EFFECTS OF PHARMACEUTICALS PRESENCE IN AGROECOSYSTEM ON VOLATILE AROMA CONTENT IN TOMATO FRUIT

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Abstract. The aim of this work was to assess the potential impact of two commonly used pharmaceuticals (azithromycin and ibuprofen) on tomato quality. The experiments were carried out upon controlled exposure box experiments at different amount of azithromycin (0.5 – 5 $mg \cdot kg^{-1}$) and ibuprofen (50 – 500 $\mu g \cdot kg^{-1}$). The results showed that tomato fruits have a higher uptake rate for ibuprofen (6.3 – 11.2 %) compared to the azithromycin that was between 2.2 – 3.2 %. However, pharmaceutical active compounds uptake amount was lower in tomato fruits compared to those detected in grown medium. Both, ibuprofen and azithromycin impact the sensory quality of tomato due to changes in volatile aroma content, especially of those derived of fatty acids (C6 and C5).

Keywords: azithromycin, ibuprofen, uptake, tomato, volatile aroma content

INTRODUCTION

The use of sewage sludge as a fertilizer is a common practice in agriculture. In Europe, more than 40 % of the produced sludge is applied on soils (Fijalkowski et al., 2017). The Sewage Sludge Directive, (86/278/EEC) that rules sewage sludge application, states that there is reduced probability of heavy metals and pathogens incorporation into soil profile (European Commission, 1986). Previous works highlight the presence of several hazardous elements in wastewater treatment plants end products (Clarke and Smith, 2011). Pharmaceuticals are in this group of hazardous elements and can be found in wastewater treatment effluents (Brown and Wong, 2018; Paiga et al., 2019; Kolecka et al., 2019), sludge by-products (Ivanova et al., 2018; Martin et al., 2012). These components are very resistance to degradation. As consequence of the increasing use of pharmaceutical products for humans and animals, their content in wastewater is growing (Pan and Chu, 2017; Verlicchi and Zambello, 2015) and consequently in agroecosystems (Biel-Maeso et al., 2018).

Recent studies found that pharmaceutical active compounds can be uptake by crops (Calderon-Preciado, 2011), such as potatoes (Conde-Cid, 2018), lettuce (Azanu et al., 2018). The impact of pharmaceutical active compounds on crops development and quality, are issues of growing concern since it has been reported that some pharmaceutical compounds can cause biochemical transformation of carbon and nitrogen in soil (Du and Liu, 2012), changing nutrient availability (Hammad et al., 2018), germination (Minden et al., 2017) and root growth (Carvalho et al., 2014; Pan and Chu, 2016). Very few researches have been carried out about the impact of pharmaceuticals on crops quality therefore, there is a need for more research and identify these impacts. Tomato (Solanum lycopersicum L.) is an important and widely consumed vegetable crop (FAO, 2015) due to its excellent source of flavonoids, carotenoids, vitamins, antioxidants and phenolics (Zou et al., 2018). Aroma, a non-visual quality parameter for fruit, is an important factor that influences consumers. In addition, it is a product widely consumed. The aim of this work is to study the potential impact of two pharmaceuticals compounds, azithromycin and ibuprofen on tomato fruits quality, specifically the uptake rate and the potential impact on volatile aroma content.

MATERIAL AND METHODS

Experiment design: Analytical grade azithromycin (≥ 95 %), ibuprofen (≥ 98 %), and organic solvents were purchase from Sigma-Aldrich Chemical Co. (St. Louis, MO., USA). Physicochemical properties of studied pharmaceuticals can be found in Table 1.

Table 1

Physicochemical properties of the two studied pharmaceuticals

Pharmaceutical	**Azithromycin*)**	**Ibuprofen*)**
IUPAC name	(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-11-[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-2-ethyl-3,4,10-trihydroxy-13-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,6,8,10,12,14-heptamethyl-1-oxa-6-azacyclopentadecan-15-one	2-[4-(2-methylpropyl)phenyl]propanoic acid
Chemical formula	$C_{38}H_{72}N_2O_{12}$	$C_{13}H_{18}O_2$
Pharmaceutical class	Macrolide antibiotics	Nos-steroidal anti-inflammatory drugs
CAS	117772-70-0	15687-27-1
Molecular weight ($g \cdot mol^{-1}$)	748.996	206.29
Log K_{OW}	4.02	3.97
Acid/Base	Base	Acid
pKa	8.74**)	4.91

*) provided data are from Pubchem (https://pubchem.ncbi.nlm.nih.gov/)

**) at 25 °C

The experiment material was cherry tomato (Lycopersicum esculentum). Germination was performed at 25 °C for 7 days. 21-day old seedlings were transferred to 5000 mL black plastic box on that exposure experiments were performed. A randomized block design with three replicates conducted in 9 boxes (3 different exposure amounts x 3 replicates) for both pharmaceuticals, ibuprofen and azithromycin were carried out. Control boxes were used to evaluate potential impacts caused by exposure at different amount of studied pharmaceuticals. A scheme of the experiment is shown in Figure 1.

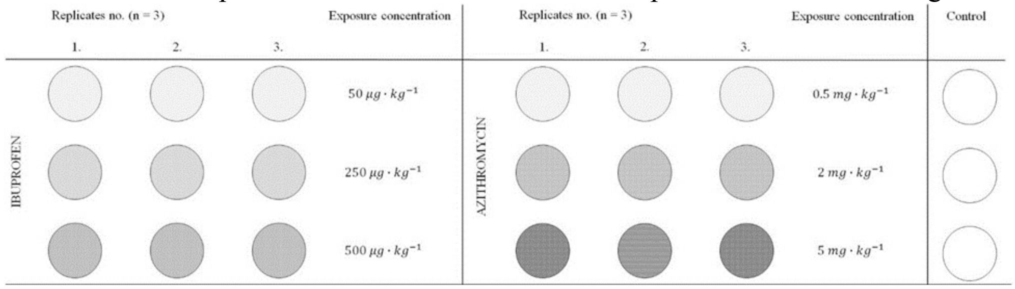

Fig. 1. Schematic diagram of artificial exposure experiment

Tomato samples were left to reach maturity in both standard and control box before analysis. The plant fruits were collected at maturity when exposure experiment has ended.

Artificial contamination was performed with the amounts used in other studies (see Figure 1). This was performed once through pharmaceutical incorporation in soil before seedlings were transferred. Details on the physicochemical properties of the soil are given in Table 2.

Table 2

Soil physicochemical properties. Mean values of three different batch analysis are provided with corresponding standard deviation (SD)

Soil properties	
Type	Clayed loam
pH value (in water)	7.3 ± 0.1
Organic carbon (%)	2.8 ± 0.3
Nitrogen (%)	0.3 ± 0.04
Cation exchange capacity (meq/100 g)	28.5 ± 3.8
Water holding capacity (g/100 g)	40.2 ± 3.4

***Laboratory analysis*:** Ibuprofen and azithromycin were extracted according to Aznar et al. (2017) and Thangadurai (2015). The extracts were analyzed by GC-MS (Agilent, USA) with electron impact mode (70 eV). The chromatographic column was as Phenomenex HP-5MS (30 m, 0.25 mm x 0.25 μm). The recoveries of the target compounds were 68 – 121 % (azithromycin) and 76 – 124 % (ibuprofen), respectively. The limit of quantification of the target pharmaceutical compounds ranged from 0.021 – 0.035, and 0.026 – 0.081 ug/kg in soil and tomato extracts, respectively. Seven concentrations of individual antibiotic were used (0.5 – 500 ug/L ibuprofen; 0.1 – 1000 ug/L and 100 – 5000 ug/L azithromycin) to calculate calibration curves ($r^2 > 0.9962$ for ibuprofen and $r^2 > 0.9975$ and 0.9954 for azithromycin).

To analyze the aroma volatile compounds, tomato samples were chopped and mixed until a paste was formed. For each sample, 2 ml of quenching agent solution (mix of 0.5 mol·L^{-1} EDTA in 0.625 mol·L^{-1} NaOH) were added to limit false aroma artifacts formation through oxidative and enzymatic reactions. Volatile aroma compounds were extracted using HS-SPME according to Lee et al. (2019). Fruit bioconcentration factor (BCF) was calculated as a ratio of the pharmaceutical's concentration in the fruit divided by the concentration in soil.

RESULTS AND DISCUSSION

***Azithromycin and ibuprofen in soil*:** Levels of incorporated azithromycin and ibuprofen concentration in soils are presented in Table 3. Azithromycin concentration in soil ranged from 41.6 – to 56.7 %, while ibuprofen incorporation rate in soil was higher (58.7 – 65.1 %). Theoretical contamination amounts were selected based on literature, representing a median of reported amounts in soils amended with wastewater treatment plant derived sludges (Ivanova et al., 2018; Martin et al., 2012; Calderon-Preciado et al., 2011).

Table 3

Incorporated amount of studied pharmaceutical in soils from exposure boxes

Pharmaceutical	Applied contamination level	Incorporated amount			
		Min (µg·kg^{-1})	Max (µg·kg^{-1})	Median (µg·kg^{-1})	RSD* (%)
Azithromycin	0.5 mg·kg^{-1}	222.8	261.2	239.8	4.5
	2 mg·kg^{-1}	865.2	1359.5	1195.6	16.6
	5 mg·kg^{-1}	1888.9	2405.1	2015.5	7.5
Ibuprofen	50 µg·kg^{-1}	22.1	35.1	29.7	8.5
	250 µg·kg^{-1}	144.8	178.5	163.1	6.3
	500 µg·kg^{-1}	288.2	365.1	315.8	5

Uptake of azithromycin and ibuprofen by tomato: Detected uptake amount of azithromycin by tomato fruit varied between 8.9 – 146.2 µg·kg^{-1} that represents an uptake percent between 1.8 – 3.6%. Ibuprofen was detected in tomato fruits in range of 2.8 – 63.2 µg·kg^{-1} with an uptake rate between 5.4 – 12.6 %, which was higher compared to azithromycin. For both pharmaceuticals, details on detected amounts for all performed experiments (exposure amounts *vs*. experiment boxes) are given in Table 4.

***Pharmaceuticals bioconcentration factor for tomato fruits*:** Bioconcentration factor (BCF) for both studied pharmaceuticals was < 1, with range between 0.082 – 0.209 for ibuprofen and 0.037 – 0.074 for azithromycin. Figure 2 summarizes BCF data for azithromycin (Figure 2.a) and ibuprofen (Figure 2.b). This illustrates variability in bioconcentration patterns for studied pharmaceuticals, both within, and between pharmaceutical contaminants, and between exposure concentrations.

Correlation analysis: Based on the Pearson correlation test, BCF value of all exposure experiments was normally distributed except case for azithromycin (2 mg/kg) where the skewness value was higher than the unit (1.412) and for ibuprofen (50 µg·kg-

[1]), where skewness value was 1.096, indicating that in these cases BCF values are positively skewed towards the lower concentration.

Table 4

Ibuprofen and azithromycin uptake by tomato under different exposure levels

Pharmaceutical	**Azithromycin**					
Exposure	**Box 1**		**Box 2**		**Box 3**	
concentration ($mg \cdot kg^{-1}$)	**Range** ($\mu g \cdot kg^{-1}$)	**Uptake*** (%)	**Range** ($\mu g \cdot kg^{-1}$)	**Uptake*** (%)	**Range** ($\mu g \cdot kg^{-1}$)	**Uptake*** (%)
0.5	8.9-12.9	2.2	10.7-16.8	2.8	13.8-18.2	3.2
2	37.5-51.2	2.2	43.5-55.2	2.5	48.2-56.2	2.7
5	98.5-125.3	2.2	114.2-146.2	2.6	105.6-136.2	2.4
Pharmaceutical	**Ibuprofen**					
Exposure	**Box 1**		**Box 2**		**Box 3**	
concentration ($\mu g \cdot kg^{-1}$)	**Range** ($\mu g \cdot kg^{-1}$)	**Uptake*** (%)	**Range** ($\mu g \cdot kg^{-1}$)	**Uptake*** (%)	**Range** ($\mu g \cdot kg^{-1}$)	**Uptake*** (%)
50	4.2-5.8	9.5	3.3-5.2	8.6	2.8-3.6	6.3
250	19.5-23.2	8.7	13.4-20.1	7	14.4-23.4	7.5
500	41.2-56.8	9.7	49.2-63.2	11.2	44.6-59.2	10.3

Contaminated tomato fruits volatile aroma content: The influence of pharmaceutical contamination on plants has been highlighted in previous studies, although it is not yet clarified whether the negative effects on plants are due to the direct actions of pharmaceutical products (Minden et al., 2017; Carvalho et al., 2014; Pan and Chu, 2016), or to the indirect actions on soil microorganisms that affect plant-microorganisms symbiosis (Hammad et al., 2018; Carvalho et al., 2014). In tomato, aroma content increases during fruit ripening and their abundance changed differentially. Representative volatile aroma compounds of tomato fruits are hexenal, hexenol, cis-3-hexenol, trans-3-hexenol, trans-2-hexenal, 3-methylbutanol and 3-methylbutanal (Wu et al., 2018; Wang and Seymour, 2017). Previous results observed that for tomato fruits, more than 400 primary volatile aroma compounds have been identified during ripening of a healthy tomato fruit (Song and Banghert, 2003; Zou et al., 2018). Usually these are attributed to the carotenoid, terpenoid, fatty acid and to the amino acid pathway, these being associated with aroma volatile biosynthesis and of that carotenoids, fatty acids and amino acids act as precursors (Zou et al., 2018). In present study, 61 volatile aroma compounds were identified of that 12 were fatty acid-derived volatiles including C6 and C5 groups. Identified volatiles derived from apocarotenoids, phenolics, and branched chain compounds were in number of 7, 9, and 9, respectively. Of terpenes, 10 compounds were identified while other volatiles were 15 compounds. Comparing volatile aroma compounds content with those from control experiment we could conclude that presence of active pharmaceutical compounds in grown media could impact potentially negative way their content, those affecting tomato fruit sensory quality. In Table 5 is presented the contingency table with statistical data for Chi-square and Fisher's exact test for major volatile aroma groups of tomato fruits derived from exposure and control experiment.

a. Box BCF for different exposure amount of azithromycin

b. Median, maximum, minimum, and upper and lower quartile values and ranges of calculated BCF for different exposure amount of ibuprofen

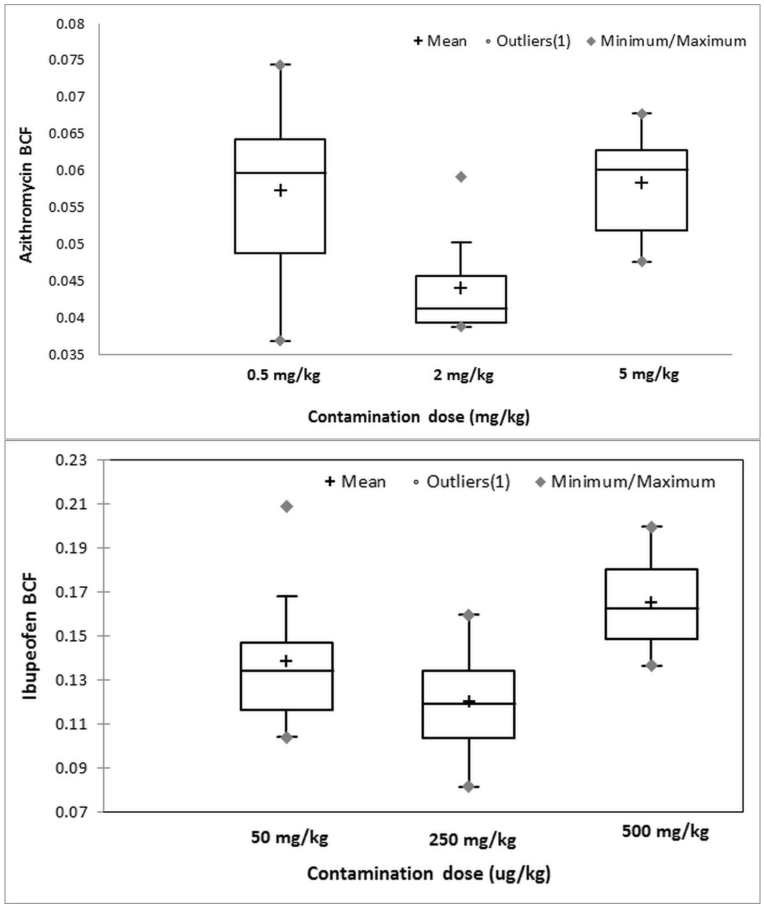

Fig. 2. Main statistical parameters of calculated BCF for different exposure amounts of pharmaceuticals.

The Chi-square test (Table 5) results showed that the concentration of pharmaceuticals is significantly different. The risk to reject the null hypothesis H0 while it is true is 99.78%. Similar results were observed through Fisher's test also. These are also proved by heat map analysis of different incorporated pharmaceutical active compounds at different dose by tomato fruit and each corresponding individual volatile aroma compounds of corresponding tomato fruits (Figure 3). The heat map was drawn with the obtained Pearson's correlation coefficients of all relative values of all data in tomato fruits volatile exposed at different amount of azithromycin and ibuprofen. Blue (-2) and red (+2) color represent negative and positive correlation between volatile aroma compounds and exposed pharmaceutical amount, respectively.

Table 5

Contingency table theoretical frequency of average values of major volatile aroma compounds of tomato fruits from different exposure and control experiment

	Azithromycin	Ibuprofen	Control	Total
C6 volatiles	45.8	46.6	68.2	160.6
C5 volatiles	6.1	6.2	9.1	21.5
apocarotenoids	38.5	39.2	57.3	134.9
phenolic volatiles	3.9	4.0	5.9	13.8
branched chain volatiles	10.8	11.0	16.1	38.0
terpenes	20.6	20.9	30.6	72.1
other volatiles	8.5	8.7	12.7	29.9
Total	134.3	136.6	199.9	470.8
Chi-square (test of independence between volatile aroma groups and experimental condition)				
p-value	0.998			
alpha	0.05			
Fisher's exact test				
p-value (two-tailed)	1			
alpha	0.05			

CONCLUSIONS

The results of this study clearly showed that tomato fruits are capable to uptake different types of pharmaceutical active compounds when exposed to a contaminated medium. Tomato has the capacity to bioaccumulate ibuprofen in higher amount (0.082 – 0.209) than azithromycin (0.037 – 0.074). Although corresponding uptake amount in edible parts of the tomato were lower than those detected in grown medium, our results has shown that could have a potential impact on the sensory quality of tomato fruits through changes in volatile aroma content. The major representative aroma compounds as those derived of fatty acids (C6 and C5 volatile aromas) were far less in tomato fruits grown in exposure boxes than to those from control boxes that had no such exposure.

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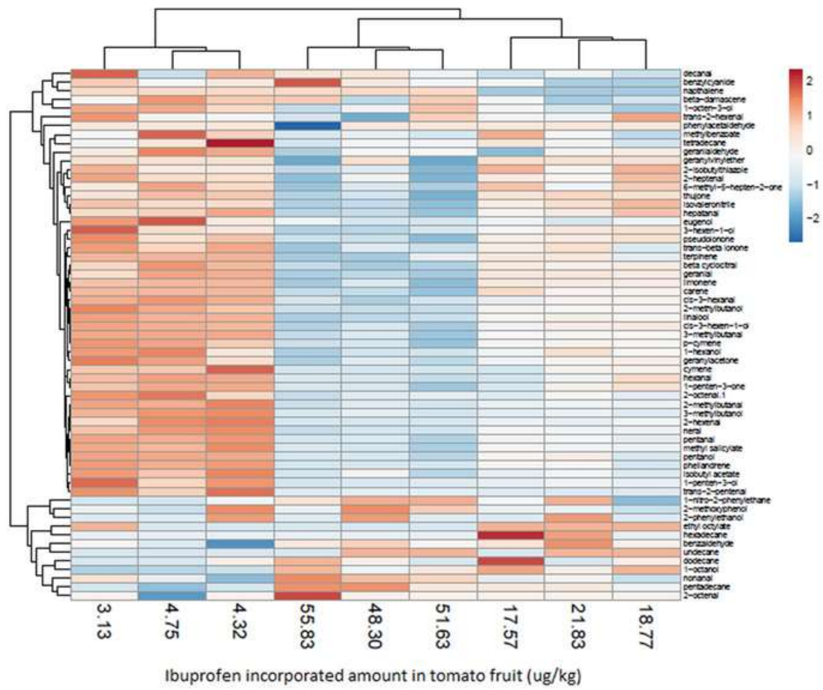

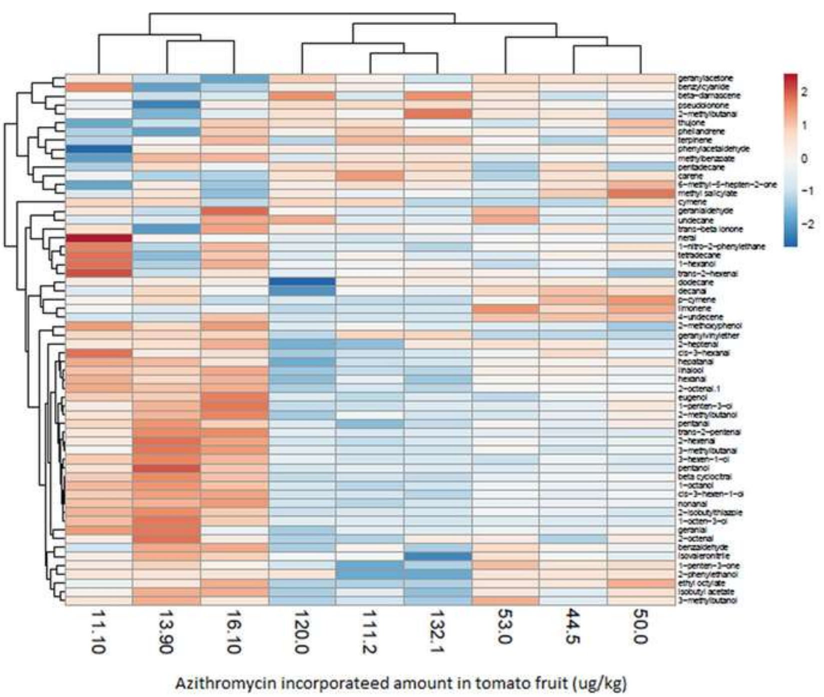

Fig. 3. Heat map of different exposure amounts of pharmaceuticals on individual volatile aroma compounds.

a.) Heat map of azithromycin incorporated amount from tomato fruit on corresponding volatile aroma compounds; b.) Heat map of ibuprofen incorporated amount from tomato fruit on corresponding volatile aroma compounds

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