook et al., 2015

Osim biljaka,
pljesni vole i ljude

9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj

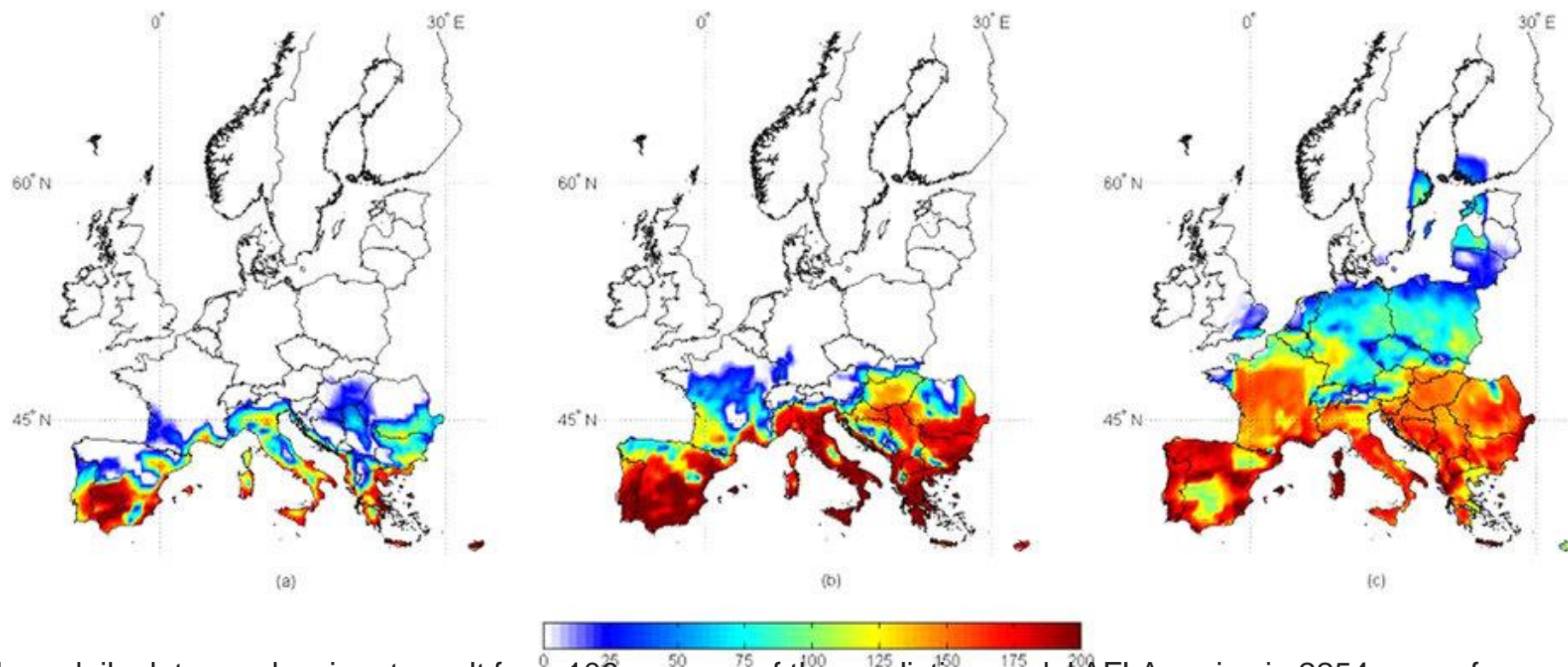
FAO: 25% uroda je kontaminirano mikotoksinima(1998)

Pinotti et al., 2016 >90 % uroda je kontaminirano mikotoksinima (2016)

SCIENTIFIC REPORTS

EN Aflatoxin B₁ contamination in maize in Europe increases due to climate change

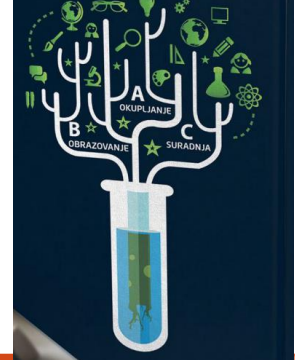
2015 P. Battilani¹, P. Toscano², H. J. Van der Fels-Klerx³, A. Moretti⁴, M. Camardo Leggieri¹,
2016 C. Brera⁵, A. Rortais⁶, T. Goumperis⁶ & T. Robinson⁶



Mean daily data used as input result from 100-year run of the predictive model AFLA-maize in 2254 geo-referenced points throughout Europe, in the 3 scenarios. The scale 0–200 refers to the aflatoxin risk index (AFI), output from the predictive model; increasing the (present (a), +2°C (b), +5°C (c)) number, the risk of contamination increases.

9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



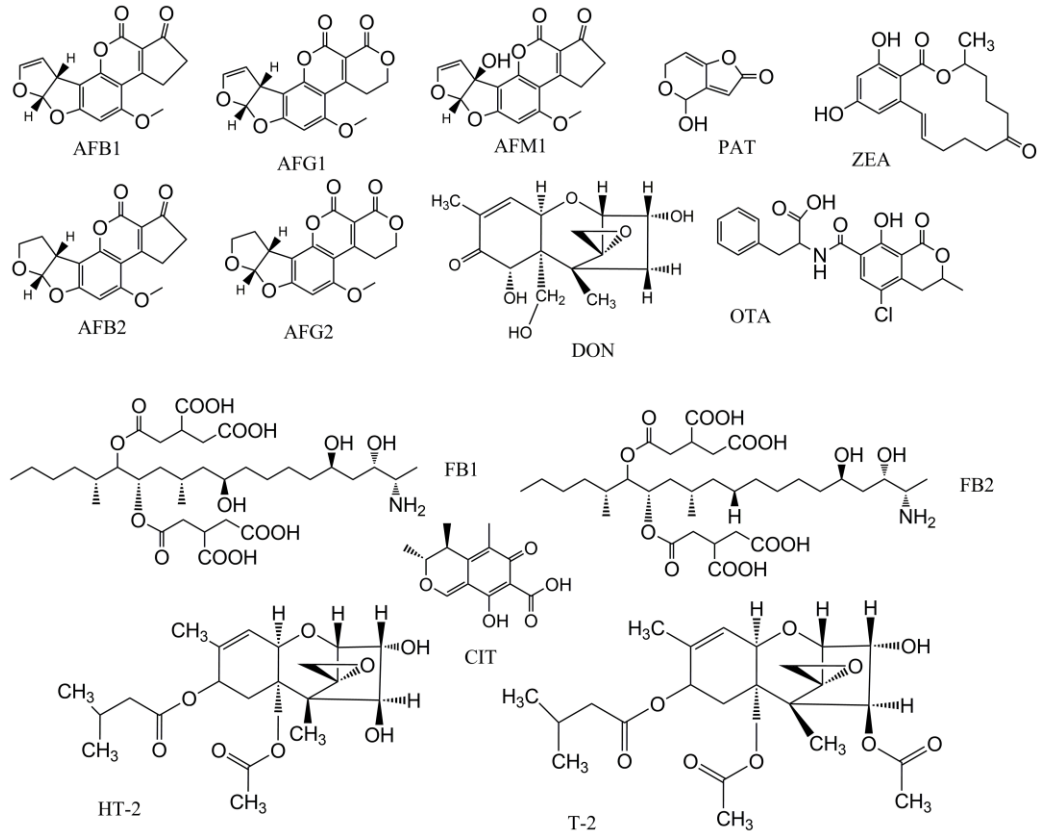
20.12.2006

EN

Official Journal of the European Union

L 364/5

COMMISSION REGULATION (EC) No 1881/2006



Regulated	Not regulated
Aflatoxins	Alternaria toxins
Deoxynivalenol	Other trichothecenes
Zearalenone	Ergot alkaloids
Ochratoxin A	Enniatins & Beauvericin
Fumonisin B1 & B2	Modified DONs
Patulin	Moniliformin
Citrinin	Sterigmatocystin...

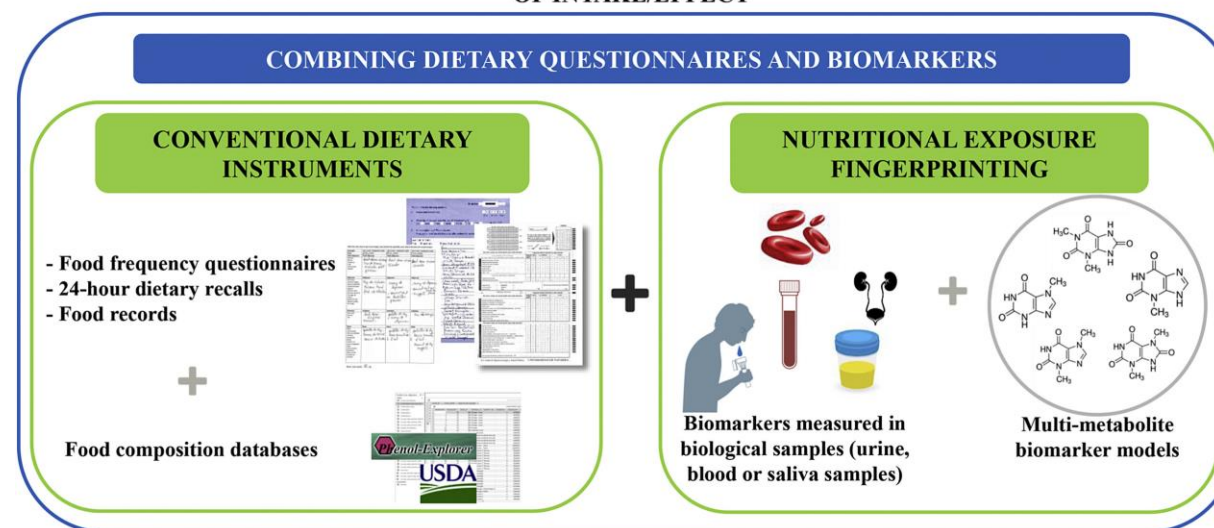
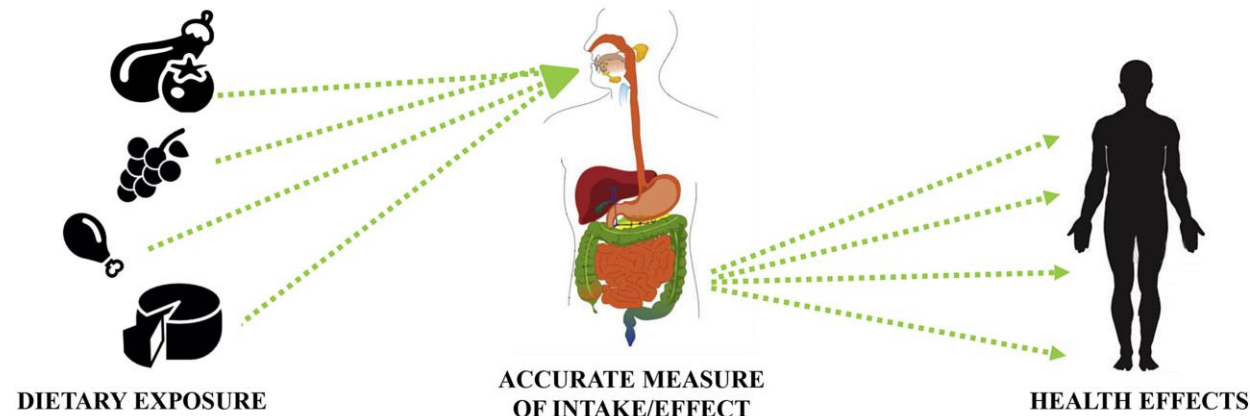
9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



Procjena rizika:

- Procjena izloženosti
 - Pojavnost mikotoksina u hrani
 - Podaci o prehrambenim navikama
- Karakterizacija opasnosti
 - Metabolizam mikotoksina
 - Toksičnost mikotoksina



Trends in Food Science & Technology xxx (2017) 1–10

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: <http://www.journals.elsevier.com/trends-in-food-science-and-technology>



Commentary

Novel strategies for improving dietary exposure assessment: Multiple-data fusion is a more accurate measure than the traditional single-biomarker approach

Mar Garcia-Aloy ^{a, b, 1}, Montserrat Rabassa ^{a, b, 1}, Patricia Casas-Agustench ^a, Nicole Hidalgo-Liberona ^a, Rafael Llorach ^{a, b}, Cristina Andres-Lacueva ^{a, b, *}

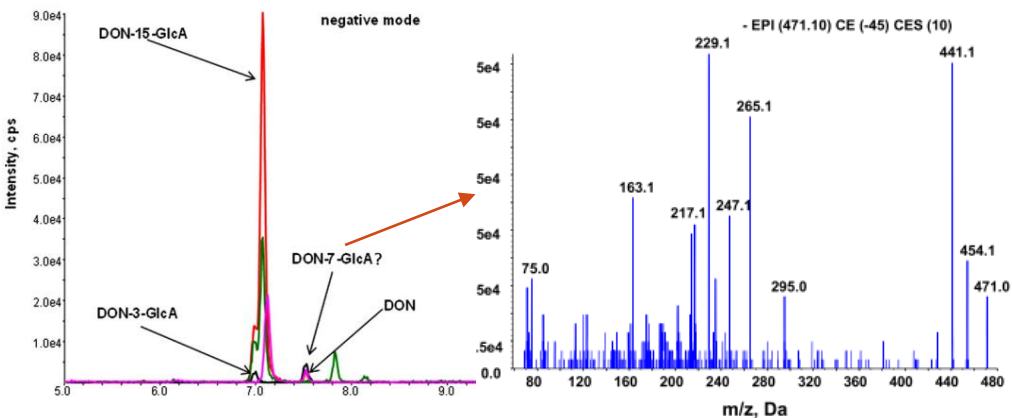
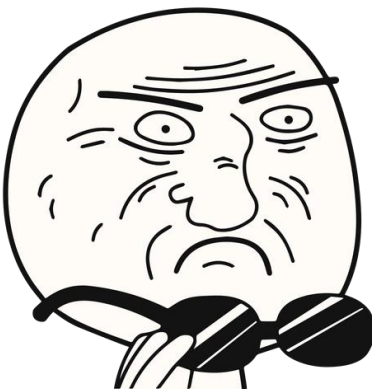
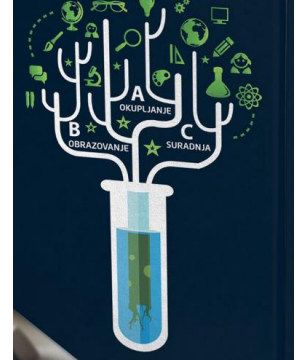



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9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj

Human mycotoxin biomarkers






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Food and Chemical Toxicology

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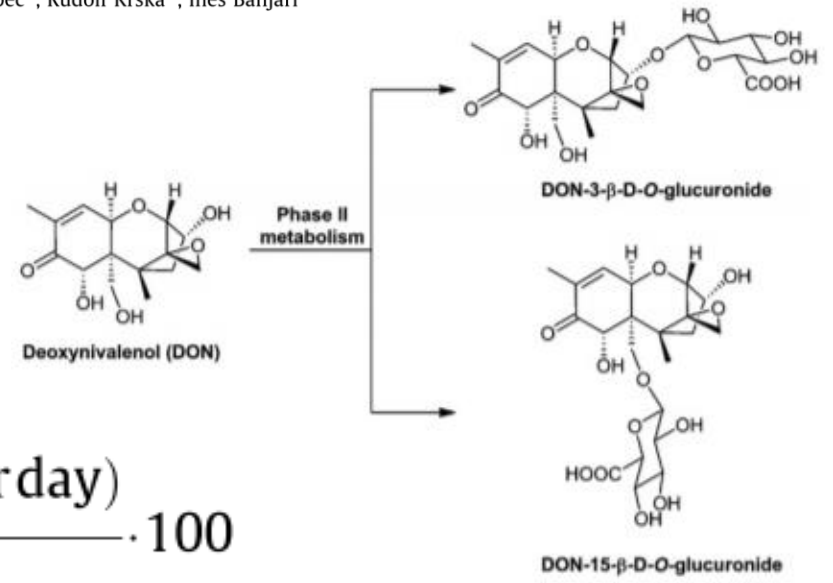
Urinary analysis reveals high deoxynivalenol exposure in pregnant women from Croatia

Bojan Šarkanj^{a,b,1}, Benedikt Warth^{b,1}, Silvio Uhlig^c, Wilfred A. Abia^{b,d,e}, Michael Sulyok^{b,*}, Tomislav Klačec^a, Rudolf Krska^b, Ines Banjari^f

Mycotoxin levels in urine samples of pregnant women from Croatia (N = 40).

	Mycotoxin [µg L ⁻¹]		
	DON-15-GlcA ^a	DON-3-GlcA ^a	DON ^a
Average	120.4	28.8	18.3
Median	55.2	10.0	6.7
Max	1237.7 ^b	298.1	275.0
<LOD	1	7	9
<LOQ	4	18	21
Number of positive (%)	39/40 (98%)	33/40 (83%)	31/40 (76%)

$$\%TDI = \frac{\left(\frac{DON-15-GlcA \cdot MW(DON)}{MW(DON-15-GlcA)} + \frac{DON-3-GlcA \cdot MW(DON)}{MW(DON-3-GlcA)} + DON \right) \cdot V(\text{urine per day})}{ER \cdot BW \cdot CF} \cdot 100$$



9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj

Human mycotoxin biomarkers



Food and Chemical Toxicology 62 (2013) 231–237

Contents lists available at [ScienceDirect](#)

Food and Chemical Toxicology

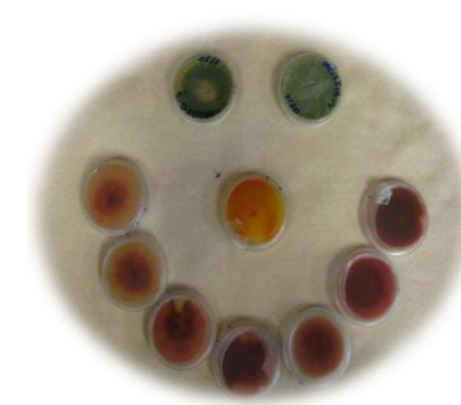
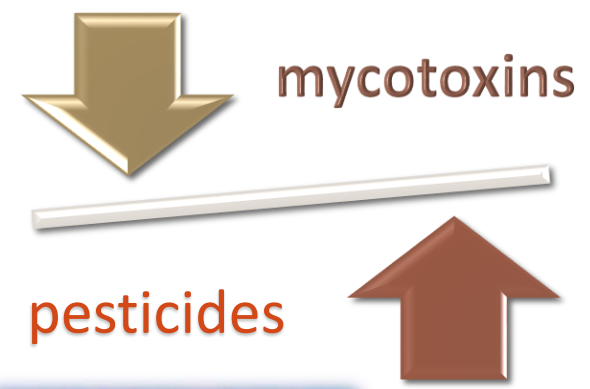
journal homepage: www.elsevier.com/locate/foodchemtox

ELSEVIER

Urinary analysis reveals high deoxynivalenol exposure in pregnant women from Croatia

Bojan Šarkanj^{a,b,1}, Benedikt Warth^{b,1}, Silvio Uhlig^c, Wilfred A. Abia^{b,d,e}, Michael Sulyok^{b,*}, Tomislav Klačec^a, Rudolf Krska^b, Ines Banjari^f

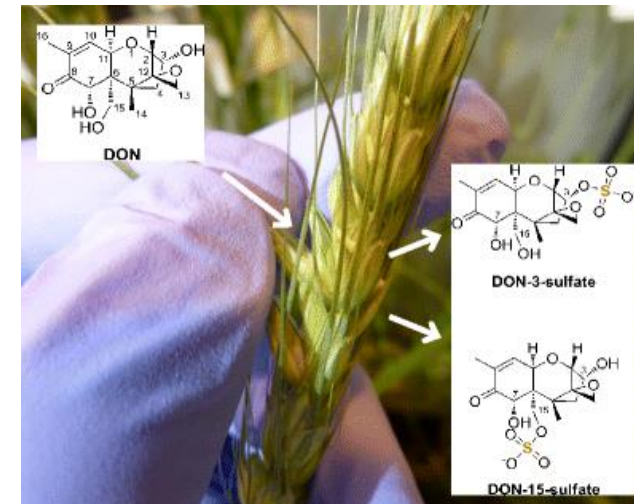
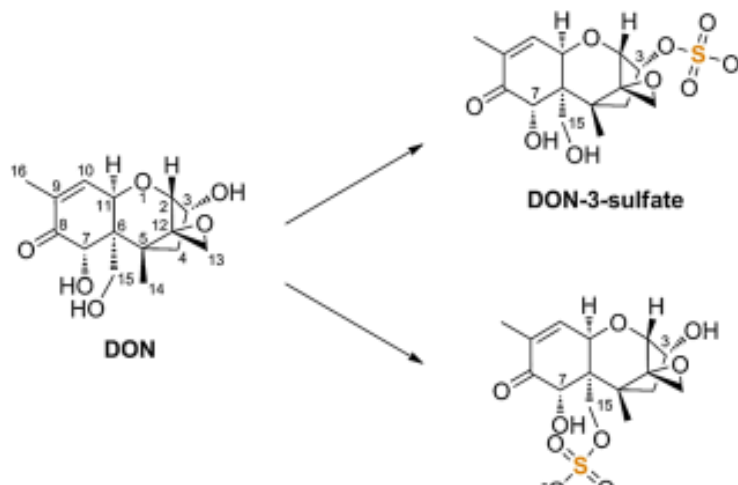
[CrossMark](#)



9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj

Human mycotoxin biomarkers



Anal Bioanal Chem (2015) 407:1033–1039
DOI 10.1007/s00216-014-8340-4

RAPID COMMUNICATION

Deoxynivalenol-sulfates: identification and quantification of novel conjugated (masked) mycotoxins in wheat

Benedikt Warth · Philipp Fruhmant · Gerlinde Wiesenberger · Bernhard Kluger ·
Bojan Šarkanj · Marc Lemmens · Christian Hametner · Johannes Fröhlich ·
Gerhard Adam · Rudolf Krska · Rainer Schuhmacher

Archives of Toxicology
<https://doi.org/10.1007/s00204-019-02459-w>

BIOLOGICS

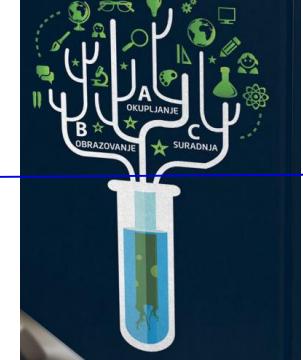
The *Fusarium* metabolite culmorin suppresses the in vitro glucuronidation of deoxynivalenol

Lydia Woelflingseder¹ · Benedikt Warth¹ · Immina Vierheilg¹ · Heidi Schwartz-Zimmermann² · Christian Hametner³ ·
Veronika Nagl⁴ · Barbara Novak⁴ · Bojan Šarkanj⁵ · Franz Berthiller² · Gerhard Adam⁶ · Doris Marko¹ 



9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



Call for data



- ▶ Upon receiving request from the EC to deliver scientific opinions on risk related to certain mycotoxins calls for data are issued by EFSA
- ▶ Collected data are extracted by the EFSA data management system and then used for writing future EFSA scientific opinions

9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj

Occurrence of mycotoxins in Cro



Occurrence of mycotoxins in Croatian cereals - CroMycoScreen

Funding: 101.448,15 € (100% EU European Structural and European Social Fund)

Duration of project: 29.6.2015.-29.9.2016.

<http://www.ptfos.unios.hr/CroMycoScreen/>

<https://www.facebook.com/groups/CroMycoScreen/>



Ministarstvo
znanosti,
obrazovanja
i sporta



9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



EFSA's most wanted:

- enniatins (**ENNs**),
- beauvericin (**BEA**),
- sterigmatocystin (**STERA**),
- moniliformin (**MON**),
- diacetoxyscirpenol (**DAS**),
- nivalenol (**NIV**),
- 3- i 15-acetyldeoxynivalenol (**3-AcDON**, **15-AcDON**),
- ergot alkaloids (**EA**),
- alternaria toxins (**AT**) and
- modified mycotoxins deoxynivalenol-3-glucoside (**D3G**).



“available occurrence data are too limited to carry out a reliable human and animal dietary exposure assessment” EFSA

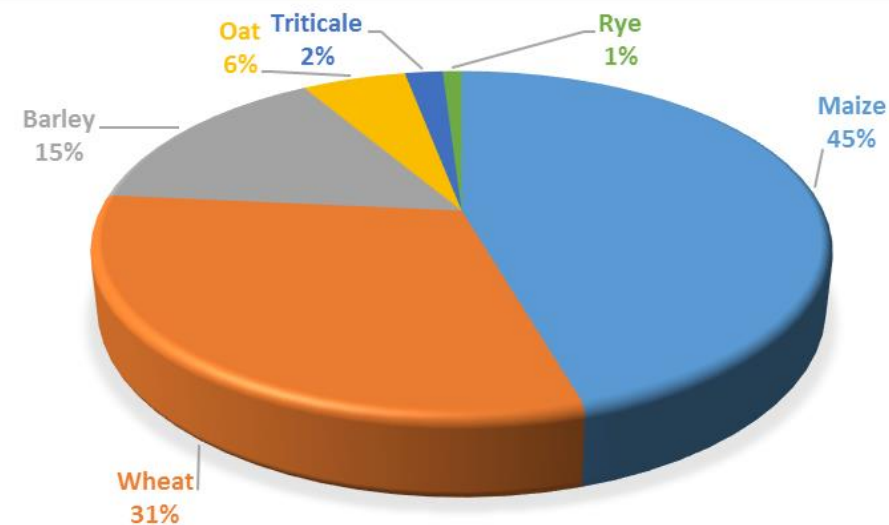
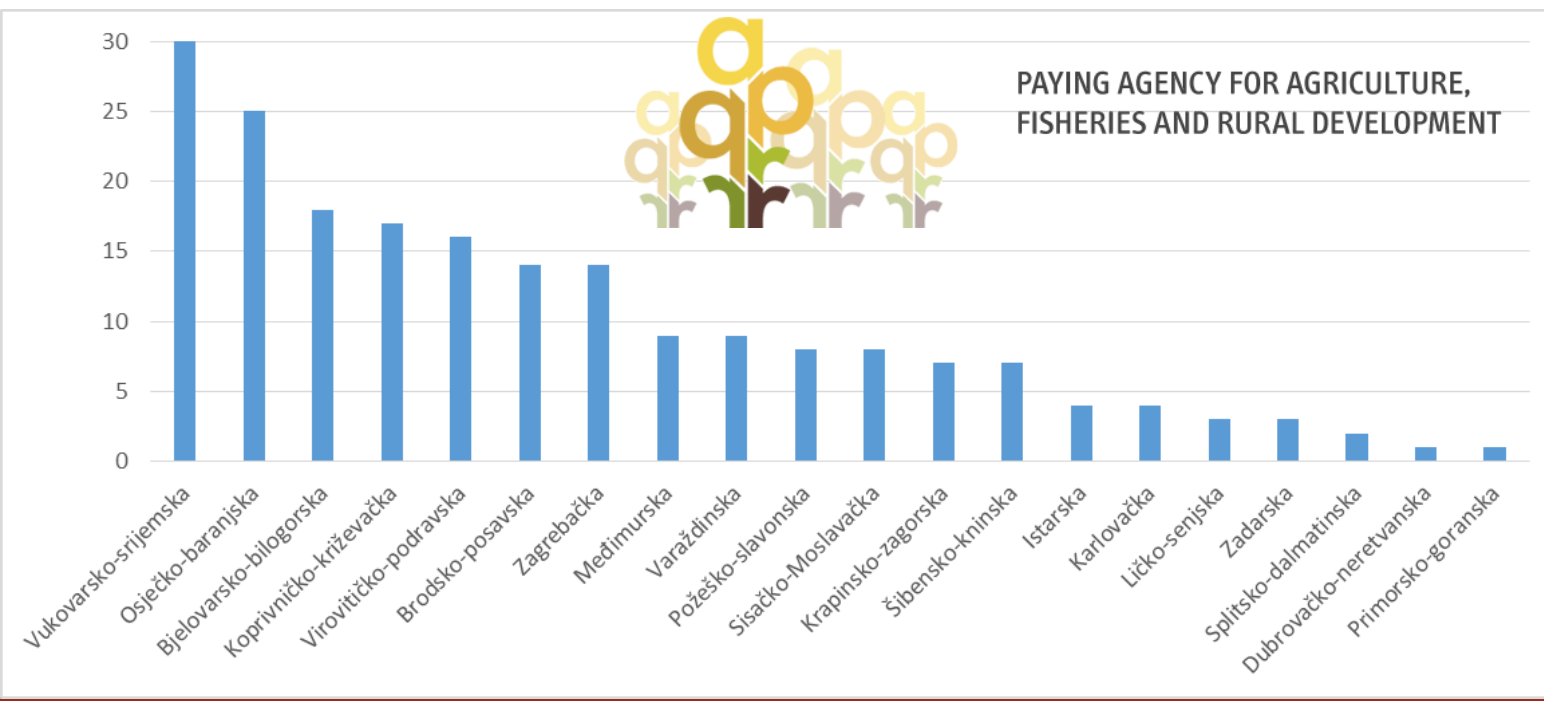
9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



200 samples of cereals:

20 for each of the Croatian counties + 180 according to their relative production rate (according to Paying agency for Agriculture, Fisheries and Rural Development)





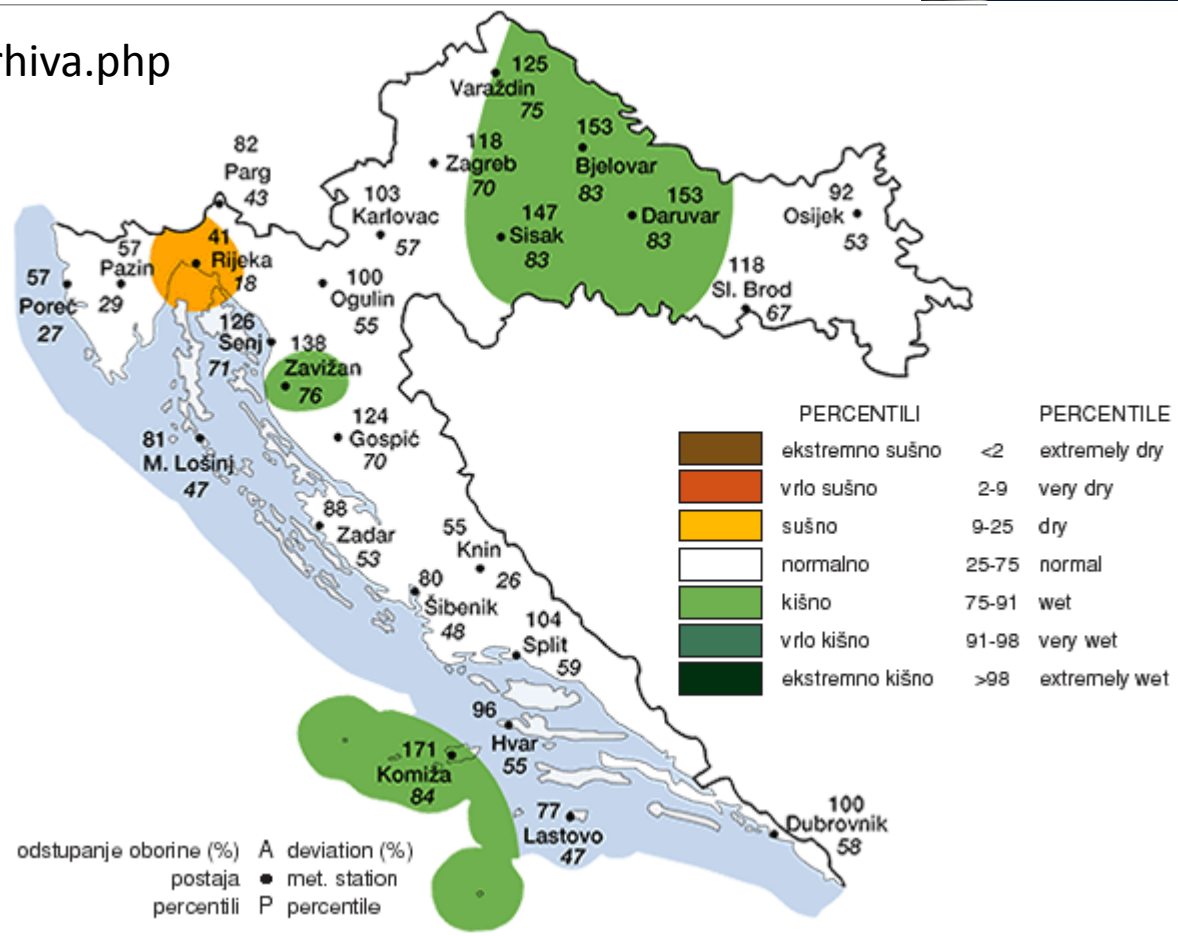
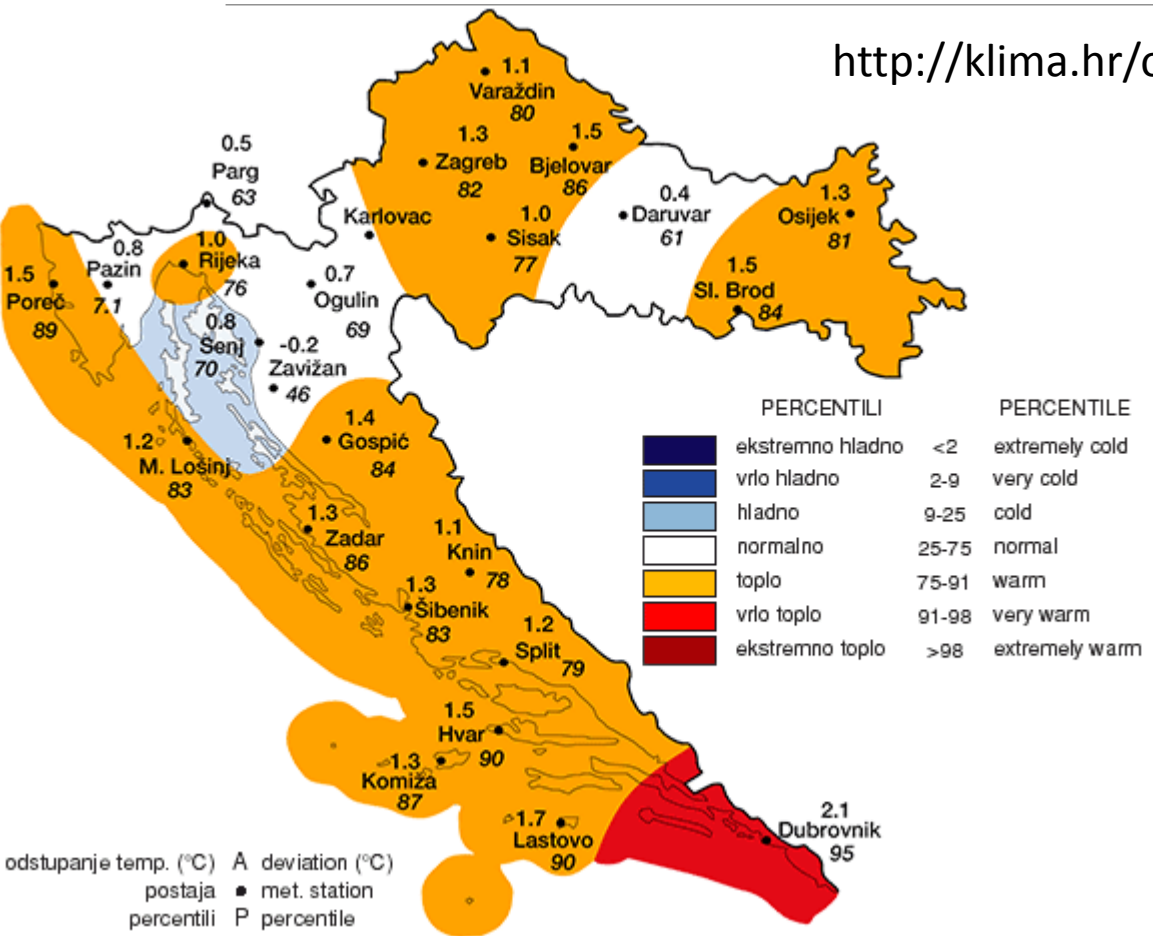
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Pojavnost nereguliranih mikotoksina u Hrvatskoj

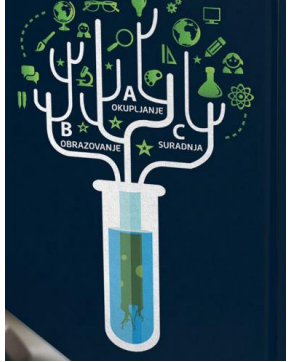


http://klima.hr/ocjene_arhiva.php

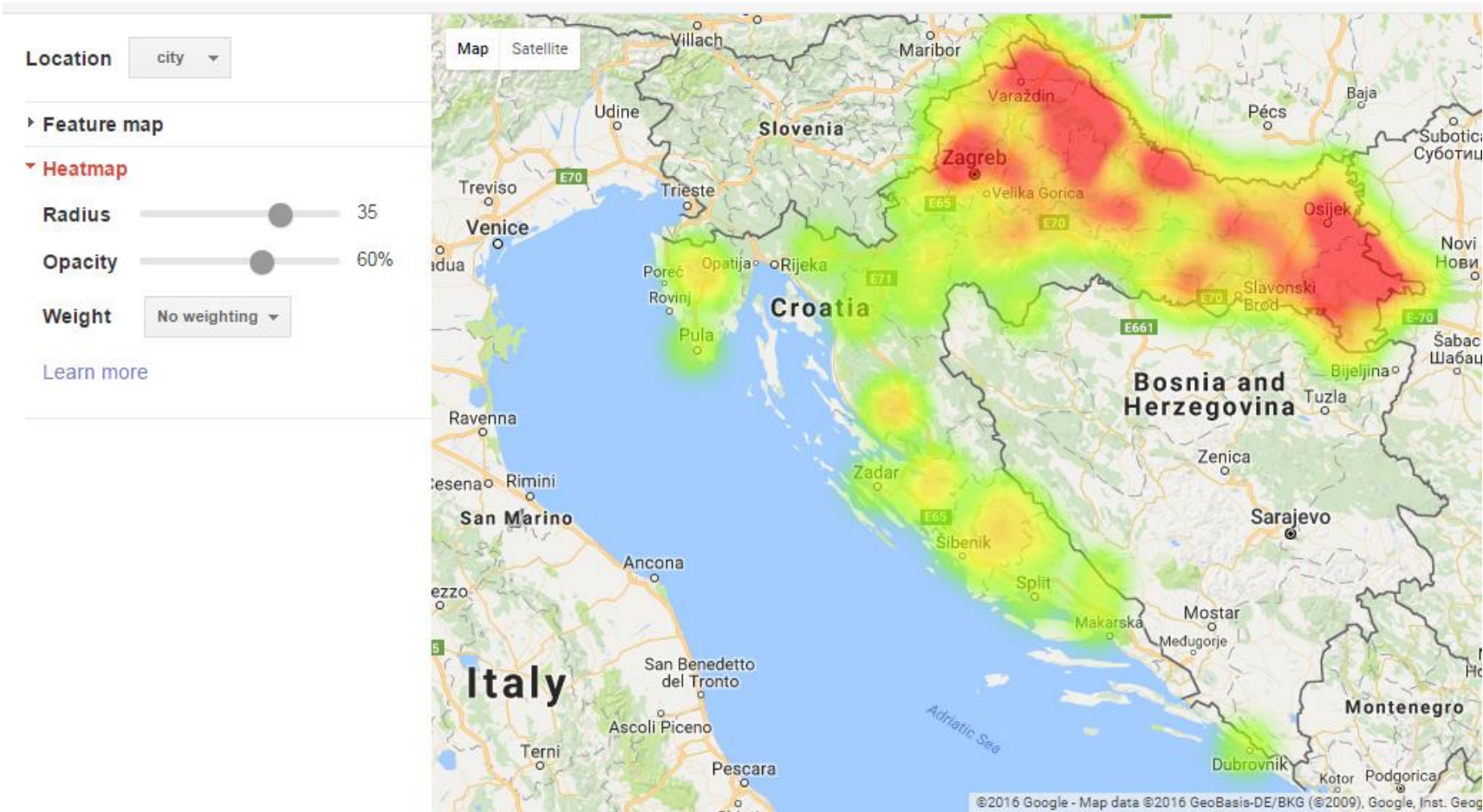


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Pojavnost nereguliranih mikotoksina u Hrvatskoj

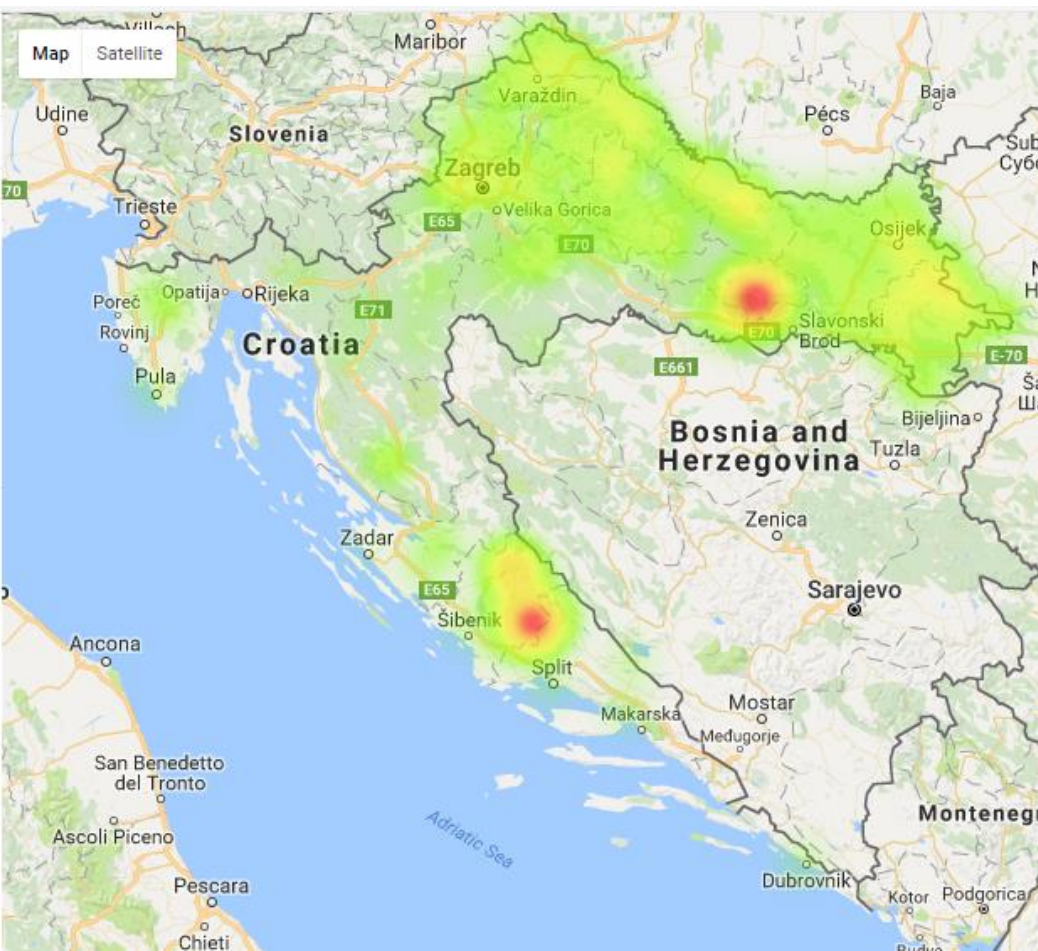
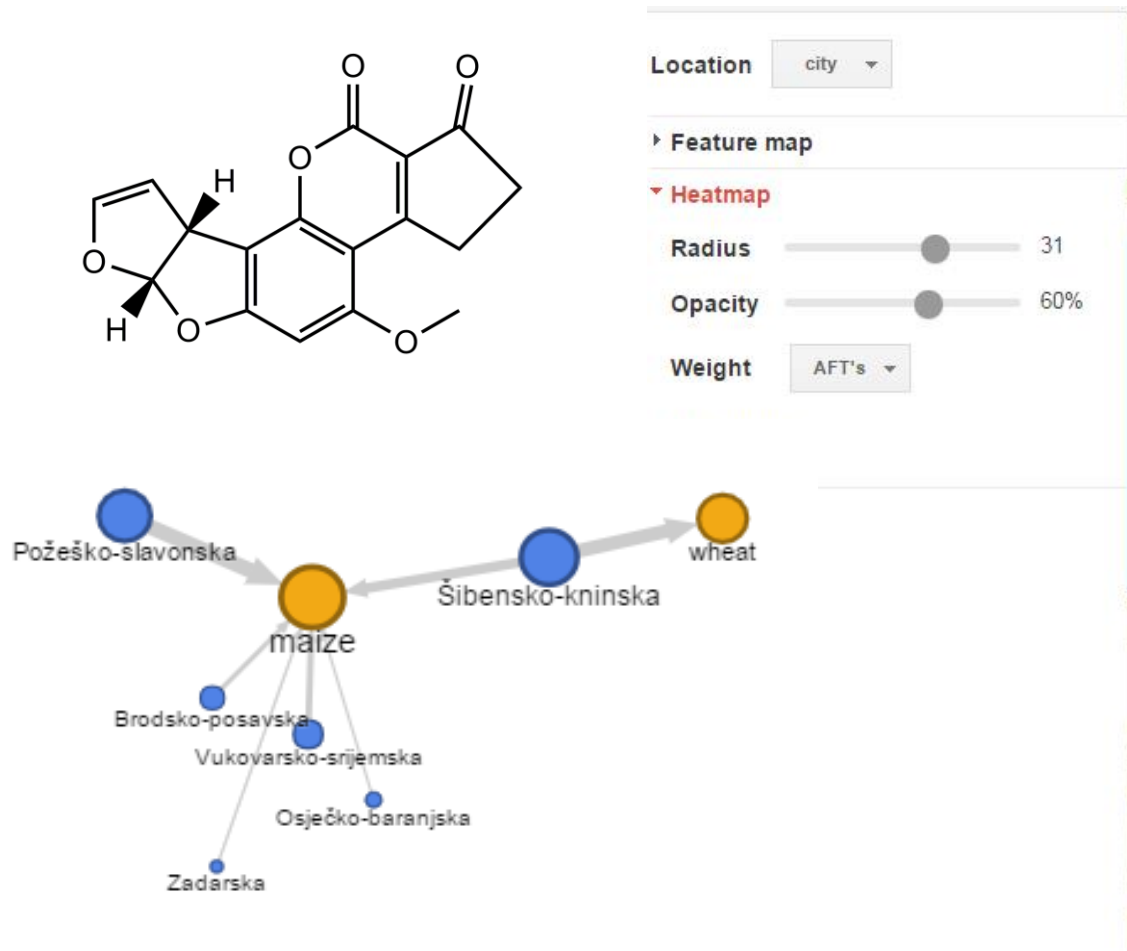
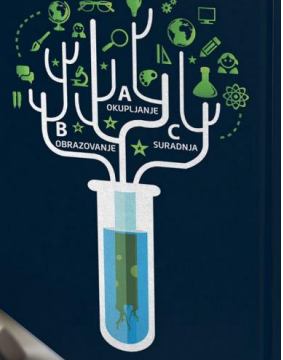


All mycotoxins



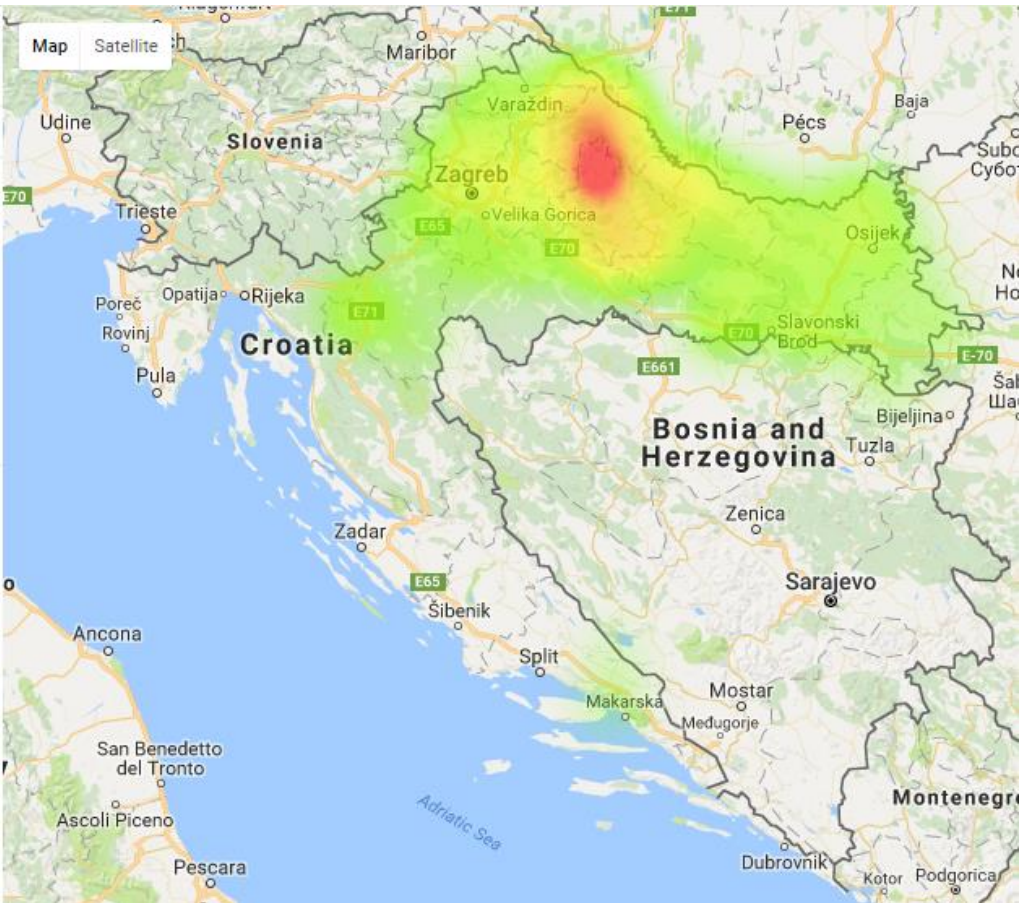
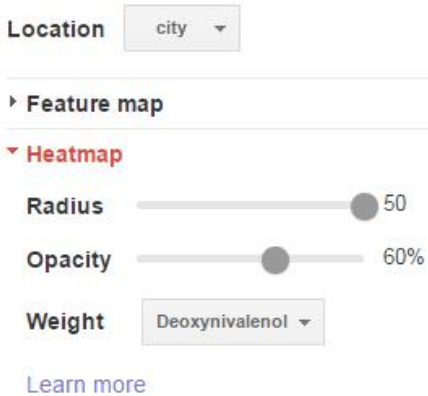
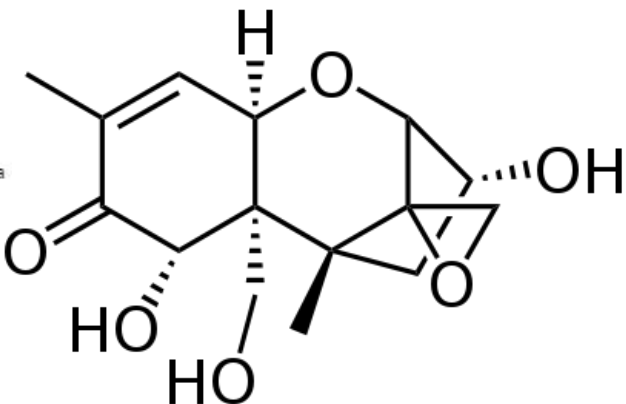
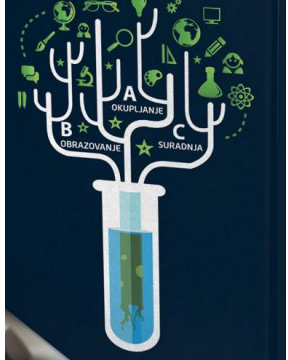
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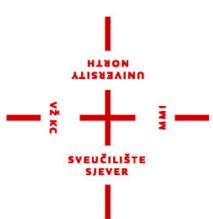
Pojavnost nereguliranih mikotoksina u Hrvatskoj



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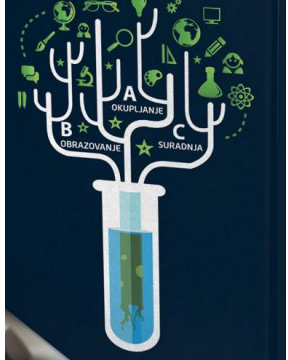




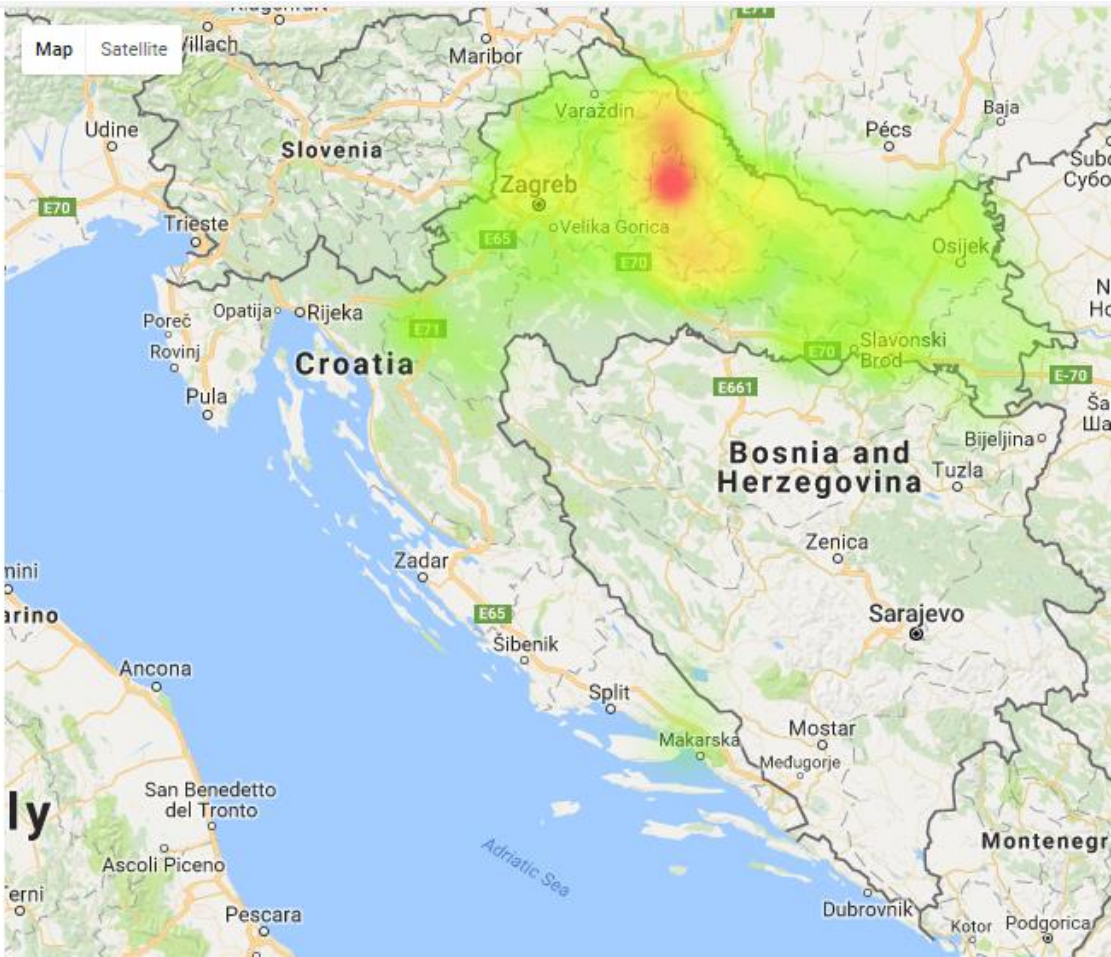
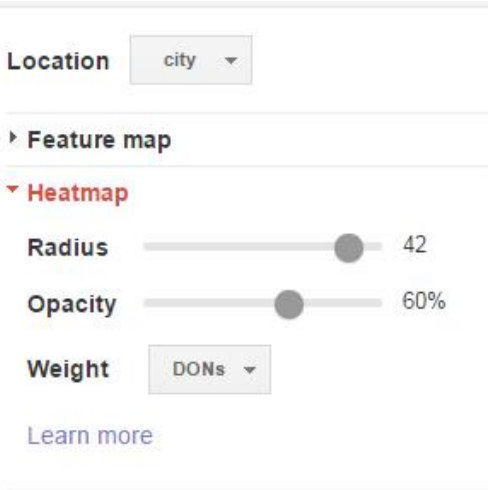
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Pojavnost nereguliranih mikotoksina u Hrvatskoj

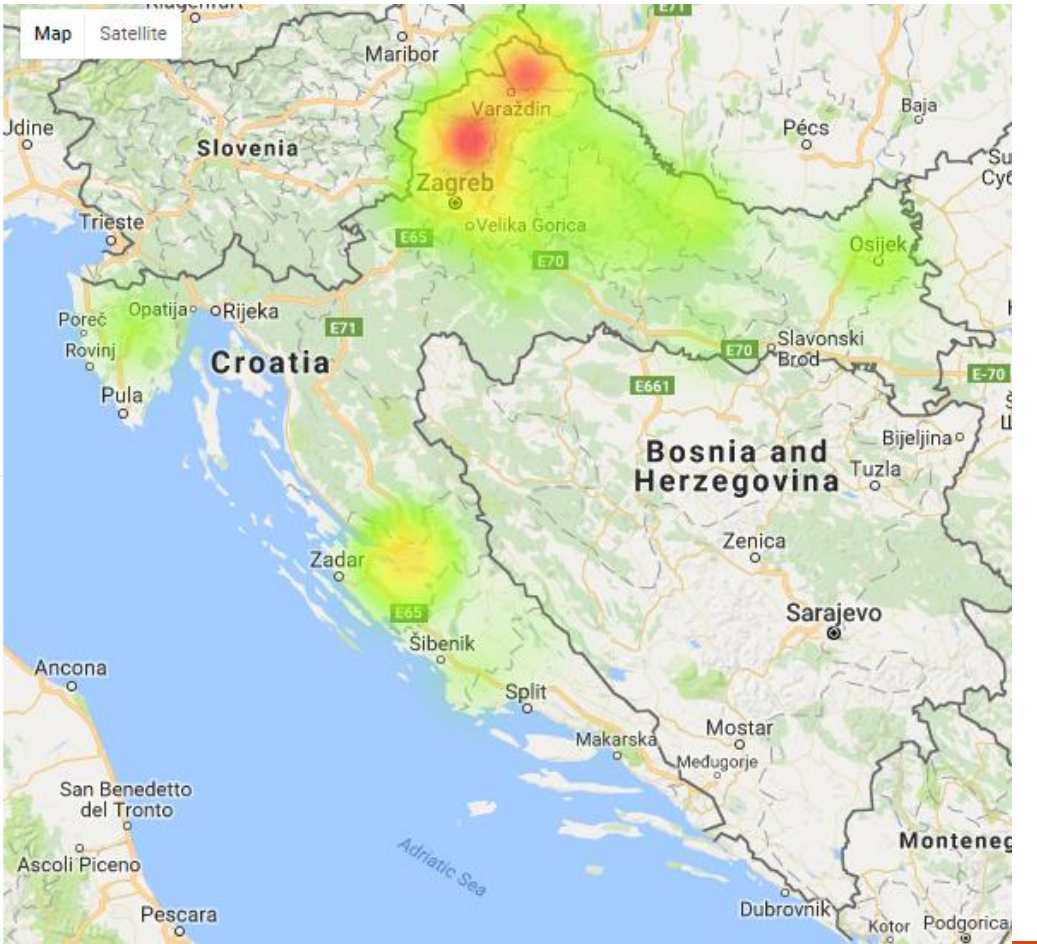
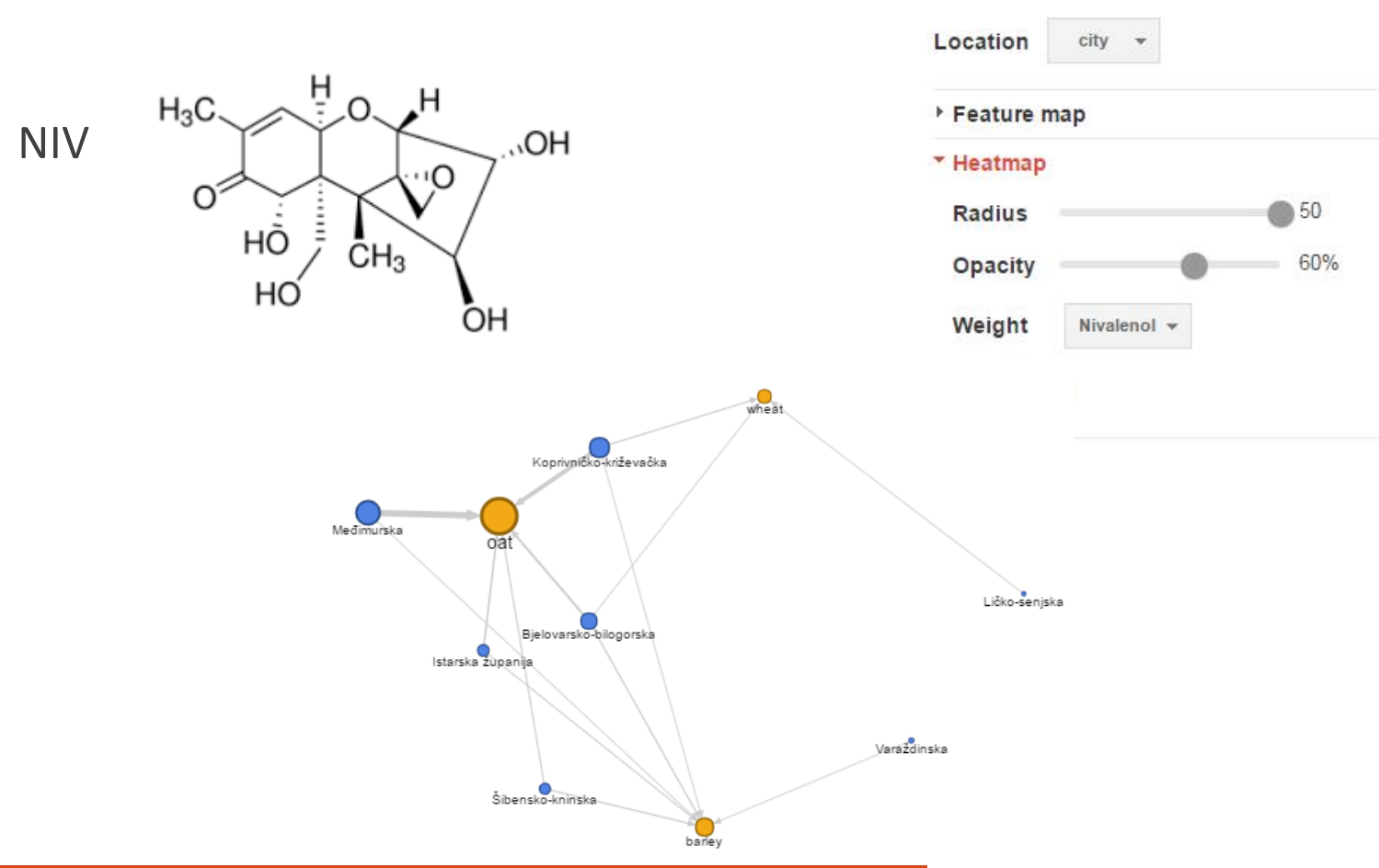
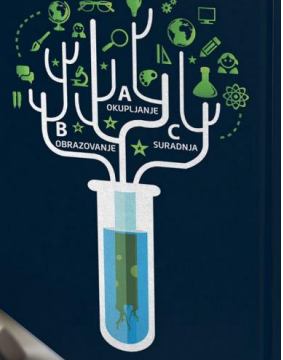


DON
DON-3-Glc
3-AcDON
15-AcDON

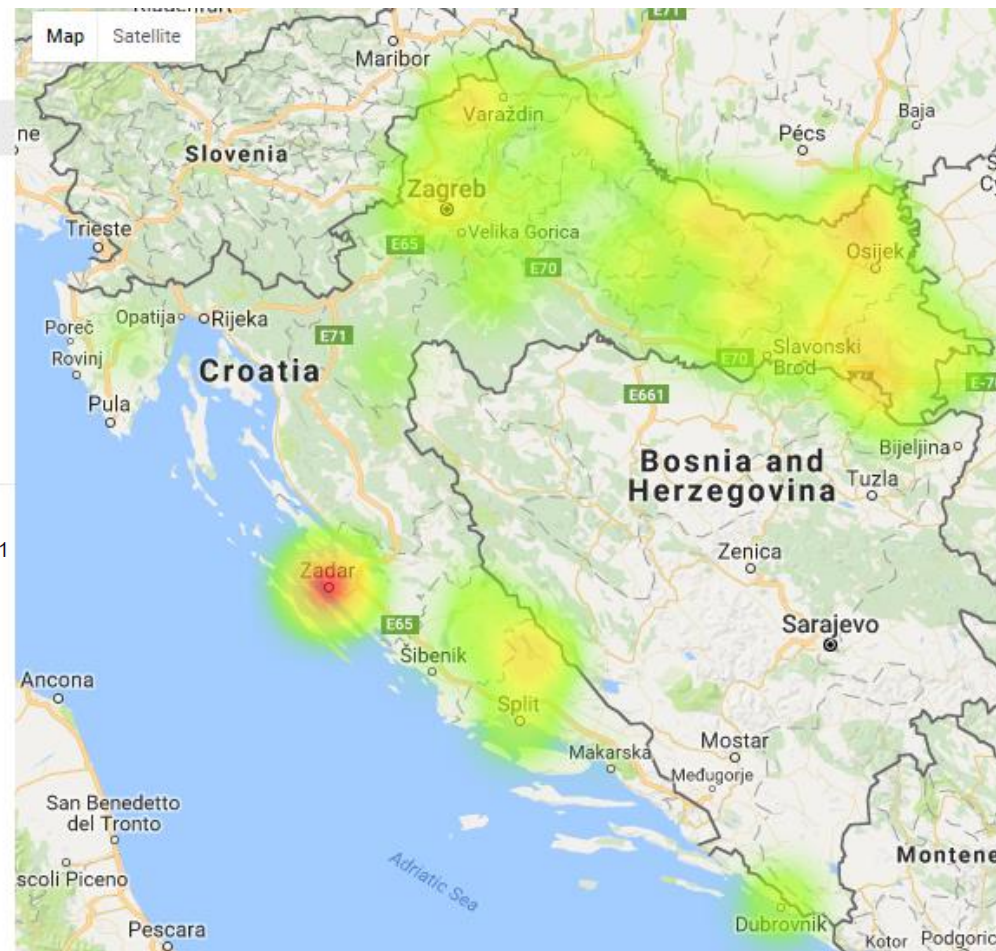
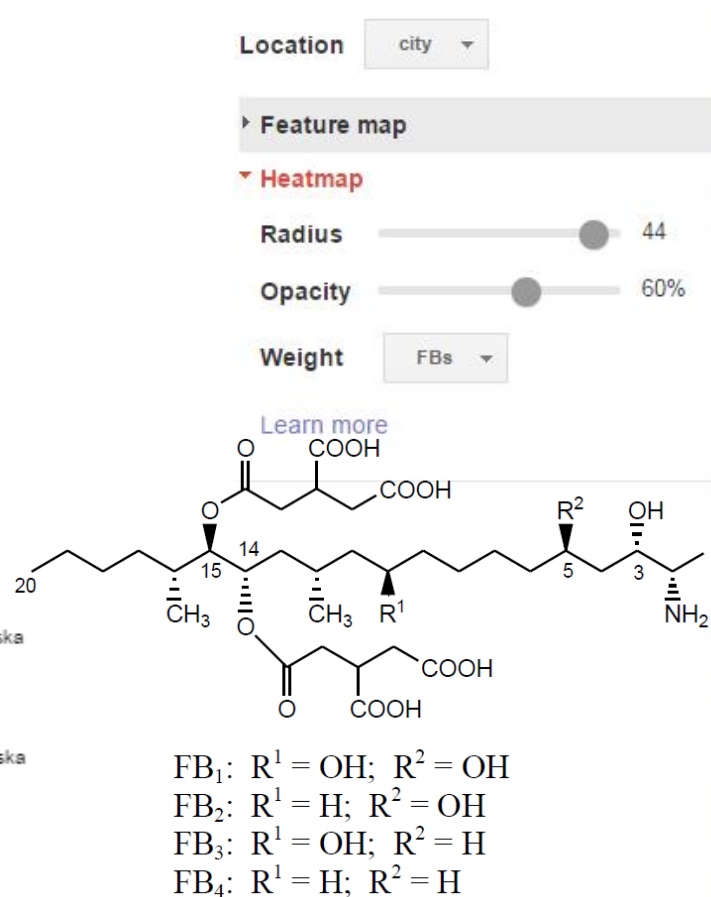


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Pojavnost nereguliranih mikotoksina u Hrvatskoj

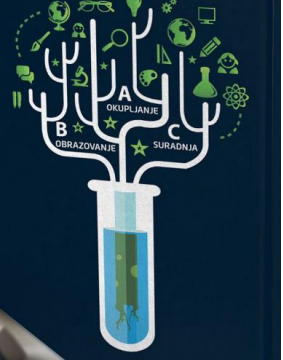


Pojavnost nereguliranih mikotoksina u Hrvatskoj

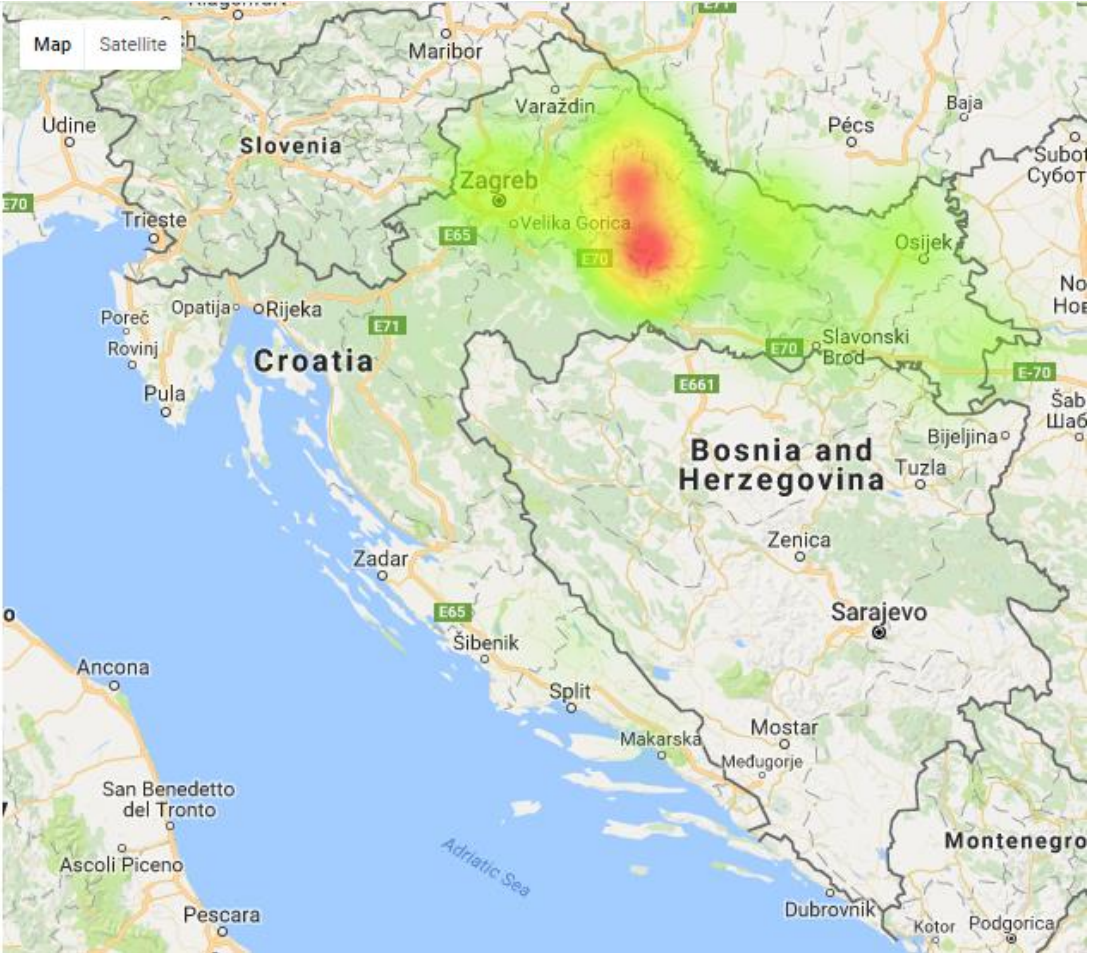
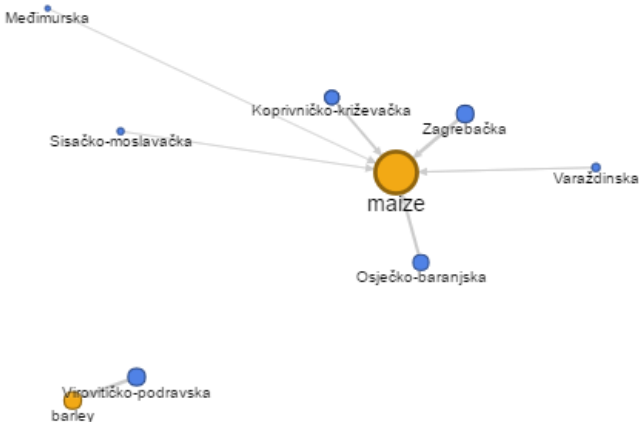
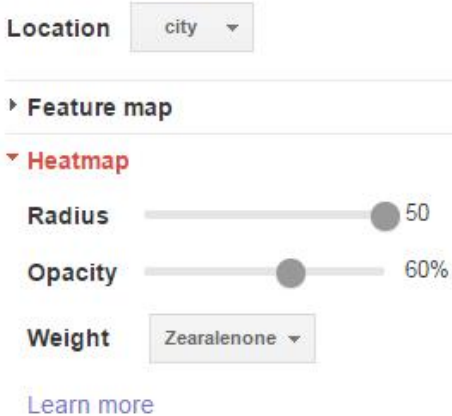
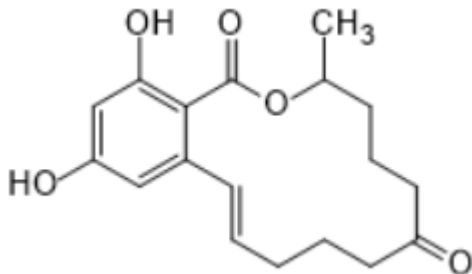


9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



Zearalenon

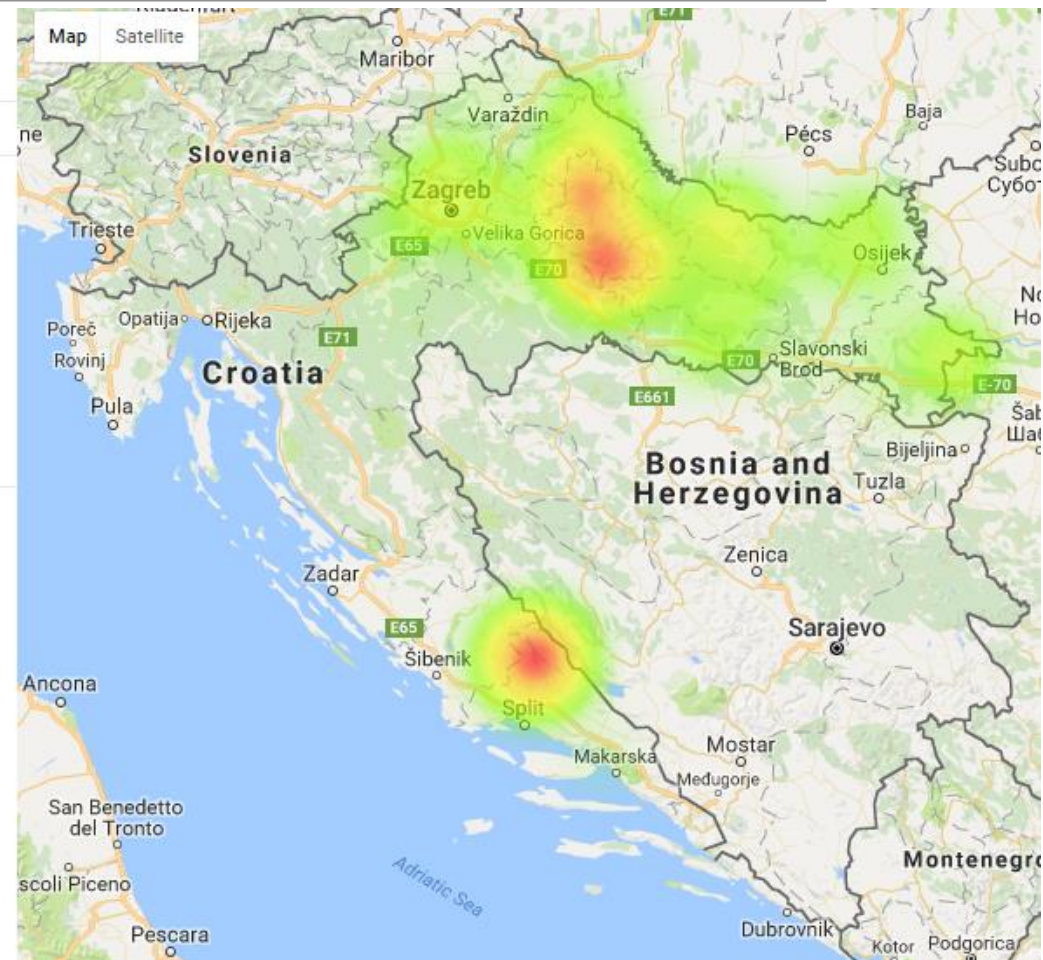
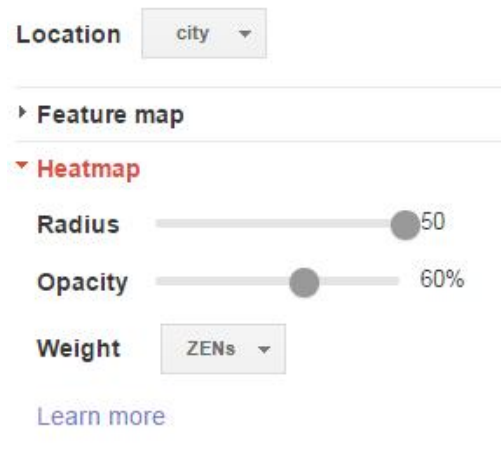


9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj

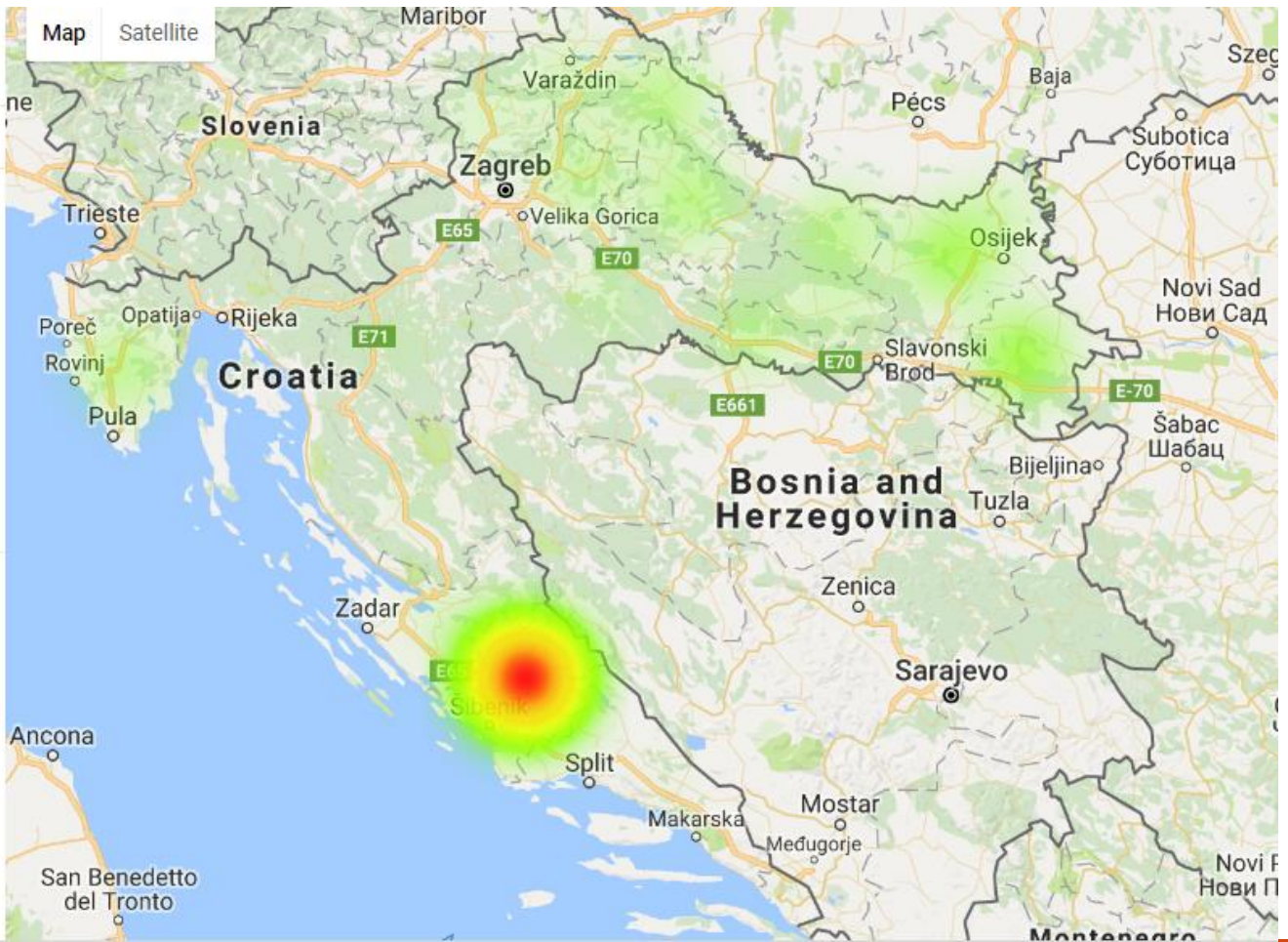
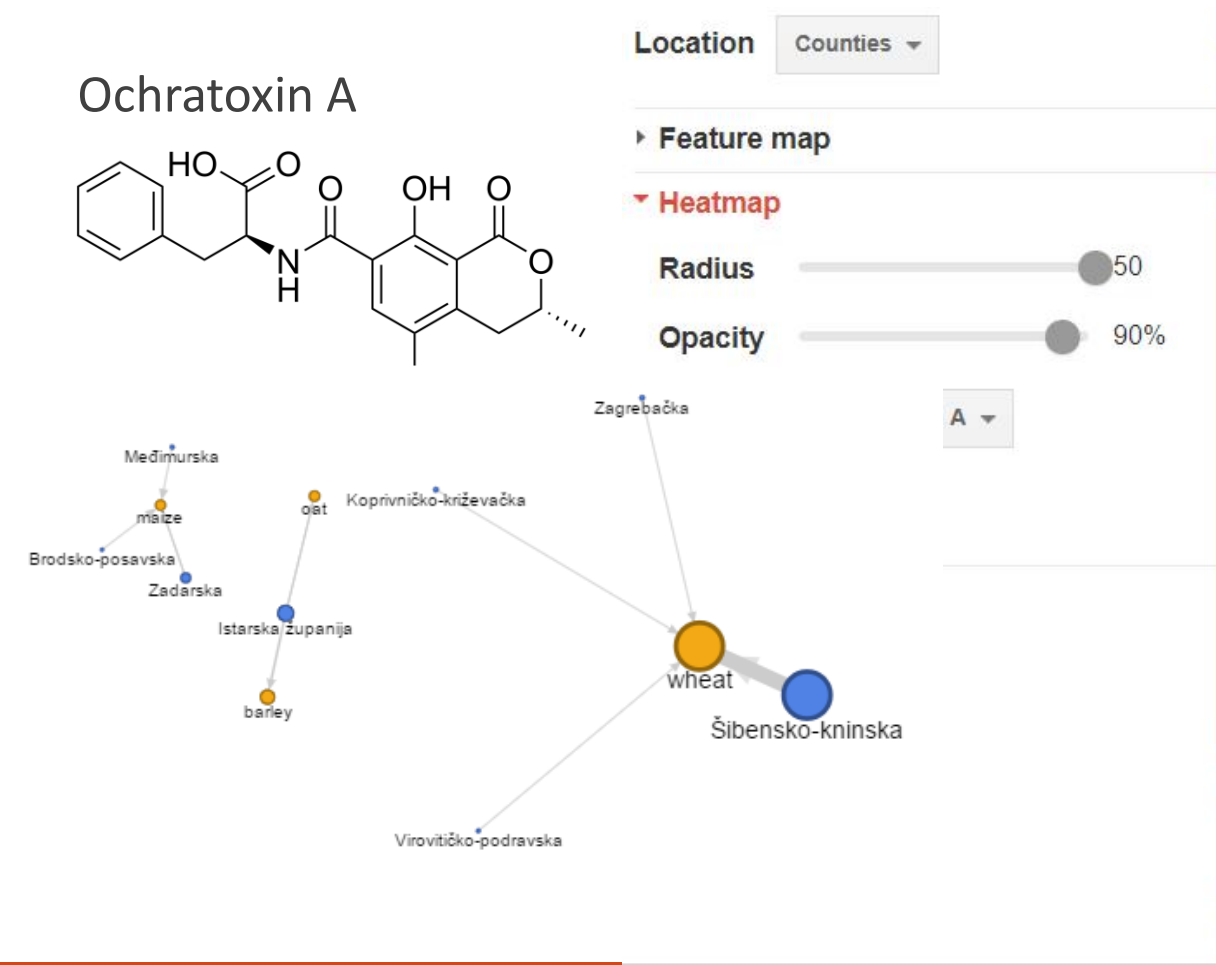
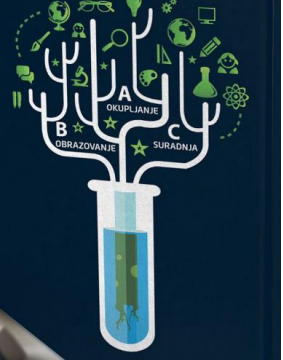


ZEL, Z-4-S, β -ZOL



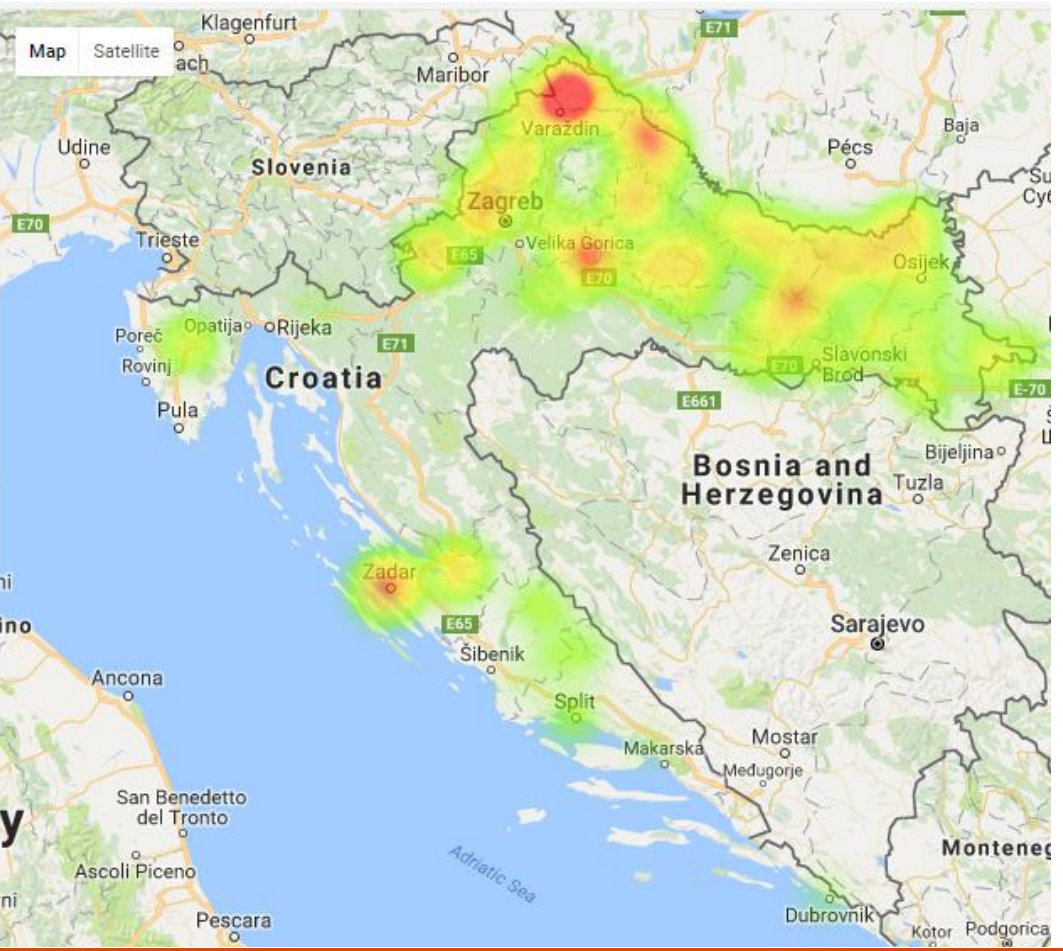
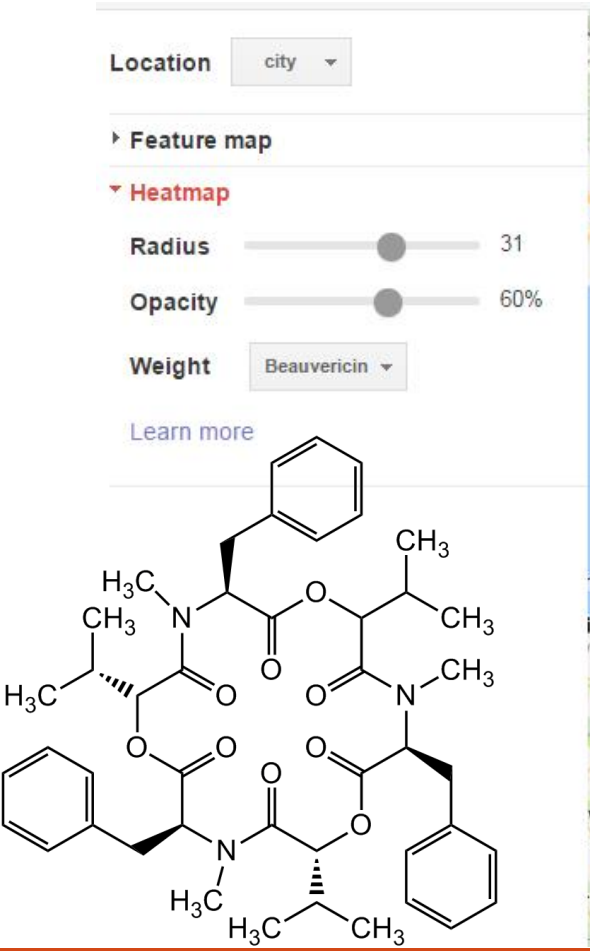
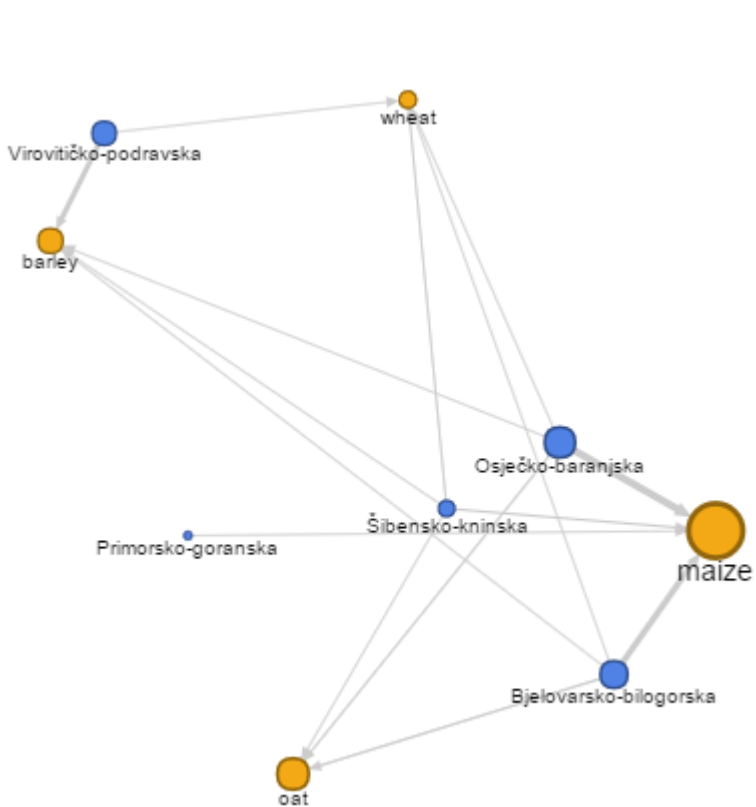
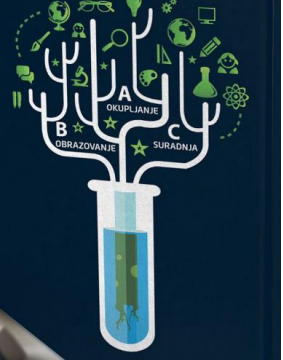
9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



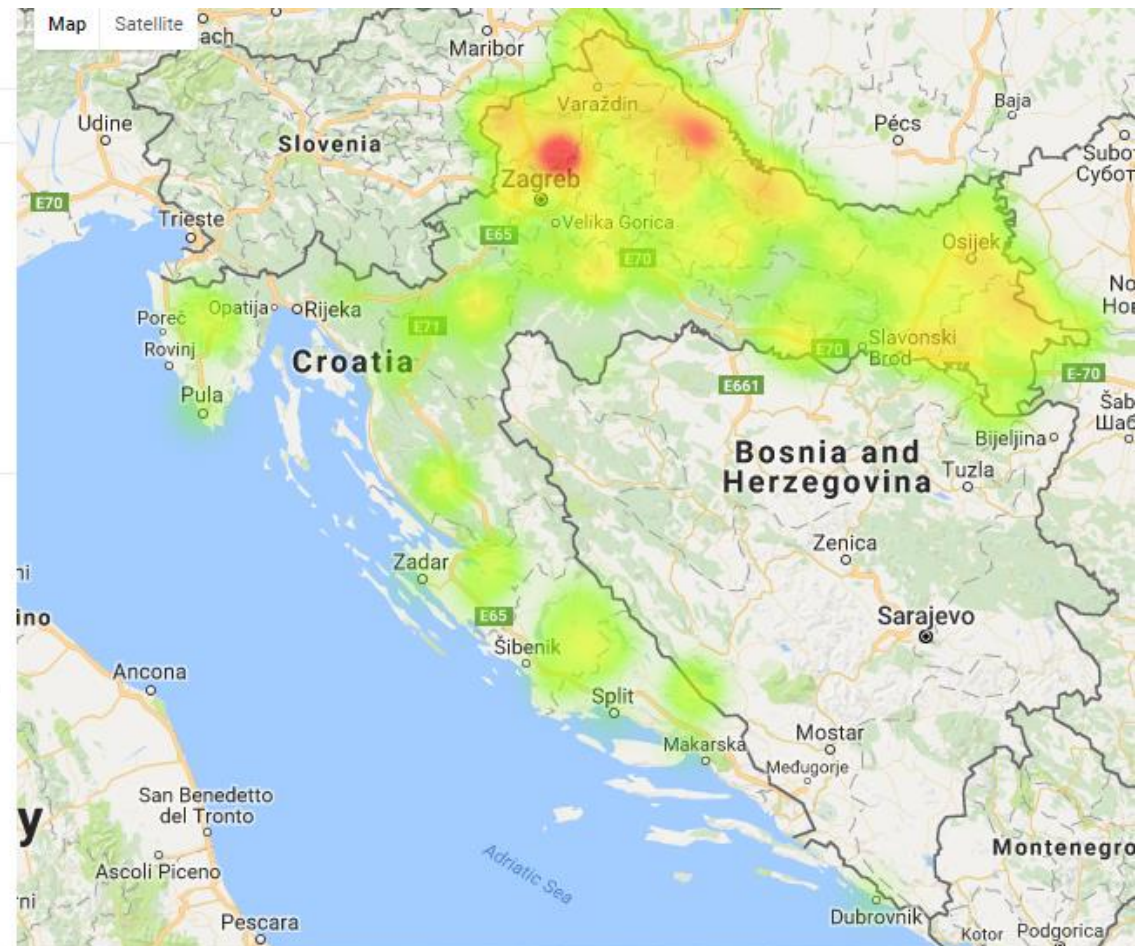
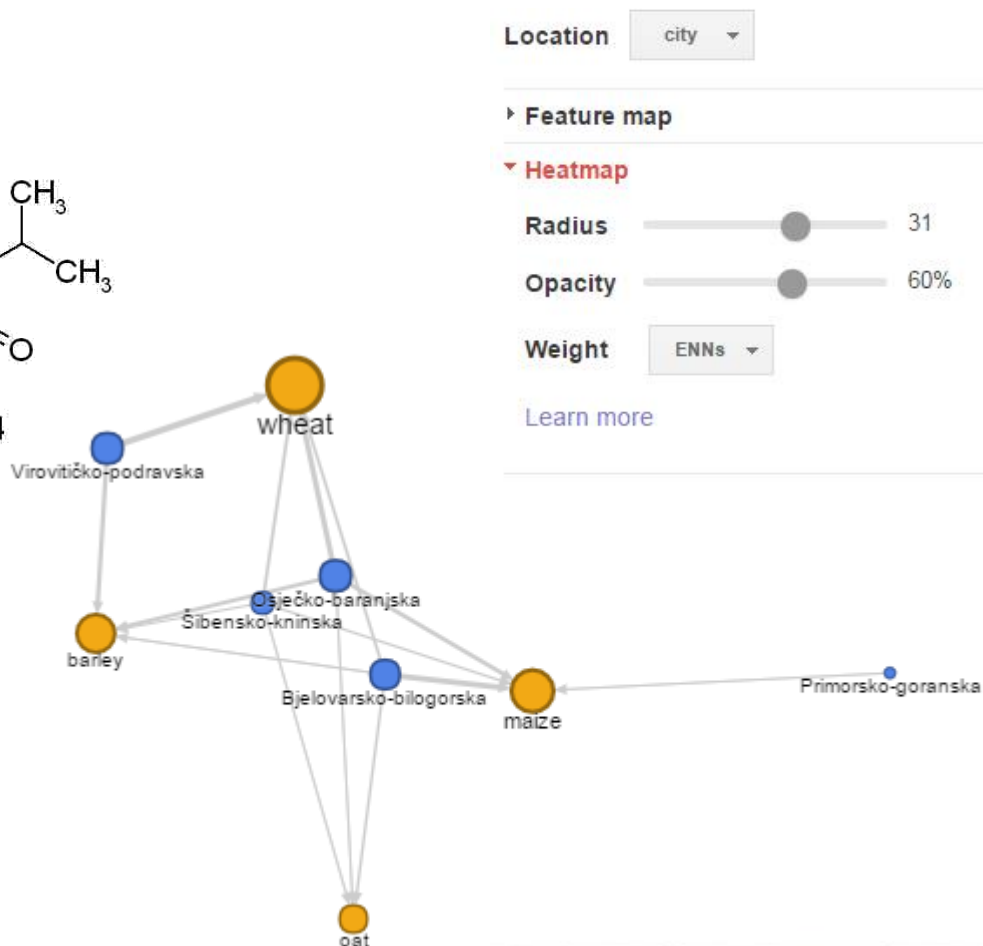
9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



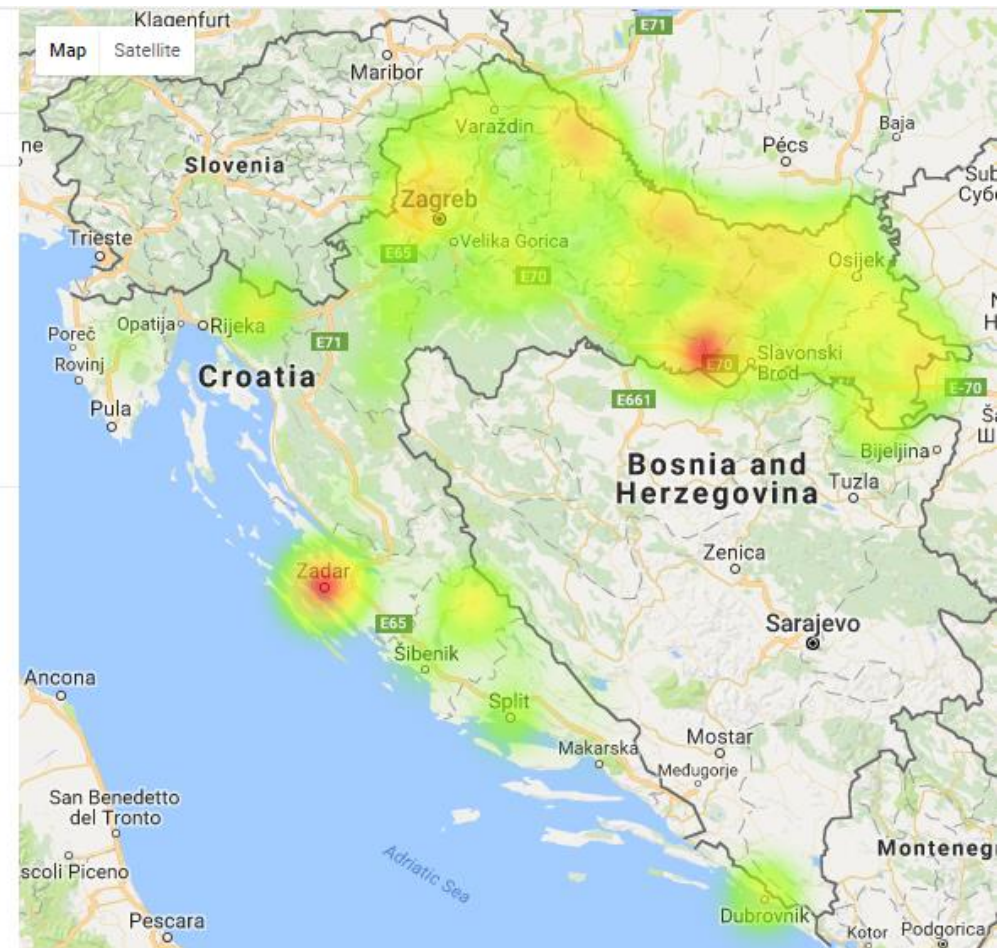
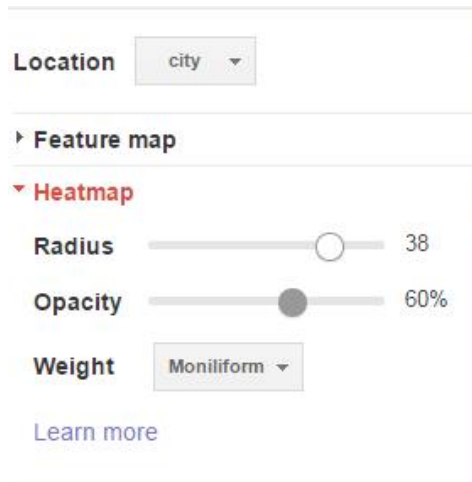
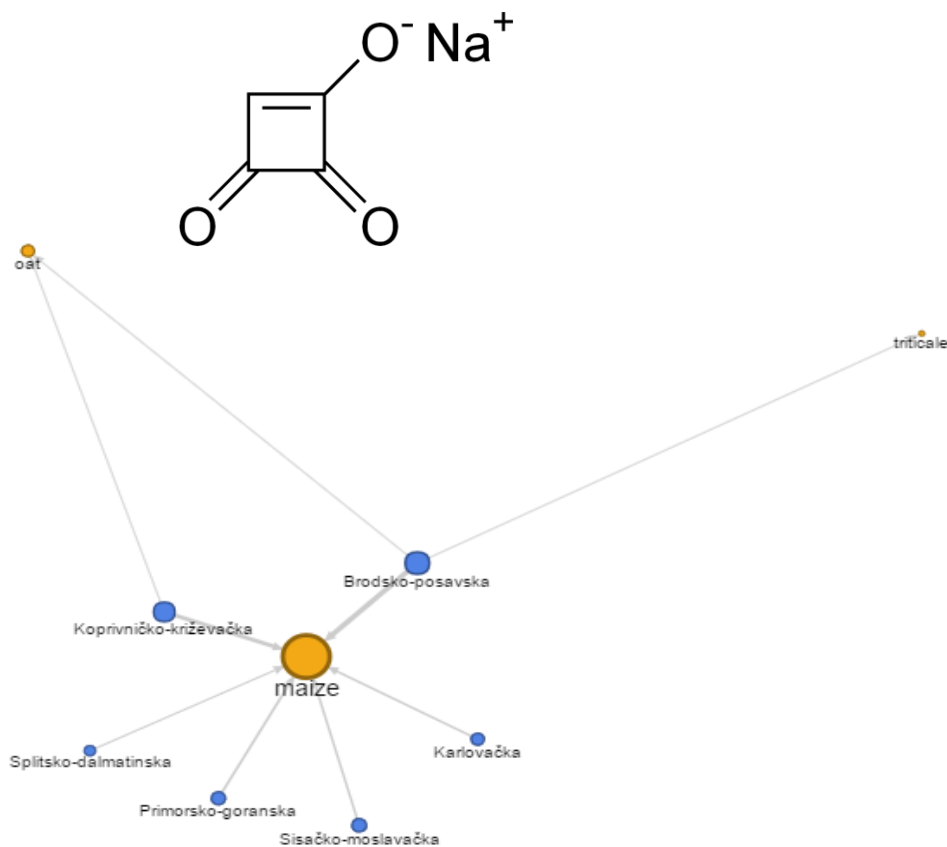
Pojavnost nereguliranih mikotoksina u Hrvatskoj

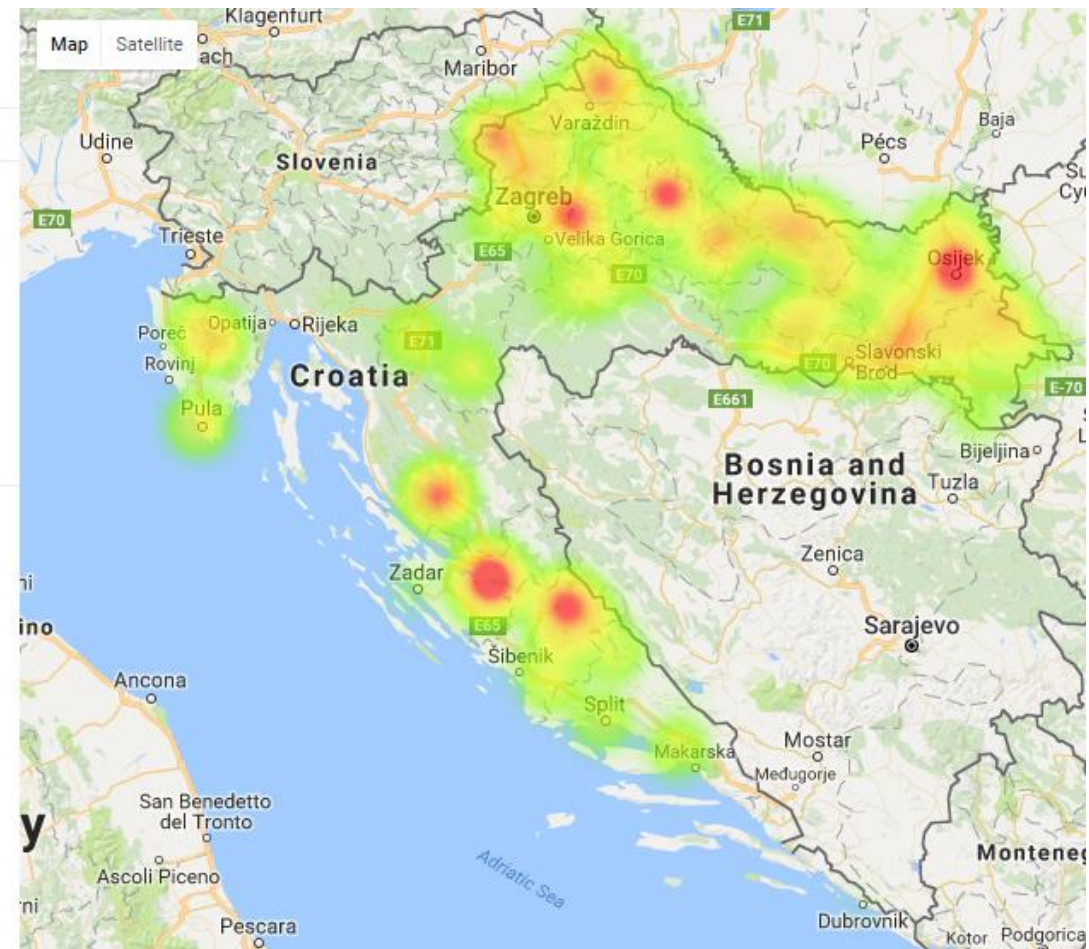
The chemical structure shows a macrocyclic lactam with four amide bonds. The ring is composed of nitrogen and carbonyl groups. Substituents include methyl groups (CH₃), isopropyl groups (CH(CH₃)₂), and R groups (R2, R4, R6). The structure is drawn in a circular fashion, with the nitrogen atoms and carbonyl groups forming the backbone of the ring.



9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

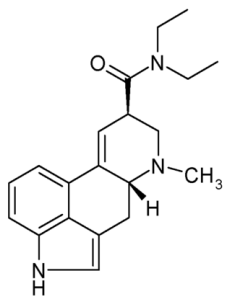
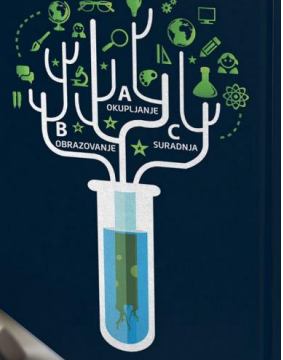
Pojavnost nereguliranih mikotoksina u Hrvatskoj



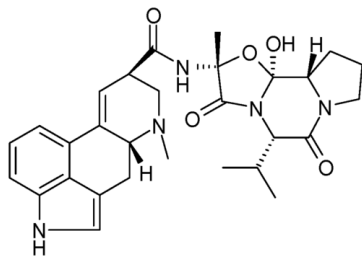


9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

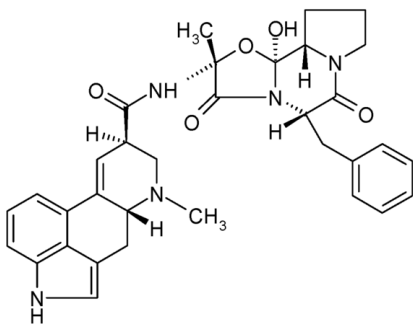
Pojavnost nereguliranih mikotoksina u Hrvatskoj



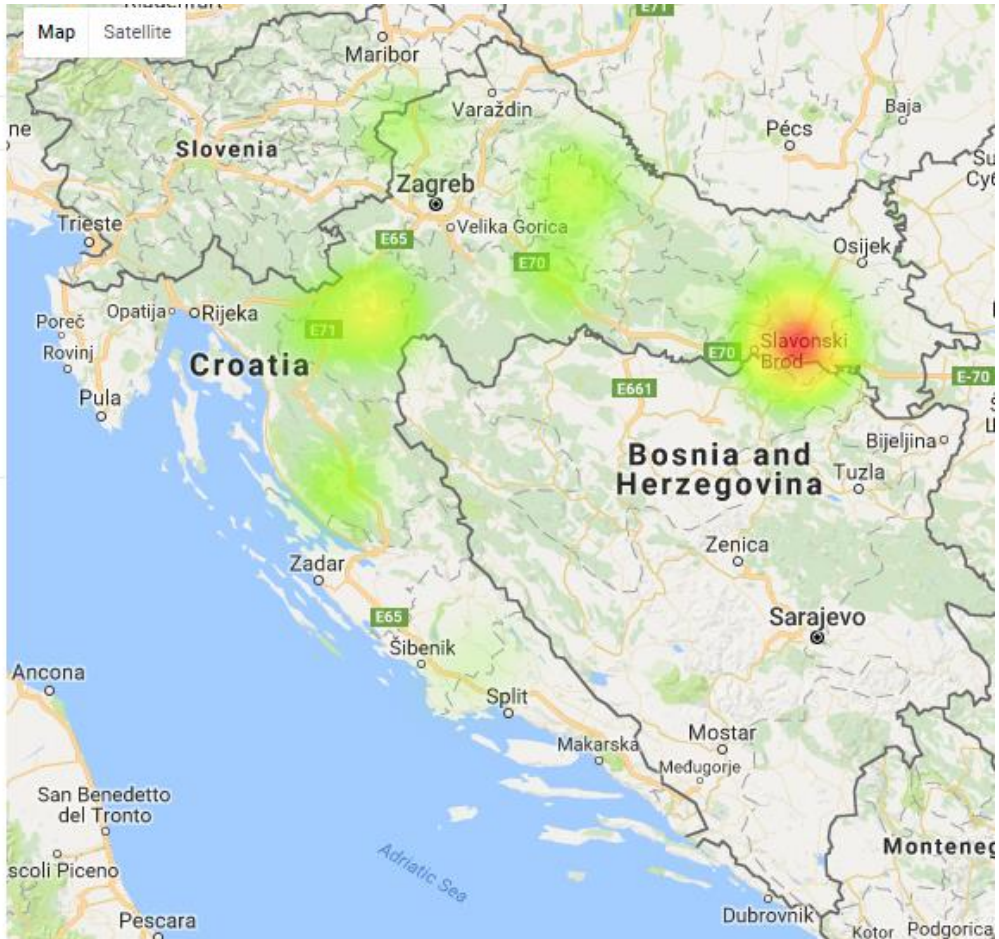
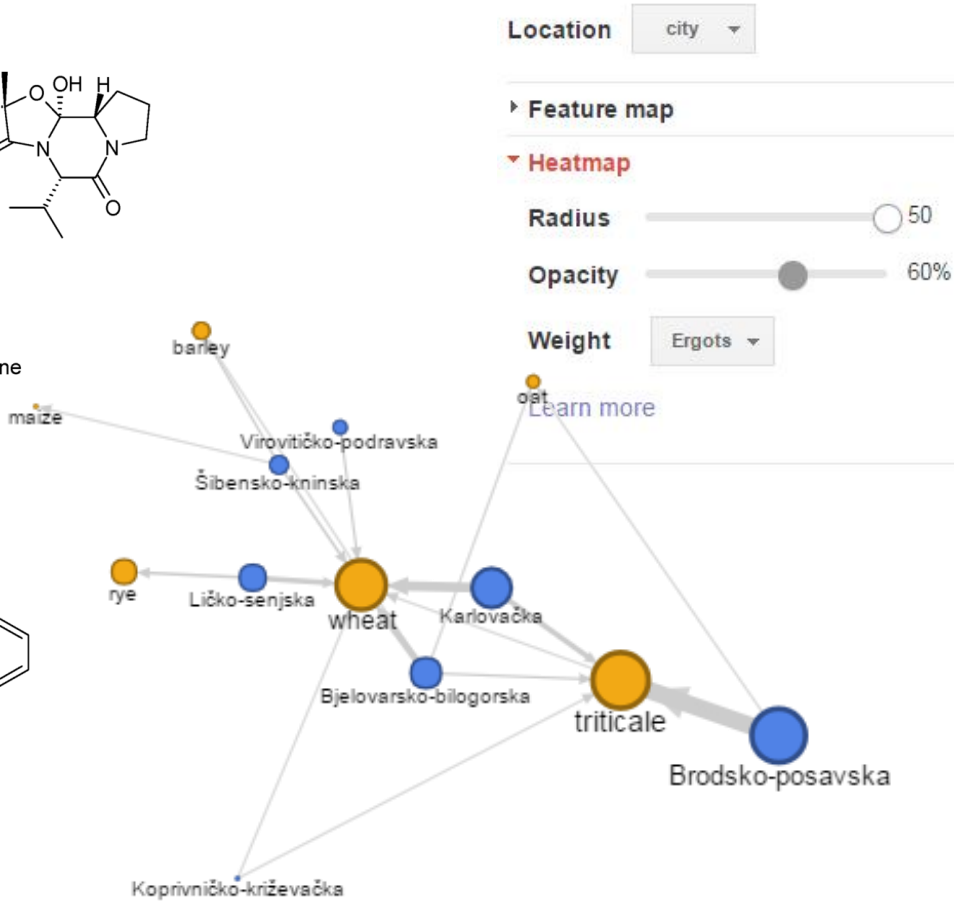
LSD



Ergovaline



Ergotamine





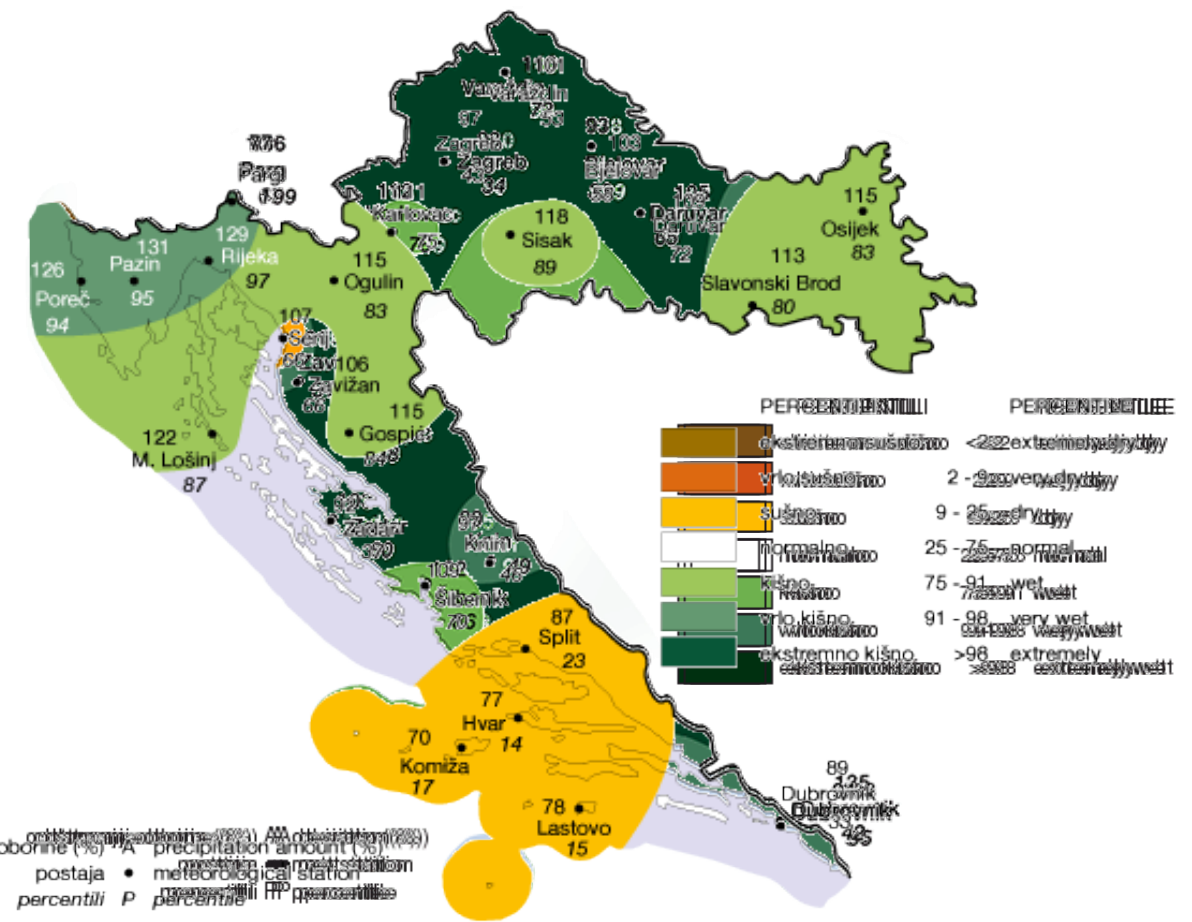
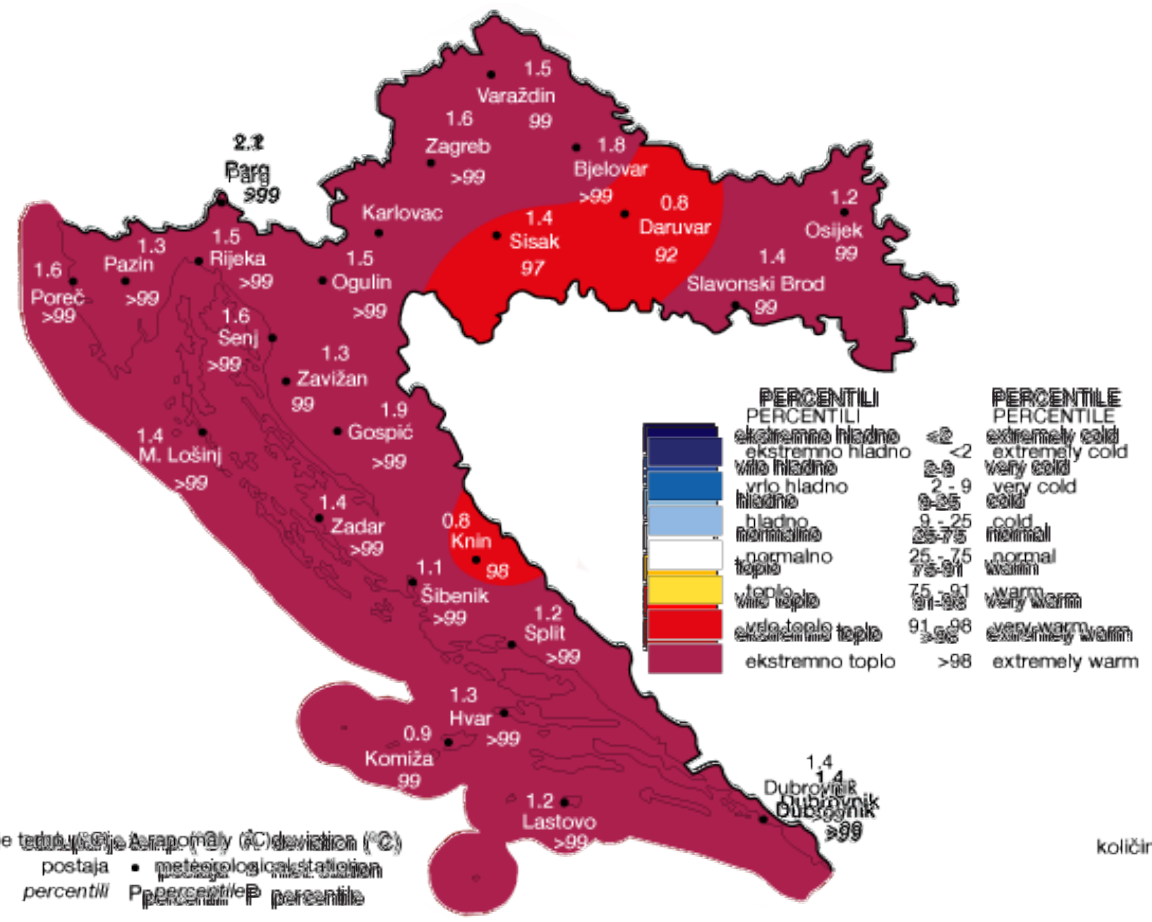
Sveučilište
Sjever

9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



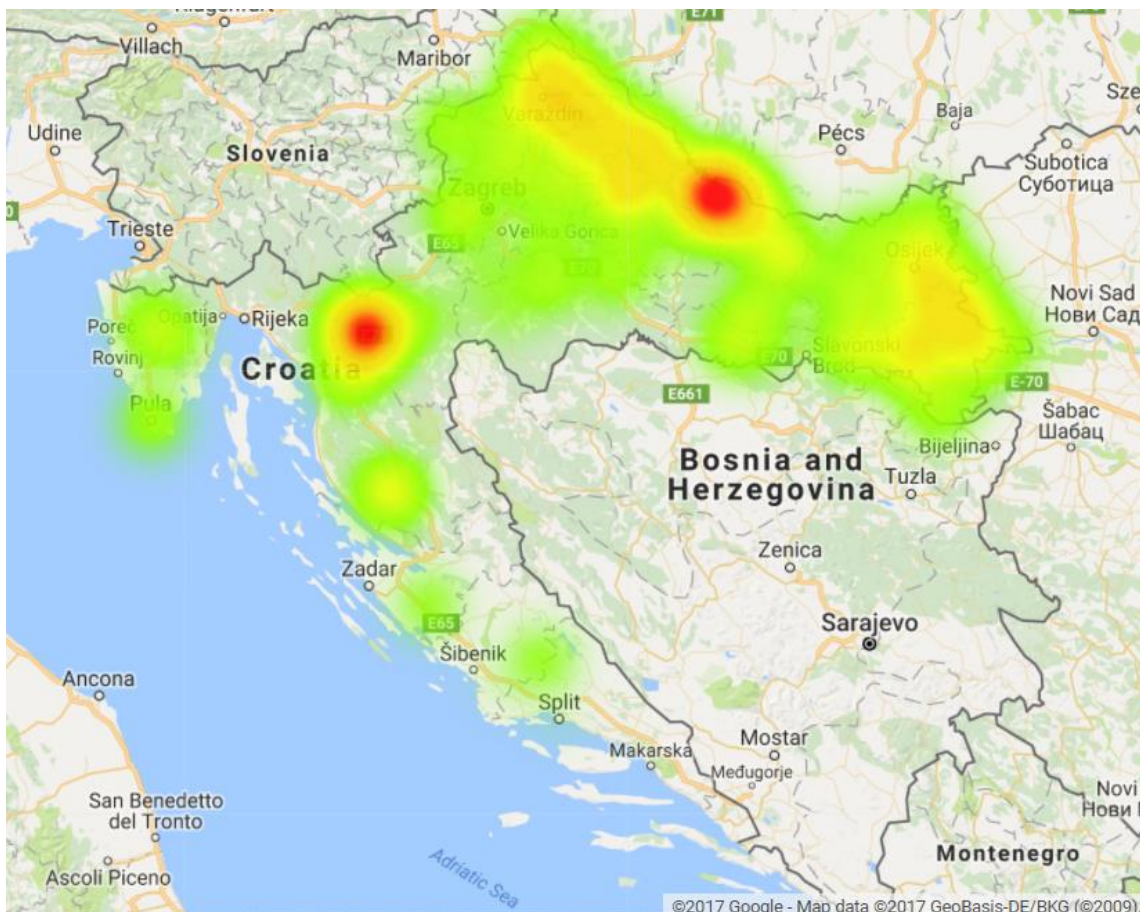
Contamination comparison through the years: 2018



9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj

Contamination comparison through the years: 2013



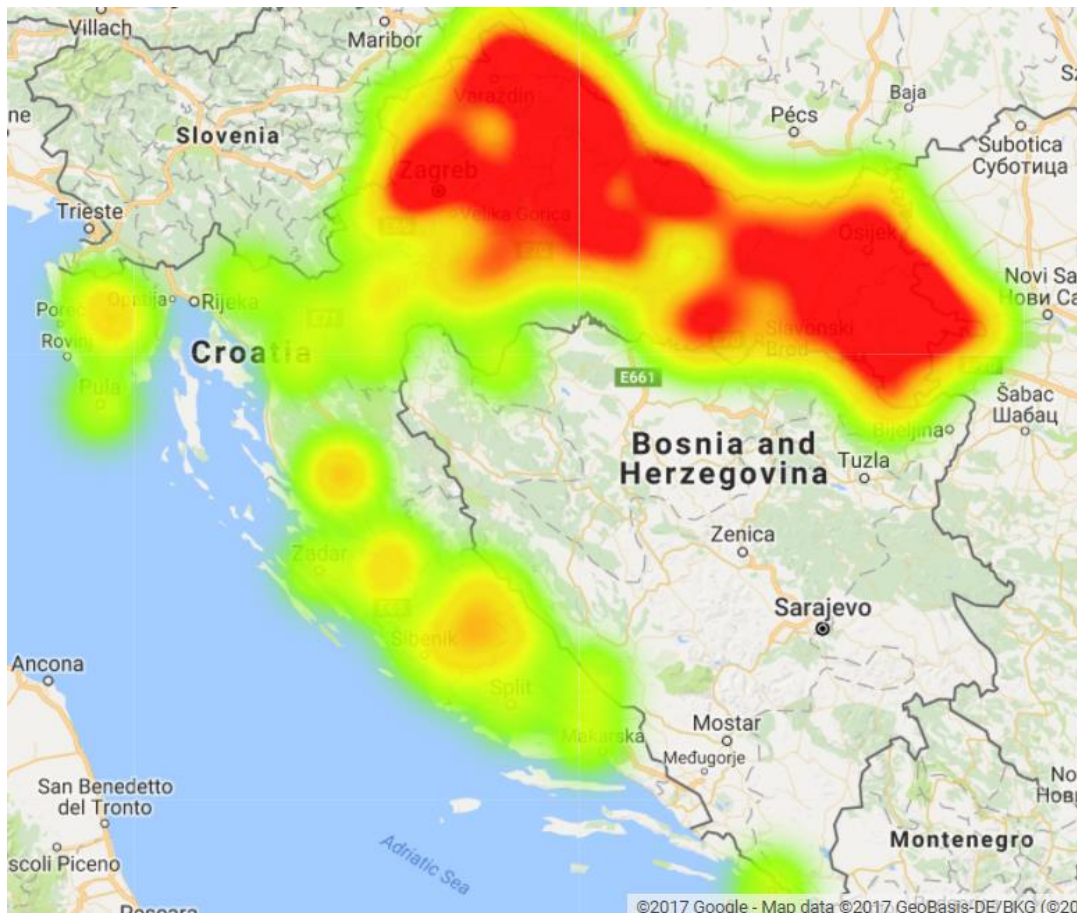
DON, Culmorin, Ergot alkaloids

All regulated mycotoxins < Limits

9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj

Contamination comparison through the years: 2014



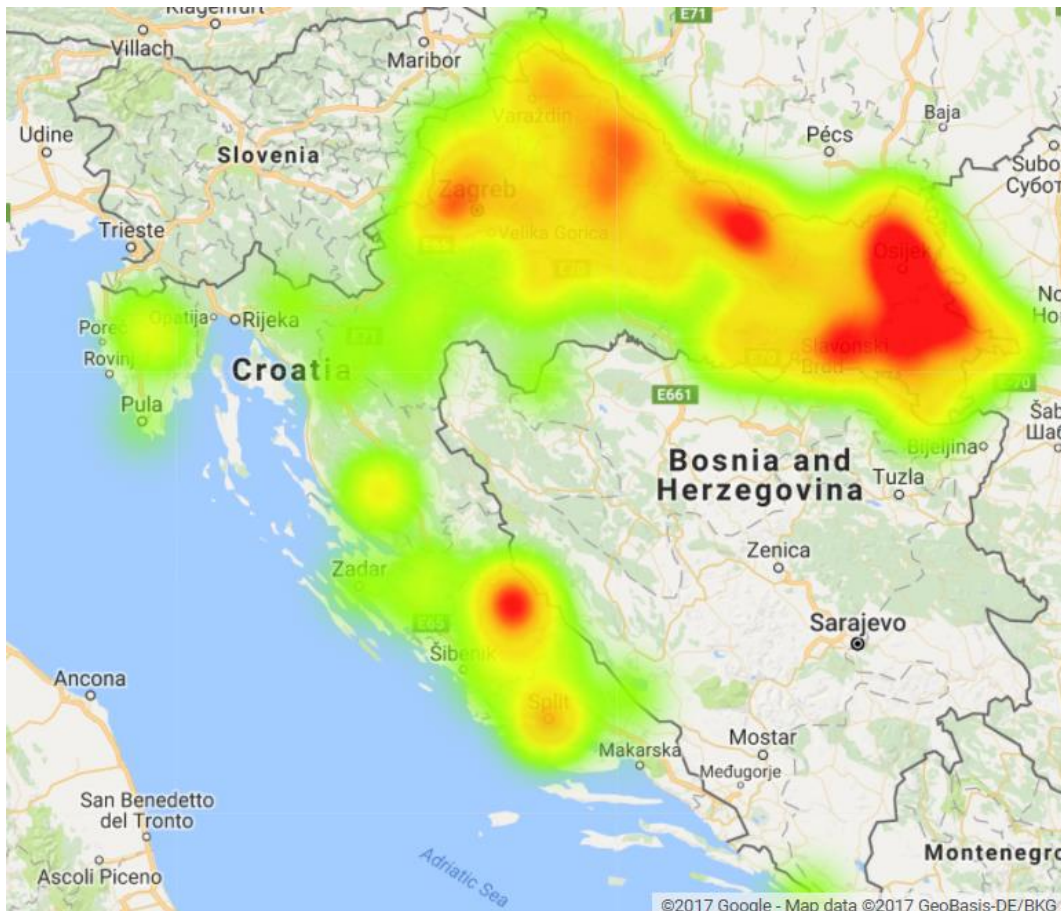
High Fusarium mycotoxins (DONs, moniliformin, emodin, culmorin...

20 % of tested samples above legal limits for human consumption according to EC 1881/2006

9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj

Contamination comparison through the years: 2015



High FB1,2,3,4, ZEN, Z4S

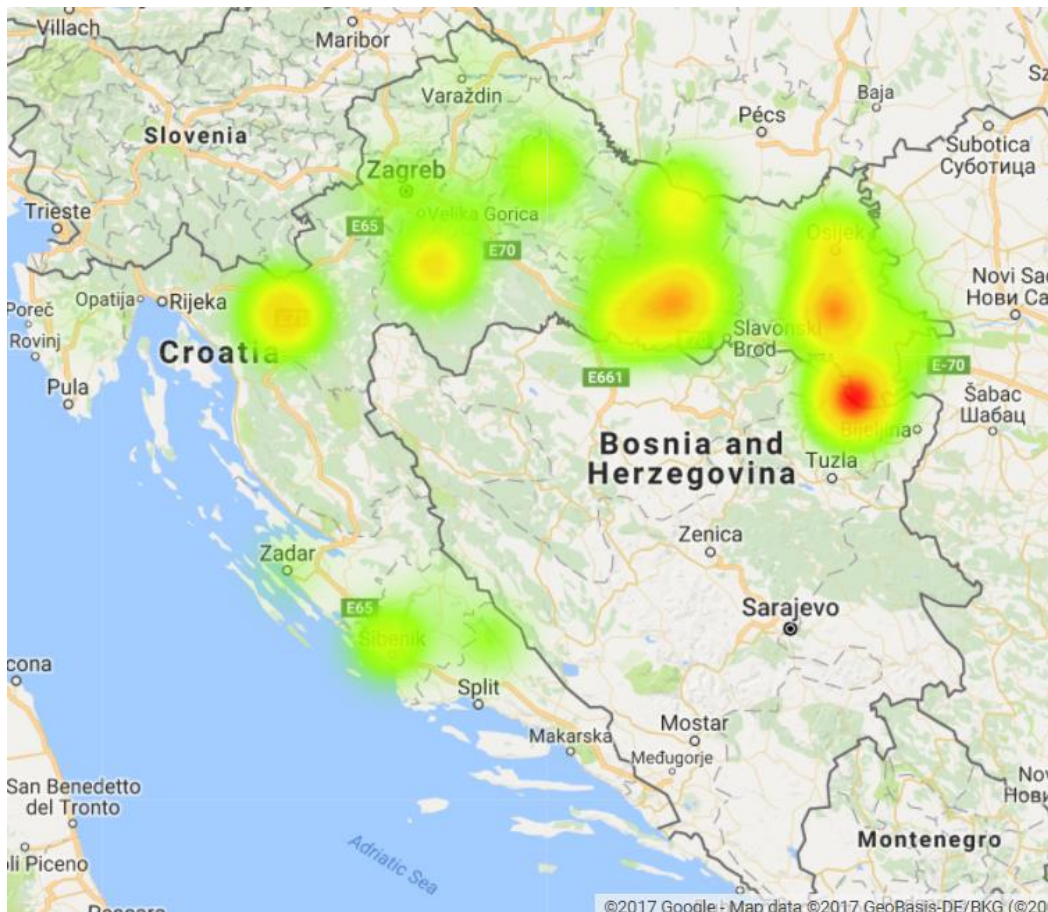
10 % of tested samples above legal limits for human consumption according to EC 1881/2006

Interesting case of AFB1, OTA & OTB in wheat

9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj

Contamination comparison through the years: 2015



Fusarium mycotoxins, (DON, FB1)

5 % of tested samples above legal limits for human consumption according to EC 1881/2006

First time masked Alternaria toxins quantified

9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



- ✓ Ukupno kvantificirano 128 toksina na Hrvatskim žitaricama (mikotoksini, biljni toksini, bakterijski toksini) -> 101 mikotoksin + 27 ostalih toksina
- ✓ 100 % žitarica kontaminirano s barem 8 (kukuruz) do 60 (ječam, kukuruz) mikotoksina
- ✓ Prvi puta napravljena lista EFSA-inih najtraženijih nereguliranih mikotoksina za Hrvatsku
- ✓ Toplinska karta & neuralna mreža kontaminacije hrvatskih žitarica
- ✓ Odnos maskiranih naprama nativnih 2 % - 87 % (prosijek 21%)

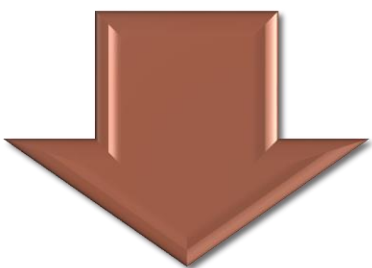


9. Stručni skup Okoliš i zdravlje i obilježavanje 30. godišnjice DKTK

Pojavnost nereguliranih mikotoksina u Hrvatskoj



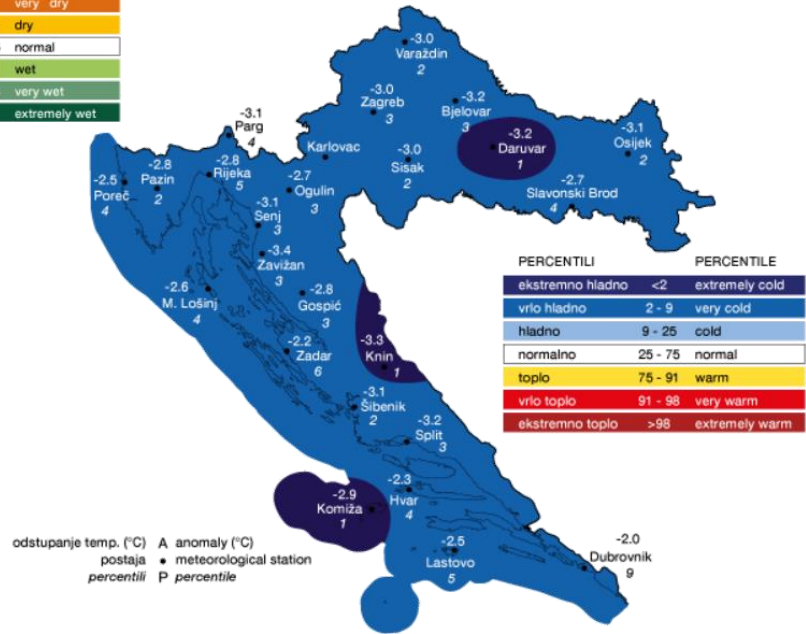
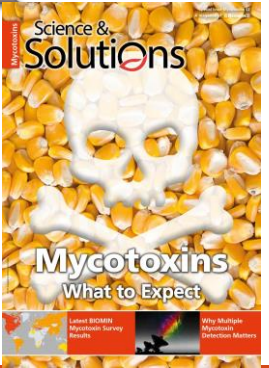
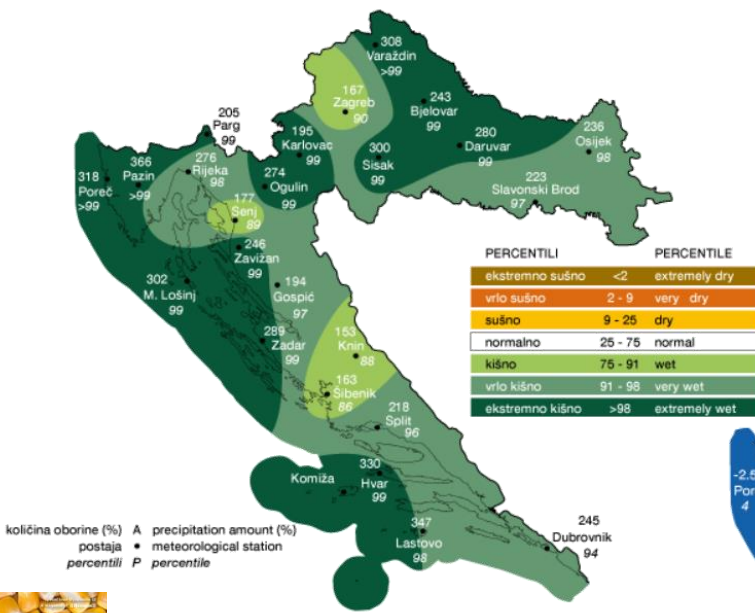
Što očekivati u 2019?



vlažno



hladno /
vruće



Hvala na pažnji

