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Uptake, translocation and transformation of three pharmaceuticals in green pea plants

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Abstract: Treated water from wastewater treatment plants that is increasingly used for irrigation may contain pharmaceuticals and, thus, contaminate soils. Therefore, this study focused on the impact of soil conditions on the root uptake of selected pharmaceuticals and their transformation in a chosen soil–plant system. Green pea plants were planted in 3 soils. Plants were initially irrigated with tap water. Next, they were irrigated for 20 days with a solution of either atenolol (ATE), sulfamethoxazole (SUL), carbamazepine (CAR), or all of these three compounds. The concentrations of pharmaceuticals and their metabolites [atenolol acid (AAC), N1-acetyl sulfamethoxazole (N1AS), N4-acetyl sulfamethoxazole (N4AS), carbamazepine 10,11-epoxide (EPC), 10,11-dihydrocarbamazepine (DHC), trans-10,11-dihydro-10,11-dihydroxy carbamazepine (RTC), and oxcarbazepine (OXC)] in soils and plant tissues were evaluated after harvest. The study confirmed high (CAR), moderate (ATE, AAC, SUL), and minor (N4AC) root uptake of the studied compounds by the green pea plants, nonrestricted transfer of the CAR species into the different plant tissues, and a very high efficiency in metabolizing CAR in the stems and leaves. The results showed neither a synergic nor competitive influence of the application of all compounds in the solution on their uptake by plants. The statistical analysis proved the negative relationships between the CAR sorption coefficients and the concentrations of CAR, EPC, and OXC in the roots (R = –0.916, –0.932, and –0.925, respectively) and stems (R = –0.837, –0.844, and –0.847, respectively).

Keywords: Atenolol; Carbamazepine; Sulfamethoxazole; Irrigation with contaminated water; Sorption in soils; Metabolites.

INTRODUCTION

It has been recognized that human pharmaceuticals are not entirely removed from wastewater in wastewater treatment plants (e.g., Golovko et al., 2014a, b; Loos et al., 2013). As a result, pharmaceuticals contaminate surface and ground waters (Loos et al., 2010). They may also pollute soils if contaminated water is used for irrigation or if sewage sludge is used as soil amendment (e.g., Thiele-Bruhn, 2003; Verlicchi and Zambello, 2015). The water environment and soils can be also polluted by veterinary pharmaceuticals from animal urine or farm waste (e.g., Charuaud et al., 2019). Pharmaceuticals present in soils can be taken up by plants (e.g., Ahmed et al., 2015; Al-Farsi et al., 2017; Christou et al., 2019; Goldstein et al., 2014; Kodešová et al., 2019a, b; Li et al., 2018, 2019a, b; Malchi et al., 2014; Montemurro et al., 2017; Mordechay et al., 2018; Shenker et al., 2011; Winker et al., 2010; Wu et al., 2013). Some of the studies (e.g., Kodešová et al., 2019b; Malchi et al., 2014; Paltiel et al., 2016) indicated a potential human health treat, if contaminated plant tissues (mainly roots and leaves) are consumed. Contaminations of plant fruits and associated risks have rarely been studied (e.g., Paltiel et al., 2016).

The mobility of a pharmaceutically active compound in soil-water and the potential availability for plants are largely controlled by the pharmaceutical's sorption into soil constituents and persistence in this environment. Sorption of pharmaceuticals in soils is driven by different mechanisms, which depend on the form of their molecules (e.g., Klement et al., 2018; Kodešová et al., 2015; Schaffer and Licha, 2015). A simultaneous sorption of differently charged compounds can be competitive (i.e., a decreased sorption of some compounds due to a competition for the same sorption sites) as well as synergistic (i.e., an increased sorption of some compounds due to their synergistic behavior) (e.g., Fér et al., 2018; Kočárek et al., 2016). There are just few studies that have focused on the soil impact on the uptake of pharmaceuticals from soils or the impact of simultaneously applied compounds to soils. The studies by Malchi et al. (2014), Goldstein et al. (2014), and Mordechay et al. (2018) indicated that the uptake of some compounds increased with decreasing organic carbon or clay content, but these trends were not proven statistically. A study by Kodešová et al. (2019a), which focused on the plant uptake of 3 pharmaceuticals (atenolol – ATE, sulfamethoxazole – SUL, and carbamazepine – CAR) from 3 different soil types, proved the statistically significant, negative relationships between the CAR sorption coefficients and CAR concentrations in roots of radishes, spinach and lamb's lettuce (no arugula), and leaves of radishes. No statistically significant relationships were found for ATE and SUL. Kodešová et al. (2019b), who studied pharmaceutical uptake from sewage sludge applied to 7 soils through spinach plants, documented close relationships between bioaccumulation factors (BAFs) and soil properties positively affecting sorption of some of analyzed compounds. They proved negative relationships between cation exchange capacity (or organic

carbon content) and BAF for CAR in leaves and roots, and negative relationships between cation exchange capacity and BAF for tramadol, citalopram, or telmisartan in roots. Distinct behaviors in two different soil groups were observed for sertraline, which was largely taken up from soils with a large base cation saturation. The sorption of organic compounds onto soil constituents reduces their amounts dissolved in pore water and thus reduces their uptake into plants (Li et al., 2019a, b). However, actual root-uptake of water and dissolved compounds is also controlled by soil water conditions (Brunetti et al., 2019). Uptake of water and available dissolved substances decreases at negative pressure heads close to zero and pressure heads above zero corresponding to soil saturation and pressure heads below the limit of decreased availability of water for plants or even below the wilting point (Feddes et al., 1978). Root-uptake can also be reduced by soil solution salinity (van Genuchten, 1987), which may also reduce plant growth and take up behavior of compounds in plant bodies (Kodešová et al., 2019b).

The study by Winker et al. (2010) documented lower concentrations of carbamazepine in ryegrass tissues when applied in a mixture with ibuprofen than when applied as a single compound solution. Christou et al. (2019) showed that while the SUL concentration in tomato fruits was reduced when applied together with trimethoprim or diclofenac, trimethoprim followed the opposite trend. On the other hand, Kodešová et al. (2019a) did not find statistical differences between the uptake of ATE, SUL, and CAR applied in single compound solutions or their mixture.

Studies by Goldstein et al. (2014), Malchi et al. (2014), Kodešová et al. (2019a, b), Montemurro et al. (2017), Mordechay et al. (2018), and Riemenschneider et al. (2017) documented that CAR can be metabolized mainly in plant leaves, and the CAR uptake and its transformation strongly depend on particular plant physiologies. Kodešová et al. (2019a) statistically proved that the metabolic efficiencies of radish and arugula (both family Brassicaceae) were very low, contrary to the high and moderate efficiencies of lamb's lettuce and spinach, respectively. The very low efficiency in metabolizing CAR (as well as some other compounds) in the roots and leaves of radish was also proven by Li et al. (2018). They documented that CAR was mostly unmetabolized in radish tissue enzyme extracts, contrary, for instance, to the intensive SUL metabolism in these extracts (the SUL recoveries in root and leaf enzyme extracts after 96 hours were 34% and 72%, respectively). Similarly, Wu at al. (2016) documented an even faster metabolism of SUL and a very low metabolism of CAR and ATE in carrot cell cultures. In addition, Kodešová et al. (2019a) found statistically significant, negative relationships between the CAR sorption coefficients and CAR two metabolite concentrations (carbamazepine 10,11-epoxide, oxcarbazepine) in the roots and leaves of radishes. Similar statistically significant relationships were not found for spinach and lamb's lettuce, which contributed to the larger metabolism of all CAR species in these plants.

In the study by Kodešová et al. (2019a), the uptake, transfer, and transformation of ATE, SUL, and CAR was studied in the roots and leaves of 3 leaf vegetables and radishes. Thus, an impact of soil conditions on the compounds' concentrations in stems and fruits could not be evaluated. The current study, therefore, focused on the evaluation of the distributions of these 3 compounds and their metabolites in tissues of green pea plants, i.e., in roots, stems, leaves, and pea pods. Our previous study also showed that the statistically significant negative correlations between sorption of compounds in soils and their concentrations in plant tissues were mostly observed for roots and not so often for leaves, which could be likely due to metabolization of accumulated compounds in leaves. Therefore the main goal was to test a hypothesis that compound concentrations in stems (and pea pods) should be less impacted by compounds' metabolism than in leaves; thus, concentrations of the compounds in stems (and pea pods) can be negatively related to their sorption coefficients in soils. In addition, a hypothesis that uptake of ATE, SUL, and CAR applied in single compound solutions and in solution of their mixture should not concededly differ, was tested.

MATERIAL AND METHODS

Experimental setup

The same soils and pharmaceuticals (Table 1 and 2) were used in this study, and the same procedures were followed as described by Kodešová et al. (2019a). Briefly, soil samples were taken from topsoils of the Haplic Chernozem developed on loess (HCh), Haplic Cambisol on paragneiss (HCa), and Arenosol Epieutric on sand (AE). Soil samples were air-dried to a soil-water content of 0.1 g/g and homogenized. These soils had very diverse soil properties (Table 1) and represented different soil environments that should affect behavior of selected compounds in soils in different ways. All 3 selected compounds ATE, SUL and CAR frequently occur in wastewater in the Czech Republic (Golovko et al., 2014a, b). ATE (Beta blocker used to treat hypertension) according to its pKa value (Table 2) should occur in tested soils (according soil pH in Table 1) in cationic form and its sorption affinity to soils is high (Table 2). SUL (antibiotic that is usually applied together with an antibiotic trimethoprim, used to treat a variety of bacterial infections) should prevail mostly in anionic form in soil of higher pH, and partly in anionic and neutral form, respectively, in soils of low pH. SUL sorption in soils is low (Table 2). Degradation half-lives (DT_{50}) of both compounds (ATE and SUL) in soils are relatively low (Table 2). CAR (anticonvulsant used primarily in the treatment of epilepsy, to control seizures and to treat pain resulting from trigeminal neuralgia and diabetic neuropathy) occurs in soils in neutral form, moderately sorbs in soils (Table 2) and is very stable in the soil environment (Table 2).

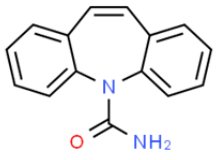
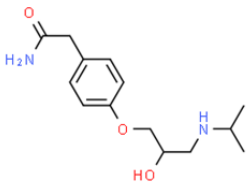
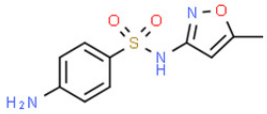
Experiments were carried out in June under greenhouse conditions (natural light, air humidity of 30%–40%, and air temperature of 20–24°C). A single plant of green peas (*Pisum sativum* L. *var. Axiphium*) was planted in a small pot (volume of 340 cm^3) in five replicates for each soil and treatment. Each plant was initially given 8 days of irrigation with tap water, followed by a period of 20 days of irrigation with solution of a single pharmaceutical (ATE, SUL, or CAR), a solution of all 3 pharmaceuticals (Table 3), or tap water. It should be mentioned that the concentrations (Table 3) were five hundred to one thousand times over environmentally relevant concentrations, e.g., in wastewater presented by Golovko et al. (2014a, b) or Loos et al. (2013). Such concentrations were used to enhance the detection and quantification of all compounds and their metabolites in all matrices. Similar concentrations were also used in our previous study (Kodešová et al., 2019a) and some other studies. For more details, see Kodešová et al. (2019a). After treatment, plants were carefully removed from soils and washed. Plant tissues (i.e., roots, stems, leaves, and pods) were separated. Since the same procedure as applied by Kodešová et al. (2019a) was followed, plant and soil samples from 5 replicates were pooled, freeze-dried, and weighed. The reason for this approach was to collect enough amount of dry plant materials for chemical analyses (i.e., at least 0.1 g of dry plant tissues, see below). As we found (Figure 1), in the case of the

green pea plant, this problem was not as acute as in our previous study for 3 leave vegetables and radish (Kodešová et al., 2019a). However, just few pods of different early stages of their development were collected, which would likely result in a high variability of measured concentrations (i.e., different time of exposure) and in some cases data would not be available for all plants. Next, all samples were ground, and concentrations of compounds (ATE, SUL, and CAR) and their metabolites [atenolol acid (AAC), N1-acetyl sulfamethoxazole (N1AS), N4-acetyl sulfamethoxazole (N4AS), carbamazepine 10,11-epoxide (EPC), 10,11-dihydrocarbamazepine (DHC), trans-10,11-dihydro-10,11-dihydroxy carbamazepine (RTC), and oxcarbazepine (OXC)] in plant tissues and soils were measured using the methods described below.

Table 1. Selected soils and their properties: organic carbon content (Cox), $CaCO_3$ content, pH_{H2O}, pH_{KCl}, content of nitrogen (N) phosphorus (P) and potassium (K), cation exchange capacity (CEC), soil hydrolytic acidity (HA), basic cation saturation (BCS), sorption complex saturation (SCS), salinity, and clay, silt and sand contents (Kodešová et al. 2019a).

Soil Type		Haplic Chernozem - HCh	Haplic Cambisol - HCa	Arenosol Epieutric - AE
Soil substrate		Loess	Paragneiss	Sand
Cox	(%)	1.74	1.57	0.46
$CaCO_3$	(%)	4.17	0.19	0.05
pH_{H2O}	–	8.2	6.0	5.6
pH_{KCl}	–	7.2	4.7	4.3
N	(mg/kg)	18.6	25.5	4.03
P	(mg/kg)	135	92.7	220
K	(mg/kg)	340	194	85.8
CEC	($mmol^+$/kg)	234.9	188.1	47.0
HA	($mmol^+$/kg)	4.5	49.9	25.5
BCS	($mmol^+$/kg)	230.4	138.2	21.5
SCS	(%)	98	74	46
Salinity	(µS/cm)	126.9	53.0	25.3
Clay	(%)	25.8	25.4	5.0
Silt	(%)	60.3	30.1	4.5
Sand	(%)	13.9	44.5	90.5

Table 2. Selected pharmaceuticals, their properties and the parameters K_F and n of the Freundlich sorption isotherms ($s = K_F c^{1/n}$, where s is the concentration sorbed onto the soil particles and c is the concentration in soil water) and dissipation half-lives DT50: HCh – Haplic Chernozem, HCa – Haplic Cambisol, AE – Arenosol Epieutric (Kodešová et al., 2019a).

Pharmaceutical	Carbamazepine			Atenolol			Sulfamethoxazole		
CAS	298-46-4			29122-68-7			723-46-6		
Molecular structure [a]									
pKa	pKa_1 = 1.0 (basic) pKa_2 = 13.9 (acidic)			9.6 (basic)			pKa_1 = 1.7 (basic) pKa_2 = 5.6 (acidic)		
Log Kow	2.25			0.16			0.89		
H-bonds Donors, Acceptors	1 1			3 4			2 6		
MW (g/mol)	236.27			266.34			253.28		
Soil	HCh	HCa	AE	HCh	HCa	AE	HCh	HCa	AE
K_F ($cm^{3/n}/\mu g^{1-1/n}$/g)	3.86	2.97	0.71	16.24	5.36	2.11	0.88	4.01	1.39
n	1.13			1.17			1.65		
DT_{50} (days)	>1 000	>1 000	>1 000	3.7	9.0	7.7	5.0	15.0	8.0

[a] blue – basic, red – acidic

Table 3. Irrigation doses and concentrations of pharmaceuticals: ATE – atenolol, SUL – sulfamethoxazole, CAR – carbamazepine, S – single-solute solution, M – tri-solute solution.

Day	Irrigation (mL)	Concentrations (mg/L)					
		ATE-S	SUL-S	CAR-S	ATE-M	SUL-M	CAR-M
8	150	1.7	0.96	1	0.72	0.64	0.49
10	150	1.7	0.96	1	0.72	0.64	0.49
11	200	1.7	0.96	1	0.72	0.64	0.49
14	150	1.3	1	0.65	0.8	0.77	0.53
16	150	1.3	1	0.65	0.8	0.77	0.53
18	200	1.1	1	0.68	0.89	0.85	0.58
21	150	1.1	1	0.68	0.89	0.85	0.58
23	150	1.2	0.78	0.64	0.87	0.88	0.58
25	150	1.2	0.78	0.64	0.87	0.88	0.58
26	100	1.2	0.78	0.64	0.87	0.88	0.58
28	Harvest	1.1	1.1	0.66	0.88	0.74	0.48

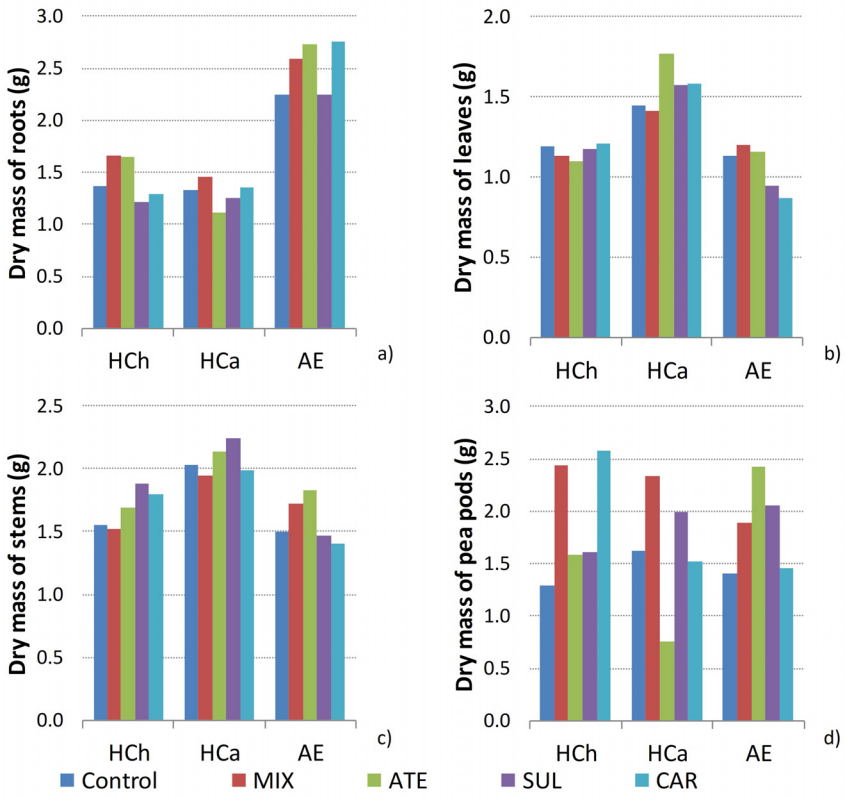


Fig. 1. Dry masses of plant parts (sums of all 5 replicates) measured for different treatments: a) roots, b) leaves, c) stems, d) pea pods, Control – irrigation with tap water, MIX – irrigation with the solution of all compounds, and ATE, SUL and CAR – irrigation with the solution of atenolol, sulfamethoxazole and carbamazepine, respectively, HCh – Haplic Chernozem, HCa – Haplic Cambisol, AE – Arenosol Epieutric.

Chemical analyses

The method for extraction of compounds (CAR, ATE, and SUL) and their metabolites (EPC, OXC, RTC, DHC, AAC, N1AS, and N4AS) from plant tissues followed a procedure previously validated for these compounds by Kodešová et al. (2019a, b). Briefly, the freeze-dried plant samples were extracted as follows: 0.1 g of sample was placed in an Eppendorf tube with a safe lock, 5 ng of internal standard, and a stainless steel ball, and 1 mL of extraction mixture 1 (acetonitrile/water, 1/1, 0.1% of formic acid) was added. Samples were consequently extracted by shaking at 1800 min^{-1} for 5 min (TissueLyser II, Quiagen, Germany). The samples were then centrifuged at 10,000 min^{-1} for 5 min (Mini spin centrifuge, Eppendorf), and the supernatant was filtered through a syringe filter (0.45 µm regenerated cellulose filters) to clean Eppendorf tube. Aliquots of 100 µl were taken and placed in an autosampler vial for LC-MS analysis.

An ultrasound-based extraction approach with two solvent mixtures was applied for the analysis of the selected compounds and their metabolites in the soil matrix (Golovko et al., 2016; Koba et al., 2016, 2017). This method was validated for 63 compounds and their metabolites in 13 soils (including compounds and soils used in this study). Briefly, 2 g of each freeze-dried soil sample was placed in a 10-mL autosampler vial, and 20 ng of internal standard was added. The samples were then extracted with 4 mL of extraction mixture 1 (acetonitrile/water 1/1, v/v acidified with 0.1% of formic acid) followed with 4 mL of mixture 2 (acetonitrile/2-propanol/H2O, 3/3/4, v/v/v, acidified with 0.1% of formic acid) in an ultrasonic bath (DT 255, Bandelin electronic, Sonorex).

The liquid chromatography-tandem mass spectrometry (LC-MS/MS) and either isotope dilution or an internal standard (IS) method with using matrix matching standard was used to determine concentrations of pharmaceuticals in irrigation doses and concentrations of pharmaceuticals and their metabolites in supernatants from plant tissues and soils. A triple-stage quadrupole mass spectrometer, Quantiva (Thermo Fisher Scientific, San Jose, CA, USA), coupled with an Accela 1250 LC pump (Thermo Fisher Scientific) and HTS XT-CTC autosampler (CTC Analytics AG, Zwingen, Switzerland), were used for the analysis of irrigation water (Koba et al., 2016). A hybrid quadrupole-orbital trap mass spectrometer, Q Exactive (Thermo Fisher Scientific, San Jose, CA, USA), operated in high-resolution product scan mode (HRPS), was used instead of a triple quadrupole for more complex soil and plant extracts. A Hypersil Gold aQ column (50 mm × 2.1 mm i.d., 5 μm particle size, from Thermo Fisher Scientific San Jose, CA, USA) was used for the chromatographic separation of the target compounds. The matrix effects were corrected using a matrix matching standard if deviation from calibration curve response factor was greater than 30%. Detail descriptions of the instrument settings can be found in article of Grabicova et al. (2018). For other details about the methods (procedures, validation of the methods etc.) please see Kodešová et al. (2019a), Golovko et al. (2016) and Koba et al. (2016, 2017). The average limits of quantification (LOQs) are shown in Table 4. Estimated uncertainty of the methods is 30%. However, both sulfomethoxazole metabolites had lower response in ESI-HRPS, which resulted in high LOQs.

Resulting concentrations of chemicals in soils and plant tissues were expressed in ng/g (dry weight) (Figure 2a, c) and also in nmol/g (dry weight). To calculate these values, the molecular weight (MW) values in Table 2 were used for the parent compounds, and the MW values of 267.33 (AAC), 295.31 (N4AS), 252.27 (EPC), 238.28 (DHC), 270.29 (RTC), and 252.27 (OXC) g/mol were used for the metabolites.

Table 4. Average limits of quantification, LOQs (ng/g), calculated from real samples analyzed in different sequences and time: ATE – atenolol, AAC – atenolol acid, N1AS – N1-acetyl sulfamethoxazole, N4AS – N4-acetyl sulfamethoxazole, SUL – sulfamethoxazole, CAR – carbamazepine, EPC – carbamazepine 10,11-epoxide, DHC – 10,11-dihydrocarbamazepine, RTC – trans-10,11-dihydro-10,11-dihydroxy carbamazepine, and OXC – oxcarbazepine.

Compound	Roots	Stems	Leaves	Pods	Soils
ATE	0.41	0.41	0.43	0.24	6.03
AAC	1.10	1.73	2.05	1.10	4.67
SUL	9.77	13.6	22.2	11.5	2.97
N1AS	24.0	28.5	50.4	23.2	1.19
N4AS	34.2	36.2	70.9	37.3	2.15
CAR	0.99	2.80	1.38	1.17	1.41
EPX	0.65	1.50	0.76	0.93	0.91
OXC	4.13	3.58	4.69	3.33	0.62
RTC	6.65	5.48	6.23	5.40	4.41
DHC	2.07	1.31	1.35	1.11	1.48

Data evaluation

The data expressed in nmol/g were next used to calculate molar fractions of the measured parent compounds and their metabolites relative to the total of all measured compounds (Figure 2b, d) or in a sum of measured parent compound and its metabolites (Figure 3). It should be noted that the studied compounds could be transformed also into other metabolites and transformation products. However, Koba et al. (2016, 2017) showed that concentrations of other metabolites of CAR, SUL and ATE in our soils can be very low and even negligible. Similarly, previous studies (e.g., Riemenschneider et al., 2017) dealing with the uptake of CAR, SUL and ATE did not suggest considerable fractions of other metabolites in tested plant materials.

The data expressed in nmol/g were also used to evaluate bioaccumulation of the compounds in plant tissues. Since exposure of plants to soil contamination was not constant (i.e., concentrations of repeatedly applied solutions differed (Table 3) and in-between applications compounds transformed in soils) standard bioaccumulation factors could be evaluated. Therefore, the parent compound load normalized concentrations (CLNC, 1/g) were calculated as, the concentrations in plant tissues divided by the parent compound load as follows (Kodešová et al., 2019b):

$$CLNC = \frac{C_p}{\sum_{i=1}^{N} V_i C_{sol,i}} \quad (1)$$

where C_p (nmol/g) is the solute concentration in plant tissue, V_i (cm^3) is the volume of the irrigation dose, $C_{sol,i}$ (nmol/cm^3) is the solute concentration of the parent compound in the irrigation dose, and N is the number of irrigation doses (Table 3). The CLNC values were next analyzed using STATGRAPHICS Centurion XV Version 15.2.06. Kruskal–Wallis tests were used to compare the CLNC values for the different tissues and treatments (Figure 4): 1. A data set for a particular chemical and plant tissue included the CLNC values from all soils and both treatments (i.e., application of chemical in solution of a single compound or their mixture); 2. A data set for a particular chemical and plant tissue included the CLNC values from all soils and one treatment. Simple correlations between the Freundlich sorption coefficients (K_F values in Table 1) and the CLNC values for the corresponding pharmaceutical or its metabolites were assessed using the Pearson product-moment correlation coefficient. The statistical significance was assessed according to the p-value.

RESULTS AND DISCUSSION

Plant growth

As found by Kodešová et al. (2019a), the dry masses of plant tissues and the total masses of plants (Figure 1) irrigated with solutions of a single compound or all three compounds did not differ from those irrigated with tap water. However, plant growth was largely impacted by soil type. While the masses of roots (Figure 1a) from AE were considerably larger than those from HCh and HCa (likely due to decreased availability of water in sandy soil), the masses of leaves (Figure 1b) and stems (Figure 1c) from HCa were larger than those from HCh and AE probably due to the optimal water and air conditions in HCa. Nevertheless, the masses of pods (Figure 1d) were similar for all scenarios.

Bioaccumulation of pharmaceuticals and their metabolites - Neutral molecules of CAR

The low concentrations of the CAR metabolites (Figures 2a and 2b) indicated that CAR was not considerably transformed in the soils by microbial activity or other chemical processes

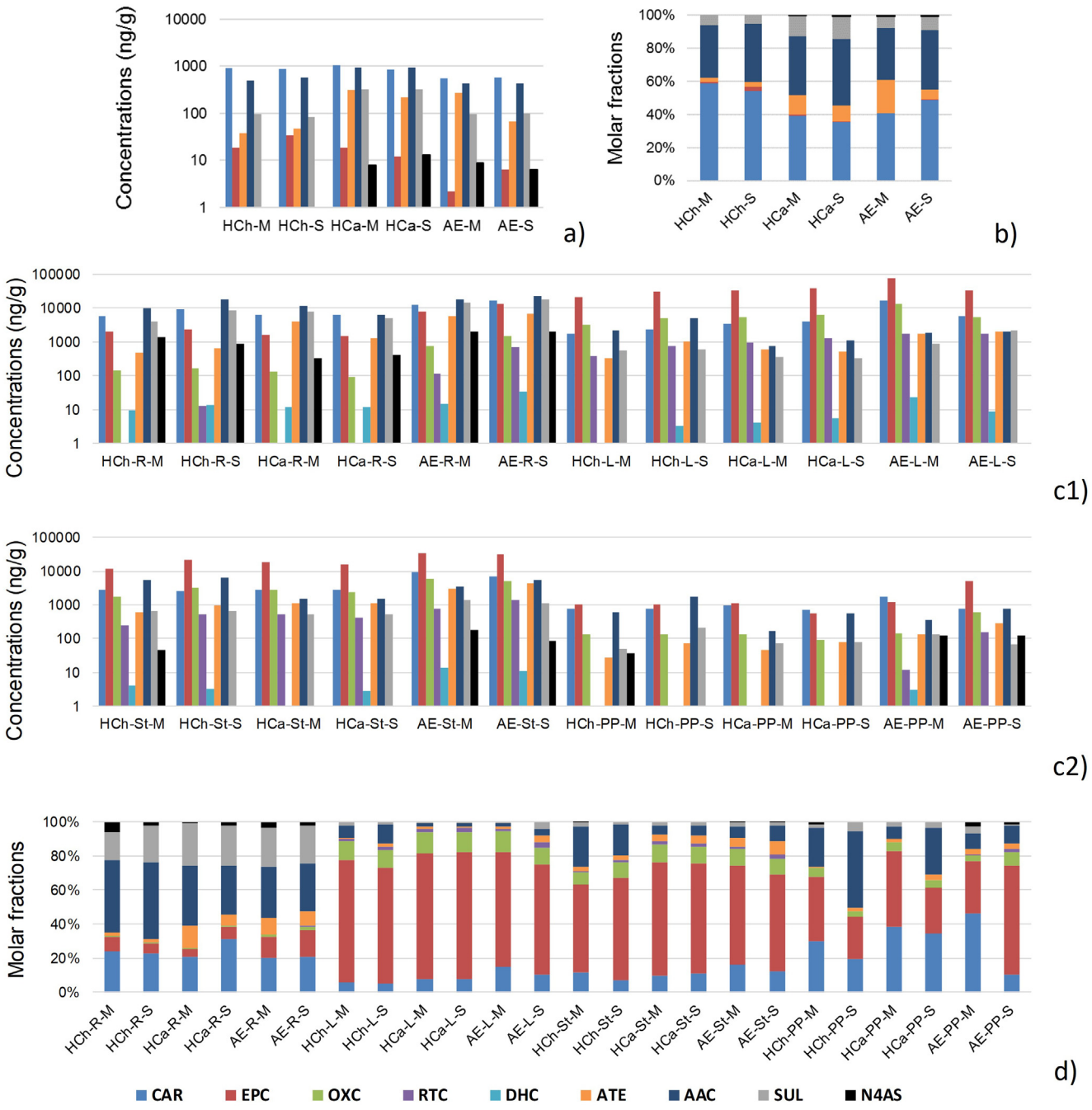


Fig. 2. Concentrations of pharmaceuticals (CAR, ATE, and SUL) and their metabolites (EPC, OXC, RTC, DHC, AAC, and N4AS) in soils HCh, HCa and AE (a), and plant tissues: (c), i.e., roots (R), stems (St), leaves (L) and pea pods (PP). Fractions of each compound in the sums of molar concentrations of all parent compounds and their metabolites quantified in soils (b) and plant tissues (d): S – single-solute solution, M – tri-solute solution, HCh – Haplic Chernozem, HCa – Haplic Cambisol, AE – Arenosol Epieutric, ATE – atenolol, SUL – sulfamethoxazole, CAR – carbamazepine, AAC – atenolol acid, N4AS – N4-acetyl sulfamethoxazole, EPC – carbamazepine 10,11-epoxide, DHC – 10,11-dihydrocarbamazepine, RTC – trans-10,11-dihydro-10,11-dihydroxy carbamazepine, and OXC – oxcarbazepine.

taking place in a soil environment (Koba et al., 2016; Kodešová et al., 2016). On the other hand, CAR was greatly metabolized in plant bodies (Figures 2c and 2d). It is widely assumed that CAR transformation in plant tissues is affected by plant cytochrome P450 enzymes (e.g., Goldstein et al., 2014; Gunnarsson et al., 2012; Malchi et al., 2014; Montemurro et al., 2017). The fractions of CAR from the sum of CAR and its metabolite molar concentrations (Figure 3a) in the roots varied between 80% and 60%, by 15% in the leaves and stems, and the fractions in the pea pods varied by 40%. The EPC fractions were dominant in the stems and leaves (70%). The relatively large fractions of OXC (15%–10%) were also measured in the stems, leaves, and pods.

Significant differences between the accumulations of different CAR species in different plant tissues were also proven by analyzing the CLNC values using Kruskal–Wallis tests and box-and-whisker plots (Figure 4a). The highest sums of concentrations of CAR and its metabolites (Figure 2) were obtained in the leaves, followed by stems, roots, pods, and soils. Our findings are consistent with the results of Riemenschneider et al. (2017), who found the largest concentrations in leaves followed by concentrations in the stems, roots, and fruits of

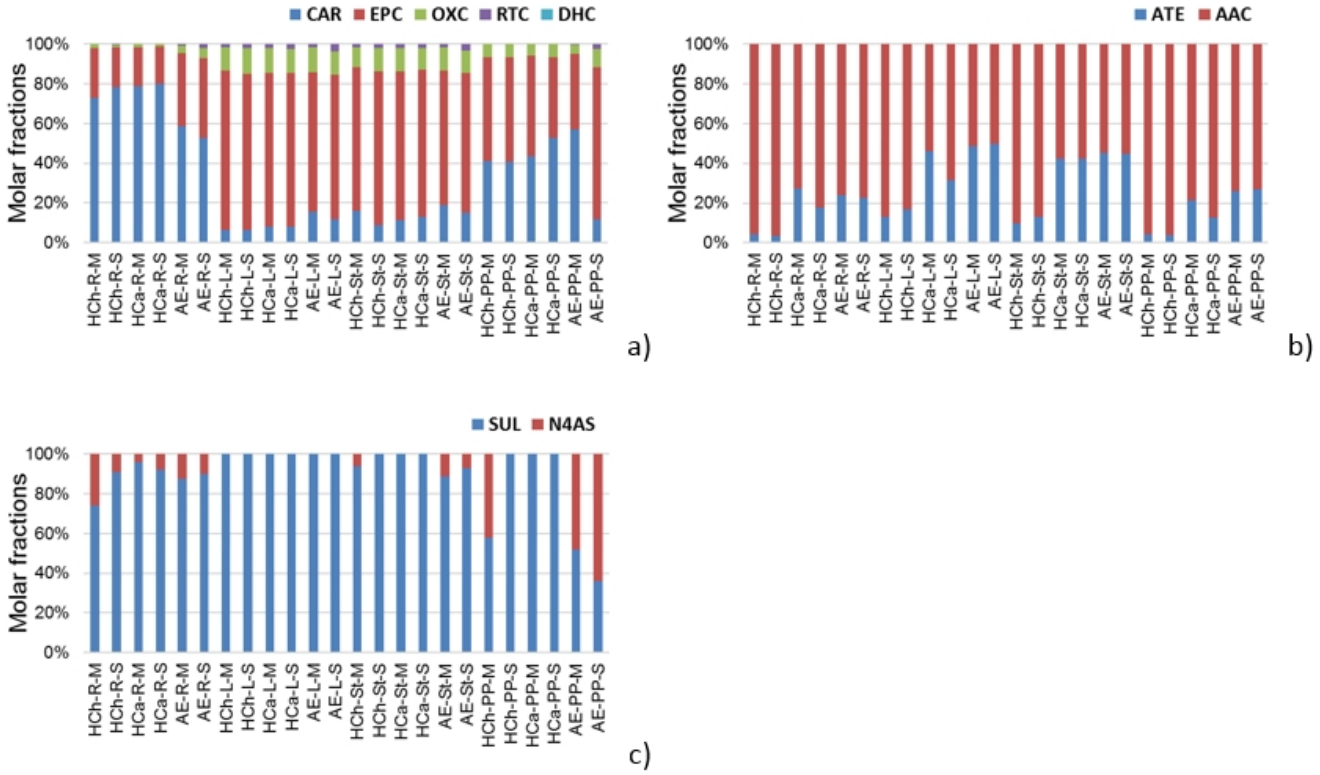


Fig. 3. Fractions of each compound in the sums of molar concentrations of the parent compound and its metabolites, i.e., sum of CAR, EPC, OXC, RTC and DHC (a), sum of ATE and AAC (b), and sum of SUL and N4AS (c) in plant tissues: R – roots, L – leaves, St – stems, PP – pea pods, S – single-solute solution, M – tri-solute solution, HCh – Haplic Chernozem, HCa – Haplic Cambisol, AE – Arenosol Epieutric ATE – atenolol, SUL – sulfamethoxazole, CAR – carbamazepine, AAC – atenolol acid, N4AS – N4-acetyl sulfamethoxazole, EPC – carbamazepine 10,11-epoxide, DHC – 10,11-dihydrocarbamazepine, RTC – trans-10,11-dihydro-10,11-dihydroxy carbamazepine, and OXC – oxcarbazepine.

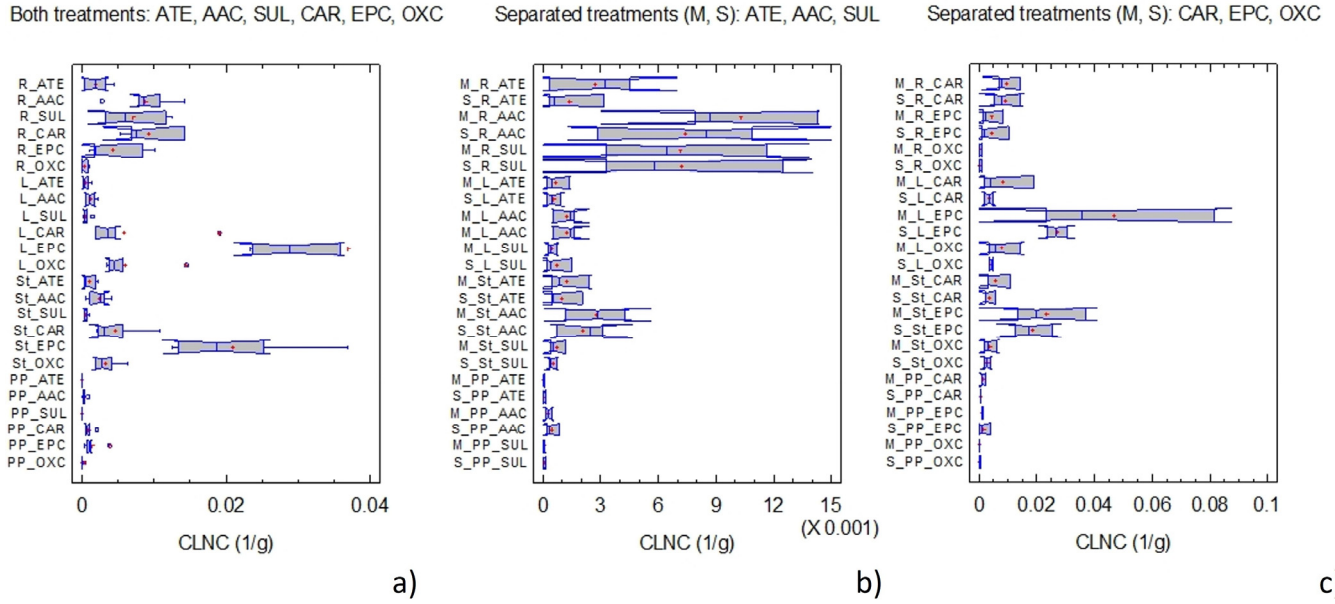


Fig. 4. (a) The CLNC values for the main compounds quantified in plant tissues (ATE – atenolol, AAC – atenolol acid, SUL – sulfamethoxazole, CAR – carbamazepine, EPC – carbamazepine 10,11-epoxide, and OXC – oxcarbazepine) from all soils and both treatments (a data set for a particular chemical and plant tissue included the CLNC values from all soils and both treatments, i.e., application of chemical in solution of a single compound or their mixture) (L_EPC data include an outlier value of 0.0815 1/g), and (b) and (c) the CLNC values for the main compounds measured in plant tissues from all soils and different treatments (a data set for a particular chemical and plant tissue included the CLNC values from all soils and one treatment either S – single-solute solution, or M – tri-solute solution): R – roots. L – leaves, St – stems, and PP – pea pods.

tomato plants. Similarly Shenker et al. (2011) found the largest concentrations in the leaves followed by the concentrations in the roots, stems, and fruits of cucumber plants. The high accumulation in the leaves of green pea plants is associated with the transpiration stream and not the restricted transfer of neutral molecules of low MW, lipophilicity, and number of H-bonds (Kumar and Gupta, 2016) through the plant bodies (e.g., Goldstein et al., 2014; Hurtado et al., 2016; Kodešová et al., 2019a, b; Malchi et al., 2014; Montemurro et al., 2017; Mordechay et al., 2018; Shenker et al., 2011, Winker et al., 2010; Wu et al., 2013) due to a passive diffusion through lipid bilayer membranes (Chuang et al., 2019). The lower accumulation in the pods is explained by the significantly shorter exposure to the contamination and a lower transpiration of pods in comparison to that in the leaves. Compared to the study by Kodešová et al. (2019a), the metabolism of CAR in green pea leaves was as efficient as in lamb's lettuce leaves.

Bioaccumulation of pharmaceuticals and their metabolites - ionic molecules of ATE and SUL

The bioaccumulations of both ionic compounds were considerably lower than the bioaccumulation of CAR. Compared to that of CAR, the roots also contained relatively large amounts of ATE (positively charged) and its metabolite AAC (Figures 2c and 2d). The $CLNC_{AAC}$ values were significantly higher than the $CLNC_{ATE}$ values and similar to the $CLNC_{CAR}$ values (Figure 4a). The fractions of ATE and AAC from the sum of their molar concentrations were 20% and 80%, respectively (Figure 3b). These findings can be explained by ATE's rapid transformation in soils (Figures 2a and 2b, and Kodešová et al., 2016), a moderately larger persistence of the AAC metabolite in soils (Koba et al., 2016), and its subsequent root uptake (Kodešová et al., 2019a). Significantly lower concentrations of ATE were found in the other plant tissues (Figures 2c and 4a). This is explained by the positive charge of the ATE molecules and their sorption onto the negatively charged cell membranes (and, thus, restricted transfer in plant bodies), which is consistent with the findings of Kodešová et al. (2019a) but is in contrast to the results of Wu et al. (2013), who found similar ATE concentrations in the leaves and roots of all plants. The fractions of ATE and AAC in the pods were similar to those in the roots (Figure 3c). The molar fractions of ATE and AAC in the leaves and stems were 40% and 60%, respectively.

Similar to ATE, considerably larger concentrations of SUL (molecules were mostly negatively charged) were measured in the roots than in the other tissues (Figures 2c, 4a), which is consistent with studies by Ahmed et al. (2015), Kodešová et al. (2019a), Malchi et al. (2014), and Wu et al. (2013). The sulfamethoxazole metabolite N1AS was not found in any matrices. Low concentrations of N4AS were quantified in all roots and some stems and pea pods. These findings differ from the results of the study by Kodešová et al. (2019a), in which neither of these two metabolites were observed in the roots and leaves of spinach, lamb's lettuce, arugula, and radishes. On the other hand, this metabolite was also found in lettuce and carrot plants by Mullen et al. (2017). The significantly lower concentrations in the above surface plant tissues, compared to that in the roots, can be explained by the negative charge of the SUL molecules and, thus, their repulsion from the cell membranes (i.e., restricted transfer in the plant bodies). Very low concentrations, or absence, of the SUL metabolites in the plants and soils can be explained by their very rapid dissipation from the soils (Figures 2a and 2b, and Koba et al., 2017). In addition, as shown by Li et al. (2018) and Wu at al. (2016), SUL (and likely also its metabolites) can be very efficiently metabolized in plant bodies (particularly in roots). The bioaccumulation of SUL in the roots (Figure 4a) was significantly larger than ATE's bioaccumulation and comparable with AAC's bioaccumulation, respectively. This finding is in contrast with the findings of Wu et al. (2013), who documented considerably lower concentrations of SUL than ATE, and to the results of Kodešová et al. (2019a), who observed similar SUL and ATE bioaccumulations. It should be noted that, contrary to SUL, the metabolism of ATE could be quite low (Wu at al., 2016). Thus, the difference between the actual uptakes of SUL and ATE (indicating the larger uptake of SUL compared to that of ATE) could be even greater.

Influence of treatment on the compound's uptake and distribution in plant tissues

No trends between the concentrations measured in the plant tissues from the different treatments (i.e., the single compound application or application of the mixture of 3 pharmaceuticals) were found (Figure 2c). Except EPC in leaves, the Kruskal–Wallis tests and the box-and-whisker plots (Fig. 4b and 4c) did not show significant differences between the CLNC values of a certain compound (i.e., CAR, EPC, OXC, ATE, AAC, and SUL) in specific plant tissues for all soils obtained under the different treatments, which is consistent with the findings by Kodešová et al. (2019a). However, it should be noted that plant tissues for an individual soil and treatment were pooled and thus difference between the CLNC values resulted from different treatments for a specific soil could not be assessed statistically. Therefore, a new study with a greater amount of plants planted in a certain soil, which would allow pooling plant tissues at least in 3 groups (i.e., replicates), is needed to prove or disprove this hypothesis.

Influence of soil on the compound's uptake and distribution in plant tissues

The correlation coefficients (Table 5) between the parent compound K_F coefficients (Table 1) and the CLNC values of the parent compound or its metabolite (merged sets of values obtained from both treatments) consistently displayed a negative influence of the sorption of the parent compound in soils on its uptake and transfer in the plant bodies.

However, statistically significant relationships were found only for the CAR concentrations in roots and stems. In these plant tissues, statistically significant, negative relationships were found also between the $K_{F,CAR}$ and CLNC values of the CAR metabolites (EPC and OXC). Similar to the study by Kodešová et al. (2016), positive relationships (but not significant) were observed between the $K_{F,ATE}$ and $CLNC_{AAC}$ values. This can be explained by the negative charge of the AAC molecule and, thus, an opposite sorption affinity to soils compared to ATE, i.e., the sorption of ATE and AAC increases and decreases, respectively, with an increasing number of negatively charged sorption sides of soil constituents (Kodešová et al., 2016).

Potential human health risks

As mentioned above applied concentrations were five hundred to one thousand times over environmentally relevant concentrations. Therefore concentrations of some compounds in leaves were very high. In the case of pods, the concentrations of CAR, ATE, SUL and their metabolites (excluding N4AS from AE scenarios) were at least 10 time lower than those in leaves.

Table 5. The correlation coefficients between the parent compound load normalized concentrations (CLNC) of particular pharmaceutical or its metabolite and the Freundlich sorption coefficient (K_F) of parent compound: CAR – carbamazepine, EPC – carbamazepine 10,11-epoxide, OXC – oxcarbazepine, ATE – atenolol, AAC – atenolol acid, and SUL – sulfamethoxazole.

Plant	CLNC	K_F, CAR	K_F, ATE	K_F, SUL
Roots	CAR	–0.916*		
	EPC	–0.932**		
	OXC	–0.928**		
	ATE		–0.805	
	AAC		–0.306	
	SUL			–0.155
Stems	CAR	–0.837*		
	EPC	–0.844*		
	OXC	–0.847*		
	ATE		–0.750	
	AAC		0.596	
	SUL			–0.304
Leaves	CAR	–0.716		
	EPC	–0.627		
	OXC	–0.629		
	ATE		–0.630	
	AAC		0.724	
	SUL			–0.315
Pea pods	CAR	–0.501		
	EPC	–0.682		
	OXC	–0.661		
	ATE		–0.724	
	AAC		0.752	
	SUL			–0.402

*p < 0.05, **p < 0.01.

In general larger concentrations were measured in plants planted in AE. Thus a greatest health risk when consuming green pea pods can be expected in case of plants planted in sandy soils.

Exposure and potential health risk associated with consumption of the pharmaceuticals and their metabolites in crops can be examined relative to acceptable daily intake (ADI) values for each substance (e.g., Kodešová et al., 2019b). Because information about long term exposure of pharmaceuticals to human health is often not available (Williams and Brooks, 2012), the ADI values can be calculated from minimal therapeutic doses (1.43 (CAR), 0.71 (ATE) and 1.43 (SUL) mg/kg of person) divided by an uncertainty factor (UF). In case they are not CMR or EDC type chemicals (Bruce et al., 2010; Bull et al., 2011; Semerjian et al., 2018), UF of 3000 can be applied. The worst case scenario can be assumed in the case of the metabolites (i.e., metabolites can have a similar impact on human health as a parent compound) and the ADI values for metabolites can be calculated assuming the ADI values for the parent compound and molar masses (Kodešová et al., 2019b). Daily consumption (DC) of fresh green pea pods by a child (25 kg) and an adult (70 kg) to reach ADI can be calculated using the measured concentrations and the mean percentage of pod dry mass of 18% (calculated from fresh and dry masses of pods obtained from different scenarios). Assuming this approach and concentrations in pea pods (Figure 2c), the DC values for children ranges from 0.12 to 0.30 kg for CAR, 0.05–0.42 kg for EPC 0.37–2.3 kg for OXC, 0.37–3.8 kg for ATE, 0.06–0.63 kg for AAC, 1.0–4.4 kg for SUL, and 1.8 kg for N4AS and AE. Some of these values are close to a possibly consumable amount of pods. In the case of adults the DC values would be 2.8 time higher. However, it can be expected that in the case of the environmentally relevant concentrations, the DC values should be more than 2 orders of magnitude higher and thus a potential health risk is likely very low. Nevertheless, it should be mentioned that pods were harvested in an early stage of their development. Concentrations could be higher after longer-time exposure. Additional studies should be carried out with environmentally relevant concentrations of various compounds to elucidate a potential health treat related to these compounds uptake to fruits.

CONCLUSION

This study confirmed high (CAR), moderate (ATE, AAC, SUL), and minor (N4AC) root uptake of the studied compounds by green pea plants, the unrestricted transfer of the CAR species into the different plant tissues and the very high efficiency in metabolizing CAR in the stems and leaves of green pea plants. As anticipated, the results showed neither competitive nor synergic effects of the simultaneous application of the compounds on their uptake by these plants. However, this phenomenon should be further studied using a larger number of plants planted in an individual soil, which would allow a statistical assessment for a certain soil environment. The results indicated the negative impact of the parent compounds' sorption affinity on their uptake by the plants, which was statistically proven for CAR, EPC, and OXC in the roots and stems. Thus, our results partly confirmed our main hypothesis, that the concentrations of some compounds in the roots and stems (but not in the pea pods) can be negatively dependent on their sorption affinities to soils.

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