A CUP OF TEA: TRANSFER OF MYCOTOXINS FROM SPIKED MATRIX INTO INFUSION

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INTRODUCTION

Camellia sinensis and herbal tea are daily basic food products. In general, their consumption depends on traditions, occupation and age. Turkey, Libya, Morocco, Ireland and the UK demonstrate the highest per capita consumption, China, the Russian Federation, Japan and India are in the list of the most important consuming countries [1]. Estimates of average chronic daily intake per capita are: 446 mL (*C. sinensis* black tea; all population; the RF) [2]; 124 mL (*C. sinensis* tea) and 61 mL ("herbal and other non-tea infusions"; all adults; the EU) [3].

Recent surveys reveal occurrence of AFLs, and STC up to dozens of μg/kg; OTA, ZEA and FBs – up to hundreds μg/kg; DON, MPA and *Alternaria* toxins – up to several mg/kg in these kinds of foods [4-12]. Regulations concern mainly AFLs and OTA: maximum level (ML) was set in the RF for AFL B1 in tea (5 μg/kg), in Argentina for AFL B1 and AFLs (5 and 20 μg/kg correspondingly), in Japan, China, Sri Lanka and India in the category "all foods" [13]. AFLs and OTA are regulated by the EU in ginger, liquorice root and extracts. MLs for AFLs in herbal drugs were set by European Pharmacopeia, while herbal teas and food supplements are not subjected to control (except mentioned above selected species) [12]. Risk assessments for mycotoxins in beverages are traditionally carried out basing on their concentration in dry matrix. Meanwhile, input is due to infusion only. The present study was aimed at evaluation of transfer rate of mycotoxins.

Factors affecting transfer: mycotoxins chemical and physical properties; matrix; water characteristics (usually temperature, hardness and pH); time

METHODS

Model: two grams of spiked *C. sinensis* green tea "clean" matrix were infused with 100 mL of water heated to 99.9˚C for 30 minutes

Studied factors: mycotoxins chemical and physical properties; water characteristics (hardness and pH)

Micromycetes	Mycotoxins/spiking level, μg/kg:
Aspergillus spp. *Penicillium* spp.	AFLs (B1, B2, G1, G2), OTA – 80; STC -200; MPA – 6,000;
Fusarium spp.	DON – 10,000; ZEA – 1,000; T-2, HT-2 – 800; FB1, FB2* – 400; ENN B, ENN A, BEA – 2,000;
Alternaria spp.	TTX – 200; ALT – 1,000; AOH, AME – 2,000

* - may be produced by *Aspergillus* spp. also

Water	TDS*, mg/L	pH
Distilled (DW)	4.4	3-9 (8 points with a unit step)
Deep-well (DW-W)	155	
Natural mineral water (NMW)	238	

* - water hardness: total dissolved solids

Detection

HPLC-MS/MS with HESI+

DON, ENNs and BEA

Titan C18, 100 × 2.1 mm, 1.9 μm

ACN gradient

Other studied mycotoxins

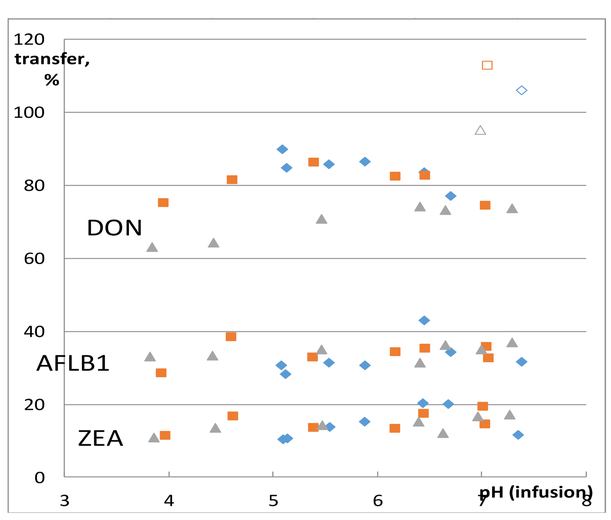
Ascentis Express F5, 100 × 3.0 mm 2.7 μm; MeOH gradient

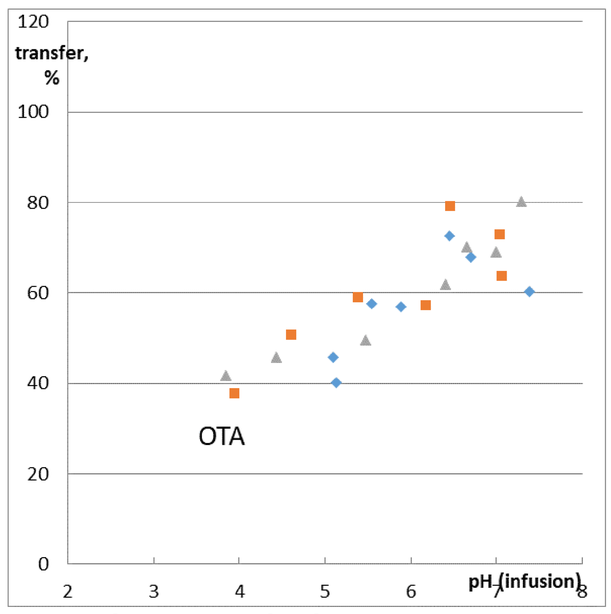
Details: sample preparation: centrifugation of infusion; three replicates for each point (TDS, pH), quantification using "matrix-matched" calibration

RESULTS

Water characteristics: TDS and pH*

◆ - DW; ■ - D-WW; ▲ - NMW

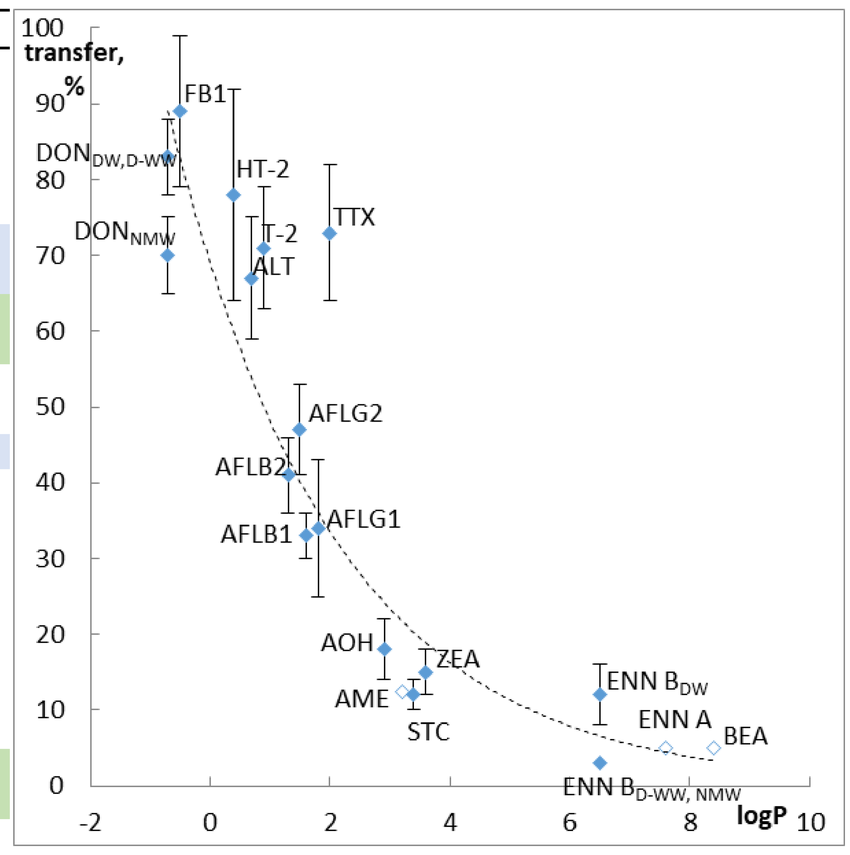




* - transfer vs pH of infusion is presented

Mycotoxins characteristics: acidic/basic properties and polarity

Mycotoxin		pKa	XlogP3-AA [1]	Transfer, %
AFL B1	neutral	17.8 [2]	1.6	33±3
AFL B2	neutral		1.3	41±5
AFL G1	neutral		1.8	34±9
AFL G2	neutral		1.5	47±6
STC	neutral		3.4	12±2
OTA	amphoteric	3.29 / -2.20 [2]	4.7	38-80*
MPA	acidic	4.2	3.2	38-86*
$DON_{DW,D-WW}$	neutral	11.91 [2]	-0.7	83±5
DON_{NMW}				70±5
ZEA	acidic	7.41 [2]	3.6	15±3
FB1	amphoteric	3.64 / 9.29 [2]	-0.5	89±10
FB2	amphoteric	3.64 / 9.25 [2]	1.2	40-103*
HT-2	neutral	13.26 [2]	0.4	78±14
T-2	neutral	13.23 [2]	0.9	71±8
ALT	acidic	7.5 [3]	0.7	67±8
TTX	acidic	5.33 [3]	2	73±9
AOH	acidic	7.16 [2] 7.63 [3]	2.9	18±4
AME	acidic	6.99 [2] 7.71 [3]	3.2	<12.5**
$ENN\ B_{DW}$	basic	-1.08 [2]	6.5	12±4
$ENN\ B_{D-WW,NMW}$			6.5	3
ENN A	basic	-0.96 [2]	7.6	<5**
BEA	neutral	18.8 [2]	8.4	<5**



[1] PubChem DataBase (for neutral molecules); [2] Lauwers M., *et al.* DOI: 10.3390/toxins11030171;
[3] Tolgyesi A., *et al.* DOI: 10.1080/19440049.2015.1072644;
* - transfer is pH-dependent; ** - was not quantified in infusions (maximum transfer evaluated using LOQ).

Highlighted blue - transfer is pH-dependent; green – TDS affects transfer rate; all others – polarity is decisive factor

Transfer from naturally contaminated herbal tea samples: STC – 7-13%, OTA – 83%, MPA – 23-96%, ZEA – 30%, TTX – 59-84%; AOH – 28-61%, AME – 11%, ENN A, ENN B < 0.03%, BEA – 0.3-0.4% [14]

CONCLUSIONS

within the studied model

$$\text{transfer rate, \%} = f\{\ \text{polarity};\ \text{infusion pH (compounds with} -\text{COOH});\ \text{TDS (not significant for the most of studied mycotoxins})\ \}$$

Transfer, %	> 60%	30-50%	<20%	pH-dependent
Mycotoxins	DON, T-2, HT-2, ALT, TTX, FB1	AFLs	ZEA, STC, AOH, AME, ENNs, BEA	OTA, MPA, FB2

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