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Uptake, Accumulation and Impact of Antiretroviral and Antiviral Pharmaceutical Compounds in Lettuce

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Abstract

While the contamination of agroecosystems with pharmaceutical compounds has been reported, the fate of these compounds, particularly uptake into plants remains unclear. This lack of environmental fate data is evident for a critical class of pharmaceuticals, the antivirals and antiretrovirals (ARVDs). Thus, this study evaluated the root uptake of the antiretroviral compounds nevirapine, lamivudine and efavirenz, and the antiviral compound oseltamivir in lettuce. The lettuce was hydroponically grown in a nutrient solution containing the four ARVD pharmaceutical mixture in the 1-100 µg L^{-1} concentration range. The measured bioaccumulation showed that efavirenz and lamivudine accumulated to the highest and lowest degree, at concentrations of 3463 ng g^{-1} and 691 ng g^{-1} respectively. The translocation factor between the root and leaf for nevirapine was greater than 1. The highest concentration of the pharmaceutical mixture had a physiological impact on the lettuce. Potential toxicity was evidenced by a statistically significant 34 % ($p = 0.04$) mean reduction in root and leaf biomass in the 100 µg L^{-1} ARVD mix exposed lettuce, compared with the controls. This study advances knowledge of the fate of ARVDs in agroecosystems, in particular, plant root – ARVD interaction and the resulting potentially toxic effects on plants.

Keywords: Antiretroviral; Antiviral; Uptake; Pharmaceuticals; Accumulation; Toxicity

1 Introduction

Plant uptake of active pharmaceutical ingredient (API) molecules has been reported in food and non-food crops. Exposure to APIs can either be under a controlled environment (hydroponic and pot trial experiments) (e.g. Goldstein et al., 2014; Ahmed et al., 2015; Hurtado et al., 2016; Kodešová et al., 2019; Tian et al., 2019) or under field conditions on soils amended with contaminated surface water, un/treated wastewater, sewage sludge and biosolids (e.g. Prosser et al., 2014; Paz et al., 2016; Ben et al., 2018; Carter et al., 2018; Bagheri et al., 2019). Uptake studies, however, have primarily focussed on antibiotics, antidepressants, analgesics, anti-epileptics, hormones and non-steroidal anti-inflammatory therapeutic based drugs. Past studies and reviews (Navarro et al., 2014; Wegst-Uhrich., 2014; Wu et al., 2015; Bártíková et al., 2016a; Zhang et al., 2017; Madikizela et al., 2018; Christou et al., 2019) reveal that there is limited information on an equally significant therapeutic class of drugs, the antivirals and antiretrovirals (ARVDs). A global review of the environmental and ecotoxicological effect of antiretrovirals by Madikizela et al., (2018), acknowledges this deficiency, recommending plant ARVD uptake studies.

The environmental occurrence of ARVDs is ostensibly less frequent and manifests at trace concentration levels in high and upper-middle-income countries and hence of minimal concern (De Voogt et al., 2009; Sui et al., 2012, Funke et al., 2016). This scenario has presumably contributed to the low number of associated ARVD plant uptake investigations as the majority of uptake studies are undertaken in these countries. The consumption of ARVDs and the extent of their contamination in the Sub-Saharan Africa region (particularly Kenya and South Africa) compared with Europe is detailed by Fekadu et al., (2019) and Nannou et al., (2020) in fresh surface waters and wastewaters respectively. Recent antiretroviral data from Lusaka, Zambia, report on the occurrence of three antiretrovirals, lamivudine nevirapine and zidovudine in the aquatic environment at concentrations in the range of 60 - 118,970 ng L^{-1} (Ngumba et al., 2020). The three compounds were also detected in source-separated urine at concentrations in the range 7740 – 12800 µg L^{-1}, which is several magnitudes higher than measured environmental concentrations. Considering that source-separated urine is re-used as fertilizer (Ledezma et al., 2015), it is imperative to gain knowledge on plant-ARVD interaction.

The therapeutic objective of ARVDs is to counter a range of viruses in the host body (De Clercq and Li, 2016; Nanno u et al., 2020). Therefore, an untargeted wide-scale release of ARVD into the environment may alter ecosystem functioning and potentially induce antiviral resistance in microorganisms (Jain et al., 2013; Bártíková et al., 2016; Nannou et al., 2020). Concerning ensuing activity and toxicity of ARVDs, a Q-SAR modelling study revealed that

ARVDs were highly potent. In a class of 50 therapeutic APIs, ARVDs were predicted to be among the top eight most hazardous drugs (Sanderson et al., 2004). Besides the public health concern, APIs may also induce toxic effects on plants by affecting their physiological and development processes (Hillis et al., 2011; Liu et al., 2013; Christou et al., 2018; Sun et al., 2018; Rede et al., 2019). For example, Sun et al., (2018) exposed cucumber to a mixture of 17 APIs at concentrations of up to 1000 µg L^{-1} and found that the leaves exhibited burn-like features. At present, there is a limited understanding of potential ARVD effects on plants. A review of the impact of APIs on plants presented by Bártíková et al. (2016). It outlines the toxic effects brought by antibiotics, anti-parasitic drugs, hormones, growth promoters and antifungals on plants but contains hardly any information on the impact of ARVDs on plants.

Plant-API uptake studies with an African perspective include Amos Sibeko et al. (2019). They detected the non-steroidal anti-inflammatory drugs (NSAIDs) naproxen, ibuprofen and diclofenac in water hyacinth (*Eichhornia crassipes)* in river water in South Africa. Naproxen showed the highest concentration (12 ng g^{-1}). Mlunguza et al., (2020) reported on the uptake of three antiretrovirals (emtricitabine, tenofovir disoproxil and efavirenz) in water hyacinth in a water reservoir in South Africa. The concentrations of antiretrovirals in the hyacinth reed varied from 0.97 to 29.6 ng g^{-1}.

Although Mlunguza et al. (2020), reported on plant uptake of ARVDs, a structured experimental approach is necessary to elucidate plant root-ARVD interactions comprehensively. In this hydroponic study, the uptake of three antiretroviral APIs (nevirapine, lamivudine and efavirenz) and one anti-influenza antiviral API (oseltamivir) into lettuce (*Lactuca sativa*) was investigated. Selection of the APIs was based on their frequency of detection from previous (Ngumba et al.,2016; K'oreje et al., 2016) studies. The advantage of hydroponic experiments is that they provide test conditions to rapidly screen and identify priority APIs that exhibit the highest uptake potential, which can inform in-depth plant–API fate studies. The major drawback, however, is that hydroponic studies do not provide the complexity of a real agroecosystem environment (Wu et al., 2015). Leafy vegetables accumulate organic contaminants greatest in comparison to root vegetables, cereals and fruit vegetables (Christou et al., 2019), hence the selection of lettuce. Uptake can be described either as a passive or active process (Kumar and Gupta, 2016). This study focussed on active processes (solute specific), i.e. it solely concentrated on the characteristic of the molecule rather than the existing environmental conditions.

2 Materials and methods

2.1 Reagents

Methanol (MeOH), acetonitrile (MeCN), HPLC water and 0.1 % formic acid in water were obtained from Fisher Scientific, UK, and ammonium hydroxide (NH_4OH) solution (25% v/v) and formic acid from Sigma Aldrich, UK. Nevirapine (NVP) and lamivudine (LVD) standards were obtained from Sigma Aldrich, UK, efavirenz (EFV) from Tokyo Chemical Industries, and oseltamivir (OSV) from Fisher Scientific, UK. All antiviral API standards had a purity grade of > 99 %.

2.2 Standards and stock solutions

Preparation and storage of stock solutions and standards followed the European Commission, SANTE/11813/2017 (2018) guidelines. LVD and OSV stock solutions were prepared in 50 % (v/v) MeOH: HPLC water solution. NVP and EFV stock solutions were made in 100 % MeOH. Dilutions of the four ARVD mix were prepared at concentrations of 0.1 to 100 µg L^{-1}.

2.3 Liquid Chromatography

Analyte separation was performed by liquid chromatography (Dionex Ultimate 3000, Thermo Scientific) on a PhenylHexyl (*Ace ultracore*) (2.5 µm × 100 mm) stationary phase. The column temperature was isocratic at 50 ^{o}C. The analytical run time was 9.8 min, starting from a mobile phase composition of 100 % 0.1% ammonia (in HPLC water) and 0.1 % formic acid solution (1:1, v/v), adjusted to pH 8.4, before transitioning to 100 % MeOH. The equilibration time was 2 minutes and at a flow rate of 500 µL min^{-1}.

2.4 High-resolution mass spectrometry (HRMS)

An Orbitrap HRMS system (Thermo Scientific) was used for mass detection. Ionization was via heated electrospray (HESI) at a vaporizer temperature of 300 ^{0}C. Nitrogen gas was used as the auxiliary gas. Spray voltage was operated in positive mode at + 3.5 kV with a capillary temperature of 270 ^{o}C. The scanning range covered the 100 -1000 *m/z* mass window and mass resolution set at 17500 *m/z*. Mass calibration of the mass spectrophotometer was performed 24 hours before analyses. An example chromatogram is provided in Figure 1.

2.5 Method validation/optimization

The ultrasonic-assisted extraction (UAE) approach adapted from Wu et al., (2012) was selected as the extraction method. Sample clean-up was achieved using solid-phase extraction (SPE). Since the target analytes exhibited varying physicochemical characteristics

(**Error! Reference source not found.**), it was necessary to determine the optimal extraction-pH.

The performance characteristics of the method were evaluated using extraction recoveries of the analytes from plant material, instrument and method linearity, method limit of detection, and quantification (MLoD and MLoQ) and evaluation of matrix effects (ME).

2.5.1 Extraction pH and recoveries

Fresh lettuce was obtained from a local grocer. The leaves were washed with high purity water (HPW), chopped and freeze-dried. The freeze-dried sample was then ground to powder. Recovery experiment was performed at two spiking levels, 10 ng g^{-1} and 50 ng g^{-1}. Ground lettuce (0.2 g) was placed in a 50 mL polypropylene centrifuge tube and spiked with the four ARVD external standard mix stock to attain the two desired spiking levels. Spiked samples were allowed to equilibrate for 24 hours. Ten mL of the extraction solvent, (MeOH: MeCN; 1:1, v/v) was added to the sample, and the tube placed in an ultrasonic bath operated at 50 Hz for 15 min. The resulting supernatant was collected and centrifuged at 4000 rpm for 5 min. The sample residue was re-extracted and the supernatants combined before solvent reduction to ca 500 µL under a gentle stream of nitrogen gas. The reduced extract was diluted with 20 mL HPW water and then filtered (GF/F, 0.7 µm). The optimal extraction pH was obtained by extracting set of samples (n = 3) at four pH values: pH 2.4 (adjusted using 1 % (v/v) formic acid), the native sample pH 5.4; pH 7.4 and 9.4 (1 M NH_4OH solution adjusted).

2.5.2 Solid-phase extraction

Solid-phase extraction was implemented using a 60 mL HLB sorbent (Waters, UK). Before sample loading, the sorbent was conditioned sequentially with MeOH and HPLC water (2.5 mL aliquots). The 20 mL sample extract was loaded onto the cartridge at a rate of 2 mL min^{-1}. After loading, the sorbent was dried and washed with 1 mL of HPLC water. Aliquots (2 x 2.5 mL) of the extracting solvent mix were used for sample elution. The 5 mL extract was evaporated to almost dryness and reconstituted to 1 mL using 30 % MeOH (in HPLC water). Finally, the 1 mL sample was filtered using a 0.22 µm PTFE filter and stored in at 4^{o} C in the dark before analysis.

2.5.3 Linear range, MLoD, MLoQ and Matrix effect

The LC-HRMS was calibrated at the 0.1 – 100 µg L^{-1} concentration range using matrix-matched (MM) standard solutions, prepared according to EU SANTE/11813/2017 (2018) guidelines. It followed that the amount of the solute stock in the MM standard solution was 10 % of the total standard solution volume and the remaining 90 % comprised of the unspiked plant extract. The MLoD and MLoQ were calculated according to the ICH guidelines (ICH,

1995), based on the calibration curve of the MM working standards at the native lettuce extract pH (pH 5.4). Matrix effects (ME) was evaluated by comparing the slope of the calibration curve in the matrix to the slope of the calibration curve using solvent (Eq *1*).

$$\text{Matrix effect (\%)} = \left(\frac{Slope\ of\ calibration\ curve\ in\ matrix}{slope\ of\ calibtration\ curve\ in\ solvent} - 1\right) \times 100 \quad (1)$$

2.6 Hydroponic experiments

The hydroponic system was housed in a greenhouse. The experimental approach was adapted from the OCSPPC 850.4800 Plant Uptake and Translocation Test guidelines (EPA, 2012). Young lettuce seedlings (10 days old) of the *Analora* genus were obtained from Defland Nurseries, UK. Before the actual exposure test, the plants were exposed to a dilute water-fertilizer solution for seven days to nurture and acclimatize the roots to a water-only environment. Individual seedlings were each exposed to 400 mL of the four ARVD mix standard nutrient solution, composed of water and commercial fertilizer (Flora Gro, NPK 3:1:6 at a concentration of 0.5 mL L^{-1}). The set up consisted of 6 replicates per exposure concentration. In total, there were 24 lettuce plant samples. The sample containers (glass) were covered with aluminium foil, and the top sealed to allow only the roots to be in contact with the nutrient solution. The solution was automatically aerated for 10 minutes every hour. The experiment was run for 21 days with the exposure solution renewed on days 7 and 14. Loss of water due to evaporation and evapotranspiration during the growing period did not exceed 20 % of the initial volume of the nutrient solution placed at the beginning of each experiment.

2.7 Accumulation and physiological effect of ARVDs on lettuce

After test termination, the plant samples were immediately washed with HPW and thoroughly dabbed dry. Lettuce samples were separated into roots and leaf then weighed. After that, the samples were freeze-dried before extraction and analysed according to the optimized method described in the previous sections. Potential physiological effects on the plant were assessed by comparing the biomass of the control (root and leaves) with the biomass ARVD exposed samples.

Uptake of ARVDs in this study was characterized using four parameters: bioconcentration factor (BCF), root concentration factor (RCF), leaf concentration factor (LCF) **Error! Reference source not found.**, **Error! Reference source not found.** and **Error! Reference source not found.** and translocation factor (TF). **Error! Reference source not found.** TF quantifies the movement of the organic analyte from the root to above-ground tissues.

$$BCF(mL/g) = C_{plant}/C_{exposure\ solution} \quad (2)$$

$$RCF\ (mL/g) = C_{root}/C_{exposure\ solution} \quad (3)$$

$$LCF(mL/g) = C_{leaf}/C_{exposure\ solution} \quad (4)$$

$$TF\ (L/g) = C_{leaf}/C_{root} \quad (5)$$

Where C_{leaf}, C_{root}, C_{plant}, and $C_{exposure\ solution}$ is the API concentration in the leaf, root, plant and nutrient solution, respectively (Goldstein et al., 2014; Hurtado et al., 2016; Emhofer et al., 2018).

2.8 Data analyses

Data were analyzed using Ms Excel 2016 and IBM SPSS Statistic v 24 software. One-way analysis of variance (ANOVA) and Dunnett's T3 test was used to measure statistical differences between means at the 95 % confidence interval.

3 Results and discussion

3.1 Method optimization

3.1.1 Recoveries and effect of pH

The objective of this experiment was to select the optimal extraction pH for the target analytes. Since the recovery of an analyte is concentration-dependent; while performing recovery experiments an analytically appropriate spiking range should be selected as opposed to a single spike level. (Thompson et al., 1999). Pharmaceuticals are typically detected at nanogram level in the environment, hence 10 ng $g^{-1}$and 50 ng g^{-1} level were selected as they were considered critical spike levels for the recovery test. The extraction recoveries were tested at four pH values, ranging from acidic (pH 2.0) to alkaline (pH 9.4).

2 depicts the obtained analyte absolute extraction recoveries. The recoveries were cumulatively evaluated across the two spike levels. Mean ARVD recoveries at the two spike levels did not vary significantly (p=0.55), being 68 ±22 % (± SD) and 65±19 % for the 10 ng g^{-1} and 50 ng g^{-1} level respectively. Individually, NVP exhibited the highest recoveries (mean 88 %). EFV and OSV had second and third highest recoveries at 75 % and 62 % respectively. LVD yielded the lowest recoveries an average of 42 %. pH change least influenced NVP and EFV recoveries. OSV recoveries were relatively consistent across three (pHs 2.0-7.4) with only a minimal drop at highest pH. Contrastingly, LVD recoveries varied widely, which ultimately contributed to its low mean recoveries.

Cumulatively, the highest ARVD extraction recoveries were obtained at pH 5.4 (mean of 72 %). At pH 2.0, 7.4 and 9.4, recoveries of 66 %, 66 % and 62 % respectively, were obtained. While EFV and NVP recoveries at pH 5.4 had recoveries of > 70 %, LVD and OSV at the same environment exhibited recoveries of < 65 %. Referring to EU SANTE/11813/2017 guidelines, it states that satisfactory extraction recoveries may vary between 70-120 %. Nonetheless, it clarifies that reproducible recoveries of 30-70 % are also acceptable. For this reason, pH 5.4 was selected as the optimal pH for subsequent extraction procedures. It is noteworthy to mention that pH 5.4 was also the natural extraction pH, so working at this pH meant that subsequent buffer additions were unnecessary.

3.1.2 Linearity, MLoD and MLoQ

A visual inspection of the plot of analyte signal vs concentration revealed the existence of a linear relationship. Whereas the relationship between the analyte signal and the analyte in solution is often linear, the relationship between analyte signal and the analyte in the matrix is not routinely linear due to the influence of matrix components (Kruve et al., 2015a). For this reason, linearity was assessed using analyte in the matrix. **Error! Reference source not found.** shows that analyte in matrix linearity was satisfactory at $r^2 > 0.990$. Likewise, the limit of detection is also matrix-dependent (Kruve et al., 2015b); accordingly, matrix-spiked samples were used in the determination of MLoD and MLoQ. The MLoD of the ARVD analytes varied from 1.51 to 5.61 ng g^{-1} and the MLoQ from 5.13 to 18.7 ng g^{-1} (**Error! Reference source not found.**).

3.1.3 Matrix effects (ME)

Ultrasonic extraction mechanically breaks down the sample matrix allowing the release of the target analytes (Schantz, 2006; Tadić et al.,2019). However, a drawback is its lack of selectivity necessitating intensive subsequent clean-up processes (Ros et al., 2016). For this reason, it was necessary to evaluate the effect of matrix on the detection of analytes. As indicated in **Error! Reference source not found.**, ion suppression predominated. LVD exhibited the lowest ion suppression at ≤ 30 %. NVP, OSV, and EFV, on the other hand, showed ion suppression ranging from 46–50 %. Classification of the ME was made according to Barreales-Suárez et al., (2018), i.e. ME of < 20 % considered as low, ME 20 - 40 % as a medium, ME 40-60 % as high and ME as 60 % as very high. In general, ME lay in the medium to high-level region, indicating significant signal suppression. Varying levels of ME following the UAE-SPE method have been reported. For example, ME of 26 to 29 % were noted during the analyses of antibiotics in four vegetable matrices (Tadić et al., 2019) and ion suppression of 30 to 60 % was reported in the analyses of 7 antibiotics in lettuce (Albero et al., 2019).

Overall, the data from this study is consistent with Furey et al. (2013) and Tadić et al. (2019) who suggest that signal enhancement or suppression due to the matrix is unpredictable. It presents itself unsystematically and also indiscriminately and therefore is unique for each analysis.

3.2 Presence of APIs in the lettuce plant

3A shows that the ARVDs present in the nutrient solution accumulated at varying concentrations in the roots and leaves of the lettuce plant. The four ARVDs studied had a molecular weight < 400 Da; molecules of this size may penetrate the root via the epidermis into the bulk of the root (Miller et al., 2015). At the lowest exposure concentration (1 µg L^{-1}), the accumulation of LVD, OSV, and EFV in the leaf was below the MLoD and was not quantified, whereas, for NVP and LVD, root concentrations were below the MLoD.

The overall whole plant accumulation of the ARVDs in the lettuce varied from < MLoQ to 3463 ng g^{-1} and < MLoD to 1647 ng g^{-1} for the root and leaf respectively across the three exposure levels. EFV exhibited the highest total tissue biomass accumulation (3463 ng g^{-1}) measured in the 100 µg L^{-1} exposed sample. This accumulation was five times higher than the concentration of the lowest accumulated ARVD, LVD (691 ng g^{-1}) in the same treatment level. NVP and OSV accumulations were 2625 ng g^{-1} and 1541 ng g^{-1}, respectively in the 100 µg L^{-1} exposed samples.

The extent of accumulation over the three exposure levels were such that the higher the concentration of the API in the nutrient solution, the higher the measured accumulation. This observation is consistent with Al-Farsi et al. (2017) and González García et al. (2018). They, in a separate hydroponic API exposure experiments, reported that the magnitude of accumulation was directly associated with the concentration of the pharmaceutical in exposure solution. In the current study, the mean plant concentration factor (CF) rise between the 1 µg L^{-1} and 10 µg L^{-1} treatment was 17.7 ± 9.32 (± 1 SD), while the CF between the 10 µg L^{-1} and 100 µg L^{-1} treatment was 11.7 ± 5.7 (± 1 SD). Cumulatively, the mean increased CF in the plant matrix between the lowest (1 µg L^{-1}) and highest exposure solution treatment (100 µg L^{-1}) was 189.8 ± 87.5 (± 1 SD).

The RCF, BCF and TF values discussed herein are the means across the 10 µg L^{-1} and 100 µg L^{-1} exposure levels. 3B illustrates that > 80 % of the OSV and LVD API mass fractions predominantly accumulated in the roots whereas for EFV root accumulation was > 95 %. Accordingly, the RCFs were such that EFV > OSV > NVP > LVD (0.043 > 0.013 > 0.08 > 0.05 mL g^{-1}, respectively). BCF (mL g^{-1}) was evaluated to determine accumulation into the bulk of the plant. The mean BCF across the 10 µg L^{-1} and 100 µg L^{-1} exposure region showed that

EFV had the highest whole plant tissue accumulation. The ascending order with regards to BCF was EFV > NVP > OSV > LVD at (0.044 > 0.025 > 0.016 > 0.005 mL g^{-1}).

Regarding transport within the plant, NVP exhibited the highest TF values, with 4 indicating that it was readily translocated to the leaves. Typically, a TF (L g^{-1}) > 1 suggests that a molecule can indeed migrate from the roots and its largest fraction accumulate in the above root tissues (Chuang et al., 2019). Therefore, a TF > 2 implied that NVP was two times more likely to bioaccumulate in the above root tissues, specifically the lettuce leaf in this study.

As noted in Section 1, there is little information on the plant uptake of ARVDs. For this reason, discussion in this study will refer and relate to pharmaceutical compounds of different therapeutic groups but of comparable physicochemical characteristics to the ARVDs studied here.

3.3 pH - adjusted octanol-water partitioning coefficient (Log D*ow*) influence on uptake

An API's solubility, hydrophobicity, molecular weight and ionization tendencies may influence its uptake potential (Al-Farsi et al., 2017; Chuang et al., 2019). Of all factors, the octanol-water partitioning coefficient (log K*ow*) is possibly the most extensively investigated API property influencing uptake. For non-ionic compounds, log K*ow* is linearly related to uptake (Collins et al., 2006; Prosser et al., 2014). For ionizable compounds, however, log K*ow* is adjusted to log D*ow* to reflect the environmental pH. The present study was conducted at a constant nutrient solution pH of 6.5.

Characterization of the relationship between log D*ow* and uptake of the APIs was achieved by plotting log D*ow* against log BCF, RCF, and TF, respectively (5). For BCF, exhibiting an R^2 value of 0.69 as indicated in 5A, linearity was influenced mainly by the hydrophobic and neutral NVP and EFV (see Table 1 for the Physico-chemical properties of the ARVDs of interest here). The contribution to linearity by the hydrophilic LVD and OSV (log D*ow* = -1.1 and -1.44, respectively) however could not be decisively defined. A closer inspection revealed that had the log K*ow* of OSV had remained unchanged, i.e. not transformed by a change in pH (as was with NVP and EFV), stronger linearity of R^2 = 0.97 value would have been realized. The observation further shows thatlog D*ow* of hydrophilic ionizable molecules (in this study OSV and LVD), potentially may not accurately describe uptake in plants. Thus, OSV's relatively low log D*ow* value may not be precise in describing its minimal accumulation in the lettuce.

The relationship between the RCF and log D*ow*, R^2 =0.47, is shown in 5B. It is a relatively weaker relationship compared with BCF's (R^2=0.69), so possibly other factors besides hydrophobicity appear responsible for the root API uptake (or its lack thereof). Hence, it may be more logical to relate whole plant accumulation (BCF) with hydrophobicity rather than RCF.

Chuang et al., (2019) also reported a weak relationship (R^2 = 0.293) between the RCF and log D*ow* in lettuce exposed to 13 ionizable APIs via hydroponic growth. This observation is consistent with Miller et al., (2015) who, in an extensive review on plant API uptake, concluded that no statistically significant relationship exists between LCF and hydrophobicity. It may be plausible to extend this inference to RCF.

Regarding TF, 5C shows that no relationship existed between log D*ow* and TF (R^2 = 0.017). This absence of a relationship is consistent with Chuang et al., (2019) and Li et al., (2019). Their studies established that no statistically significant relationship between hydrophobicity and TF for APIs with log D*ow* between -3 and 4.

3.4 Speciation of ARVD compounds and influence on plant uptake

The cell walls of plant root hairs are negatively-charged. The root cell wall is approximately 0.4 µm thick and composed of polysaccharides which naturally reduce the cell wall permeability to solutes. As a result, uptake of anionic molecules is constrained by the electrostatic repulsion by the root hair cells (Trapp, 2000; Miller et al., 2015; Christou et al., 2019). Physiologically the cell vacuole is larger than the cytoplasm, occupying up to 95 % of a plant cell's volume t It has a pH of 5.5 compared to pH 7 of the cytoplasm. Nonetheless, it is the cytoplasm that is in contact with the root cell wall (Trapp, 2000; Goldstein et al., 2014) and transport of molecules is predominantly via the symplastic pathway (through the cell's cytoplasm) rather than the apoplastic pathway (Goldstein et al., 2014; Pan et al., 2014; Prosser et al., 2014; Al-Farsi et al., 2017).

OSV was the only ARVD fully ionized at the pH of the growing medium (pH 6.5), being 99.8 % in the cationic form (**Error! Reference source not found.**). OSV was thus potentially electrostatically attracted to the negatively charged root hairs. In contrast, LVD, NVP, and EFV were predominantly neutral at the exposure pH. Strong retention due to sorption, however, could have impeded the permeation of the highly cationic OSV further into the roots. One may relate the characteristics of OSV to the antibiotic trimethoprim (TMP). At pH 5.8 (approximately 1 pH unit lower than this study pH), TMP was chiefly cationic (95%) and with a log D*ow* of -0.43 In Chuang et al. (2019). Lettuce was exposed to 50 µg L^{-1} of TMP in a hydroponic-based experiment. The concentration of TMP in the leaf in was < 25 % of the API's total accumulation, which is similar to the OSV leaf accumulation measured (< 20 %) in this study (3B). TMP also exhibited a TF value of 0.1, demonstrating its limited ability to be translocated to the above root tissues. Referring to 4, OSV had a comparable TF of approximately 0.1.

In contrast to OSV, NVP (log D*ow* 2.48, neutral) had the highest TF of the four ARVDs, being > 1. NVP lies in the region of moderate hydrophobicity (1 < log D*ow* < 3). Organic molecules

lying within this hydrophobic window region exhibit the highest predisposition to be transported to above root tissues (Tanoue et al., 2012; Kumar & Gupta, 2016; Li et al., 2019). Kumar & Gupta (2016) illustrated that a sigmoidal relationship exists between the transpiration stream factor (the ratio of the amount of the contaminant in the xylem to exposure medium) and log K*ow* whose maxima lies in the range between log K*ow* 2-2.5, as shown in 6. This phenomenon thus provides the most definitive account in describing the measured NVP's high TF value that certainly originated from enhanced mobility from the transpiration stream.

This observation is consistent with Chuang et al. (2019), who examined carbamazepine (CBZ) uptake in lettuce under hydroponic conditions. CBZ which has comparable characteristics to NVP (i.e., log D*ow* 2.5 and neutral charge), displayed a TF pattern that was analogous to NVP, i.e. it had the highest TF of the 13 APIs studied. González García et al. (2018) reported that the concentration of CBZ was 82 % higher in leaves compared to roots in three varieties of lettuce irrigated with CBZ-spiked treated wastewater. Similarly, Shenker et al. (2011) found that 76-84 % of CBZ accumulated in the leaf compartment of cucumber plants compared to roots in a hydroponic and pot trial experiment.

EFV showed the greatest whole plant bioaccumulation in lettuce in comparison with the other ARVDs (Figure 3B). However, > 95 % of its mass was retained in the roots, consequently presenting the highest RCF value. Characteristically, EFV was unionized and remained neutral at the experimental pH. Organic compounds with log D*ow* > 4.5 hardly experience any significant translocation to above-ground tissues (Kumar & Gupta, 2016)**.** However, for a better comprehension of the high accumulation tendencies of EFV on the roots, it is vital to carry out root EFV sorption experiments. Sorption tests enable the quantification of the fraction of EFV that adsorbed onto the root surface and the actual fraction that permeates into the bulk of the roots. Boxall et al., (2006) and Miller et al., (2015) for example, reported on a significant amount of APIs being detected on the exterior of root crops following analyses of the peels rather than the core of the plant. For this study, supposing that the bulk of the EFV permeated into the root, it then implies that the API did not migrate to reach the vascular tissues (i.e., the phloem and xylem). According to Collins et al., (2006), neutral non-ionizable organic compounds with log Kow (> 4), are primarily retained by the lipid cell components in the endodermis and do not reach the vascular tissues for subsequent transport to above root tissues. As EFV was in its neutral form and its log D*ow* = log K*ow* = 4.3 (**Error! Reference source not found.**), it therefore implied that it was highly unlikely that it partitioned into the vascular tissue and was largely retained at the endodermis. This interaction between EFV and the plant root is analogous to diclofenac (DCF). DCF, a non-steroidal anti-inflammatory drug (NSAID) with a log D*ow* of 4.5 is similar to EFV but differs in terms of p*Ka*. González García et al., (2018) measured and found that 89 % of DCF was retained on lettuce roots. Likewise,

Zhang et al., (2012) reported higher root accumulation factors (0.40-1.36 mL g^{-1}) of DCF in the roots of the macrophyte, *Scirpus validus,* compared with the shoot accumulation (0.17–0.51 mL g^{-1}). These two studies further affirm that the roots retain highly hydrophobic APIs (log D*ow* > 4).

LVD exhibited the lowest bioaccumulation in lettuce (3B). LVD also had the lowest octanol-water partition level coefficient (-1.1) before pH correction. As with OSV, minimal accumulation of LVD could be attributed to its hydrophilic nature. Low hydrophobicity i imply minimal permeation into the lipophilic root cell membranes. LVD was primarily uncharged at the test pH. LVD uptake can be compared with caffeine, which is highly soluble in water and has a log K*ow* value of -0.77. Accumulation of caffeine in cucumber leaves was observed to be lower than in the root (Goldstein et al., 2014). In contrast, Chuang et al. (2019), reported a TF value of > 1 for caffeine in lettuce. These two contrasting degrees on uptake highlight the need for more in-depth investigations into the uptake of LVD and other similar hydrophilic molecules into vascular plant tissues.

3.6 Impact of ARVD exposure on plant physiology

Accumulation of APIs in plants may induce toxicity as (at high concentration) or hormesis (at lower concentration levels) (Christou et al., 2018). Hormesis is a positive, non-distress effect experienced by a plant when exposed to small doses of xenobiotics, characterized by a non-linear dose-response relationship (Agathokleous et al., 2018). Hormesis may be characterized by an increase in the length of roots and number or size of leaves. Toxicity, on the other hand, presents as perturbations in plant growth, e.g. lowered germination rates, chlorosis, tissue deformation, reduced length or mass of the root and shoot. Also, reduced reproduction rate, and enzymatic activity (Liu et al., 2009; Hillis et al., 2011; Furtula et al., 2012; Liu et al., 2013; Bártíková et al., 2016a; Christou et al., 2018, Sun et al., 2018). The current study focussed on the visible physiological effects specifically on the measurable mass of the leaf and root.

7 shows the root and leaf mean wet weight (n=6) across the four concentration exposure levels (including the control, i.e. unspiked nutrient solution). The mean biomass (root and leaf mass) of the control sample differed (Dunnett's T3 test, p=0.039) with the 100 µg L^{-1} exposed sample. Likewise, the 1 and 10 µg L^{-1} treatment contrasted with the 100 µg L^{-1} treated sample (Dunnett's T3, p=0.012 and p=0.07 respectively). The mean root and leaf mass of the control was 1.80 ± 0.26 g (± 1 SD) and 3.10 ± 0.34 g respectively, while for the 100 µg L^{-1} exposed lettuce was 1.25±0.32 g and 2.02±0.53 g respectively, representing a mean 34 % reduction in mass.

The significant difference in tissue mass between the control sample and the ARVD exposed samples was likely an indicator of potential physiological effect induced by the ARVDs. Hillis et al. (2011), reported that root elongation of lettuce was inhibited when exposed to a mixture of 10 antibiotics at concentrations of 1, 10, 100, 1000 and 10000 µg L^{-1}. Wei et al., (2009) indicated that tetracycline applied at 1, 10 and 100 mg kg^{-1} impacted the mass of the root of ryegrass inducing a 40 % decline in weight. In the same study, shoot (leaf) inhibition was less impacted compared to the root. The physiological impacts in Wei et al. (2009) however differ with this study, in that most significant biomass variation occurred in the shoots (leaves) rather than with the roots.

Contrasting the phytotoxic effect observed in the 100 µg L^{-1} treatment, a possible hormetic influence was exerted on the 1 µg L^{-1} and 10 µg L^{-1} treated samples. An inspection of 7 shows that the two sets of treated lettuce samples had a relatively higher mean biomasses (though not significantly different, p=0.70 and p=0.96 respectively) of 18 % and 8 % respectively compared with the control. A comparable response was exhibited by *Phragmites australis* (common wetland plant) when exposed to a mixture of 3 antibiotics, ciprofloxacin, oxytetracycline and sulfamethazine in the 0.1 -1000 µg L^{-1} concentration range (Liu et al., 2013). Hormetic response (on root activity) was evident at the lower exposure level (0.1 - 10 µg L^{-1}) and not in the > 100 µg L^{-1} treatment range. Root activity in the 0.1 - 10 µg L^{-1} exposed plants displayed a negative rate of inhibition. In contrast, toxicity was dominant on the 100 and 1000 µg L^{-1} exposed plants, depicting a positive inhibition of root activity at 18 % and 36 % compared to the control.

Conclusion

This study provides an optimized protocol for determining ARVDs in biological matrices. It also provides evidence of ARVDs uptake in plants. Moreover, it shows that ARVDs interaction with plant roots can be related to other APIs of similar physical-chemical characteristics. Uptake is primarily influenced by molecules' hydrophobicity of the ARVDs, however, along the confines sigmoidal relationship. At low and high concentration levels, a mixture of ARVDs induces both hormetic and toxic effects to plants.

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Conflict of interest declaration

The authors declare NO conflicts of interests associated with any of this work.

4 Reference

Agathokleous, E., Kitao, M., Calabrese, E.J., 2018. Human and veterinary antibiotics induce hormesis in plants: Scientific and regulatory issues and an environmental perspective. Environment International 120, 489–495. https://doi.org/10.1016/j.envint.2018.08.035

Ahmed, M.B.M., Rajapaksha, A.U., Lim, J.E., Vu, N.T., Kim, I.S., Kang, H.M., Lee, S.S., Ok, Y.S., 2015. Distribution and accumulative pattern of tetracyclines and sulfonamides in edible vegetables of cucumber, tomato, and lettuce. Journal of Agricultural and Food Chemistry 63, 398–405. https://doi.org/10.1021/jf5034637

Al-Farsi, R.S., Ahmed, M., Al-Busaidi, A., Choudri, B.S., 2017. Translocation of pharmaceuticals and personal care products (PPCPs) into plant tissues: A review. Emerging Contaminants 3, 132–137. https://doi.org/10.1016/j.emcon.2018.02.001

Albero, B., Tadeo, J.L., Del Mar Delgado, M., Miguel, E., Pérez, R.A., 2019. Analysis of multiclass antibiotics in lettuce by liquid chromatography-tandem mass spectrometry to monitor their plant uptake. Molecules 24. https://doi.org/10.3390/molecules24224066

Amos Sibeko, P., Naicker, D., Mdluli, P.S., Madikizela, L.M., 2019. Naproxen, ibuprofen, and diclofenac residues in river water, sediments and Eichhornia crassipes of Mbokodweni river in South Africa: An initial screening. Environmental Forensics 20, 129–138. https://doi.org/10.1080/15275922.2019.1597780

Bagheri, M., Al-jabery, K., Wunsch, D.C., Burken, J.G., 2019. A deeper look at plant uptake of environmental contaminants using intelligent approaches. Science of the Total Environment 651, 561–569. https://doi.org/10.1016/j.scitotenv.2018.09.048

Barreales-Suárez, S., Callejón-Mochón, M., Azoulay, S., Bello-López, M.Á., Fernández-Torres, R., 2018. Liquid chromatography quadrupole time-of-flight mass spectrometry determination of six pharmaceuticals in vegetal biota. Uptake study in Lavandula dentata. Science of the Total Environment 622–623, 655–663. https://doi.org/10.1016/j.scitotenv.2017.11.244

Bártíková, H., Podlipná, R., Skálová, L., 2016a. Veterinary drugs in the environment and their toxicity to plants. Chemosphere 144, 2290–2301. https://doi.org/10.1016/j.chemosphere.2015.10.137

Bártíková, H., Podlipná, R., Skálová, L., 2016b. Veterinary drugs in the environment and their toxicity to plants. Chemosphere 144, 2290–2301. https://doi.org/10.1016/J.CHEMOSPHERE.2015.10.137

Ben Mordechay, E., Tarchitzky, J., Chen, Y., Shenker, M., Chefetz, B., 2018. Composted biosolids and treated wastewater as sources of pharmaceuticals and personal care products for plant uptake: A case study with carbamazepine. Environmental Pollution 232, 164–172. https://doi.org/10.1016/j.envpol.2017.09.029

Boxall, A.B.A., Johnson, P., Smith, E.J., Sinclair, C.J., Edward Stutt, A., Levy, L.S., 2006. Uptake of Veterinary Medicines from Soils into Plants. https://doi.org/10.1021/JF053041T

Briggs, G.G., Bromilow, R.H., Evans, A.A., 1982. Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. Pesticide Science 13, 495–504. https://doi.org/10.1002/ps.2780130506

Burken, J.G., Schnoor, J.L., 1998. Predictive relationships for uptake of organic contaminants by hybrid poplar trees. Environmental Science and Technology 32, 3379–3385. https://doi.org/10.1021/es9706817

Carter, L.J., Williams, M., Martin, S., Kamaludeen, S.P.B., Kookana, R.S., 2018. Sorption, plant uptake and metabolism of benzodiazepines. Science of the Total Environment 628–629, 18–25. https://doi.org/10.1016/j.scitotenv.2018.01.337

Christou, A., Michael, C., Fatta-Kassinos, D., Fotopoulos, V., 2018. Can the pharmaceutically active compounds released in agroecosystems be considered as emerging plant stressors? Environment International 114, 360–364. https://doi.org/10.1016/j.envint.2018.03.003

Christou, A., Papadavid, G., Dalias, P., Fotopoulos, V., 2019. Ranking of crop plants according to their potential to uptake and accumulate contaminants of emerging concern 170, 422–432. https://doi.org/10.1016/j.envres.2018.12.048

Chuang, Y.H., Liu, C.H., Sallach, J.B., Hammerschmidt, R., Zhang, W., Boyd, S.A., Li, H., 2019. Mechanistic study on uptake and transport of pharmaceuticals in lettuce from water. Environment International 131, 104976. https://doi.org/10.1016/j.envint.2019.104976

Collins, C., Fryer, M., Grosso, A., 2006. Plant uptake of non-ionic organic chemicals. Environmental Science and Technology 40, 45–52. https://doi.org/10.1021/es0508166
De Clercq, E., Li, G., 2016. Approved antiviral drugs over the past 50 years. Clinical Microbiology Reviews 29, 695–747. https://doi.org/10.1128/CMR.00102-15
De Voogt, P., Janex-Habibi, M.L., Sacher, F., Puijker, L., Mons, M., 2009. Development of a common priority list of pharmaceuticals relevant for the water cycle. Water Science and Technology 59, 39–46. https://doi.org/10.2166/wst.2009.764
Emhofer, L., Himmelsbach, M., Buchberger, W., Klampfl, C.W., 2018. Insights into the uptake, metabolization, and translocation of four non-steroidal anti-inflammatory drugs in cress (Lepidium sativum) by HPLC-MS2. Electrophoresis 39, 1294–1300. https://doi.org/10.1002/elps.201700438
EPA, 2012. Plant Uptake and Translocation Test -OCSPP 850:4800, Ecological effects test guidelines.
Fekadu, S., Alemayehu, E., Dewil, R., Van der Bruggen, B., 2019. Pharmaceuticals in freshwater aquatic environments: A comparison of the African and European challenge. Science of the Total Environment 654, 324–337. https://doi.org/10.1016/j.scitotenv.2018.11.072
Funke, J., Prasse, C., Ternes, T.A., 2016. Identi fi cation of transformation products of antiviral drugs formed during biological wastewater treatment and their occurrence in the urban water cycle. Water Research 98, 75–83. https://doi.org/10.1016/j.watres.2016.03.045
Furey, A., Moriarty, M., Bane, V., Kinsella, B., Lehane, M., 2013. Ion suppression; A critical review on causes, evaluation, prevention and applications. Talanta 115, 104–122. https://doi.org/10.1016/j.talanta.2013.03.048
Goldstein, M., Shenker, M., Chefetz, B., 2014. Insights into the uptake processes of wastewater-borne pharmaceuticals by vegetables. Environmental Science and Technology 48, 5593–5600. https://doi.org/10.1021/es5008615
González García, M., Fernández-López, C., Pedrero-Salcedo, F., Alarcón, J.J., 2018. Absorption of carbamazepine and diclofenac in hydroponically cultivated lettuces and human health risk assessment. Agricultural Water Management 206, 42–47. https://doi.org/10.1016/j.agwat.2018.04.018
Hillis, D.G., Fletcher, J., Solomon, K.R., Sibley, P.K., 2011. Effects of ten antibiotics on seed germination and root elongation in three plant species. Archives of Environmental Contamination and Toxicology 60, 220–232. https://doi.org/10.1007/s00244-010-9624-0
Hsu, F.C., Marxmiller, R.L., Yang, A.Y.S., 1990. Study of root uptake and xylem translocation of cinmethylin and related compounds in detopped soybean roots using a pressure chamber technique. Plant Physiology 93, 1573–1578. https://doi.org/10.1104/pp.93.4.1573
Hurtado, C., Domínguez, C., Pérez-Babace, L., Cañameras, N., Comas, J., Bayona, J.M., 2016. Estimate of uptake and translocation of emerging organic contaminants from irrigation water concentration in lettuce grown under controlled conditions. Journal of Hazardous Materials 305, 139–148. https://doi.org/10.1016/J.JHAZMAT.2015.11.039
ICH, 1995. Validation of Analytical Procedures: Text and Methodology, in: ICH Steering Comittee. p. 278.
Jain, S., Kumar, P., Vyas, R.K., Pandit, P., Dalai, A.K., 2013. Occurrence and removal of antiviral drugs in environment: A review. Water, Air, and Soil Pollution 224. https://doi.org/10.1007/s11270-012-1410-3
K'oreje, K.O., Vergeynst, L., Ombaka, D., De Wispelaere, P., Okoth, M., Van Langenhove, H., Demeestere, K., 2016. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. Chemosphere 149, 238–244. https://doi.org/10.1016/j.chemosphere.2016.01.095
Kodešová, R., Klement, A., Golovko, O., Fér, M., Nikodem, A., Kočárek, M., Grabic, R., 2019. Root uptake of atenolol, sulfamethoxazole and carbamazepine, and their transformation in three soils and four plants. Environmental Science and Pollution Research 26, 9876–9891. https://doi.org/10.1007/s11356-019-04333-9
Kruve, A., Rebane, R., Kipper, K., Oldekop, M.L., Evard, H., Herodes, K., Ravio, P., Leito, I., 2015a. Tutorial review on validation of liquid chromatography-mass spectrometry methods: Part II. Analytica Chimica Acta 870, 8–28. https://doi.org/10.1016/j.aca.2015.02.016
Kruve, A., Rebane, R., Kipper, K., Oldekop, M.L., Evard, H., Herodes, K., Ravio, P., Leito, I., 2015b. Tutorial review on validation of liquid chromatography-mass spectrometry methods: Part I. Analytica Chimica Acta 870, 29–44. https://doi.org/10.1016/j.aca.2015.02.017
Kumar, K., Gupta, S.C., 2016. A framework to predict uptake of trace organic compounds by plants. Journal of Environmental Quality 45, 555–564. https://doi.org/10.2134/jeq2015.06.0261

Ledezma, P., Kuntke, P., Buisman, C.J.N., Keller, J., Freguia, S., 2015. Source-separated urine opens golden opportunities for microbial electrochemical technologies. Trends in Biotechnology 33, 214–220. https://doi.org/10.1016/j.tibtech.2015.01.007

Li, Y., Sallach, J.B., Zhang, W., Boyd, S.A., Li, H., 2019. Insight into the distribution of pharmaceuticals in soil-water-plant systems. Water Research 152, 38–46. https://doi.org/10.1016/j.watres.2018.12.039

Liu, L., Liu, Y.H., Liu, C.X., Wang, Z., Dong, J., Zhu, G.F., Huang, X., 2013. Potential effect and accumulation of veterinary antibiotics in Phragmites australis under hydroponic conditions. Ecological Engineering 53, 138–143. https://doi.org/10.1016/j.ecoleng.2012.12.033

Madikizela, L.M., Ncube, S., Chimuka, L., 2018. Uptake of pharmaceuticals by plants grown under hydroponic conditions and natural occurring plant species: A review. Science of the Total Environment 636, 477–486. https://doi.org/10.1016/j.scitotenv.2018.04.297

Miller, E.L., Nason, S.L., Karthikeyan, K.G., Pedersen, J.A., 2015. Root Uptake of Pharmaceuticals and Personal Care Product Ingredients. https://doi.org/10.1021/acs.est.5b01546

Mlunguza, N.Y., Ncube, S., Mahlambi, P.N., Chimuka, L., Madikizela, L.M., 2020. Determination of selected antiretroviral drugs in wastewater, surface water and aquatic plants using hollow fibre liquid phase microextraction and liquid chromatography - tandem mass spectrometry. Journal of Hazardous Materials 382, 121067. https://doi.org/10.1016/j.jhazmat.2019.121067

Nannou, C., Ofrydopoulou, A., Evgenidou, E., Heath, D., Heath, E., Lambropoulou, D., 2020. Antiviral drugs in aquatic environment and wastewater treatment plants: A review on occurrence, fate, removal and ecotoxicity. Science of the Total Environment 699, 134322. https://doi.org/10.1016/j.scitotenv.2019.134322

Ngumba, E., Gachanja, A., Nyirenda, J., Maldonado, J., Tuhkanen, T., 2020. Occurrence of antibiotics and antiretroviral drugs in source-separated urine, groundwater, surface water and wastewater in the peri-urban area of chunga in lusaka, Zambia. Water SA 46, 278–284. https://doi.org/10.17159/wsa/2020.v46.i2.8243

Ngumba, E., Gachanja, A., Tuhkanen, T., 2016. Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. Science of the Total Environment 539, 206–213. https://doi.org/10.1016/j.scitotenv.2015.08.139

Pan, M., Wong, C.K.C., Chu, L.M., 2014. Distribution of antibiotics in wastewater-irrigated soils and their accumulation in vegetable crops in the Pearl River Delta, Southern China. Journal of Agricultural and Food Chemistry 62. https://doi.org/10.1021/jf503850v

Paz, A., Tadmor, G., Malchi, T., Blotevogel, J., Borch, T., Polubesova, T., Chefetz, B., 2016. Fate of carbamazepine, its metabolites, and lamotrigine in soils irrigated with reclaimed wastewater: Sorption, leaching and plant uptake. Chemosphere 160, 22–29. https://doi.org/10.1016/j.chemosphere.2016.06.048

Prosser, R.S., Lissemore, L., Topp, E., Sibley, P.K., 2014a. Bioaccumulation of triclosan and triclocarban in plants grown in soils amended with municipal dewatered biosolids. Environmental Toxicology and Chemistry 33, 975–984. https://doi.org/10.1002/etc.2505

Prosser, R.S., Trapp, S., Sibley, P.K., 2014b. Modeling Uptake of Selected Pharmaceuticals and Personal Care Products into Food Crops from Biosolids-Amended Soil. Environmental Science & Technology 48, 11397–11404. https://doi.org/10.1021/es503067v

Rede, D., Santos, L.H.M.L.M., Ramos, S., Oliva-Teles, F., Antão, C., Sousa, S.R., Delerue-Matos, C., 2019. Individual and mixture toxicity evaluation of three pharmaceuticals to the germination and growth of Lactuca sativa seeds. Science of the Total Environment 673, 102–109. https://doi.org/10.1016/j.scitotenv.2019.03.432

Ros, O., Vallejo, A., Olivares, M., Etxebarria, N., Prieto, A., 2016. Determination of endocrine disrupting compounds in fish liver, brain, and muscle using focused ultrasound solid–liquid extraction and dispersive solid phase extraction as clean-up strategy. Analytical and Bioanalytical Chemistry 408, 5689–5700. https://doi.org/10.1007/s00216-016-9697-3

Sanderson, H., Johnson, D.J., Reitsma, T., Brain, R.A., Wilson, C.J., Solomon, K.R., 2004. Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. Regulatory Toxicology and Pharmacology 39, 158–183. https://doi.org/10.1016/J.YRTPH.2003.12.006

SANTE/11813/2017, 2018. ANALYTICAL QUALITY CONTROL AND METHOD VALIDATION PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS.

Schantz, M.M., 2006. Pressurized liquid extraction in environmental analysis. Analytical and Bioanalytical Chemistry 386, 1043–1047. https://doi.org/10.1007/s00216-006-0648-2

Shenker, M., Harush, D., Ben-Ari, J., Chefetz, B., 2011. Uptake of carbamazepine by cucumber plants - A case study related to irrigation with reclaimed wastewater. Chemosphere 82, 905–910. https://doi.org/10.1016/j.chemosphere.2010.10.052

Stacia R, W.-U., Divina A, N., Lisa, Z., Diana S, A., 2014. Assessing antibiotic sorption in soil: A literature review and new case studies on sulfonamides and macrolides. Chemistry Central Journal. https://doi.org/10.1186/1752-153X-8-5
Sui, Q., Wang, B., Zhao, W., Huang, J., Yu, G., Deng, S., Qiu, Z., Lu, S., 2012. Identification of priority pharmaceuticals in the water environment of China. Chemosphere 89, 280–286. https://doi.org/10.1016/j.chemosphere.2012.04.037
Sun, C., Dudley, S., Trumble, J., Gan, J., 2018. Pharmaceutical and personal care products-induced stress symptoms and detoxification mechanisms in cucumber plants. Environmental Pollution 234, 39–47. https://doi.org/10.1016/j.envpol.2017.11.041
Tadić, Đ., Matamoros, V., Bayona, J.M., 2019. Simultaneous determination of multiclass antibiotics and their metabolites in four types of field-grown vegetables. Analytical and Bioanalytical Chemistry 411, 5209–5222. https://doi.org/10.1007/s00216-019-01895-y
Tanoue, R., Sato, Y., Motoyama, M., Nakagawa, S., Shinohara, R., Nomiyama, K., 2012. Plant Uptake of Pharmaceutical Chemicals Detected in Recycled Organic Manure and Reclaimed Wastewater. Journal of Agricultural and Food Chemistry 60, 10203–10211. https://doi.org/10.1021/jf303142t
Thompson, M., Ellison, S.L.R., Fajgelj, A., Willetts, P., Wood, R., 1999. Harmonised guidelines for the use of recovery information in analytical measurement (Technical Report), Pure and Applied Chemistry. https://doi.org/10.1351/pac199971020337
Tian, R., Zhang, R., Uddin, M., Qiao, X., Chen, J., Gu, G., 2019. Uptake and metabolism of clarithromycin and sulfadiazine in lettuce. Environmental Pollution 247, 1134–1142. https://doi.org/10.1016/j.envpol.2019.02.009
Trapp, S., 2000. Modelling uptake into roots and subsequent translocation of neutral and ionisable organic compounds. Pest Management Science 56, 767–778. https://doi.org/10.1002/1526-4998(200009)56:9<767::AID-PS198>3.0.CO;2-Q
Wei, X., Wu, S.C., Nie, X.P., Yediler, A., Wong, M.H., 2009. The effects of residual tetracycline on soil enzymatic activities and plant growth. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes 44, 461–471. https://doi.org/10.1080/03601230902935139
Wu, X., Conkle, J.L., Gan, J., 2012. Multi-residue determination of pharmaceutical and personal care products in vegetables. Journal of Chromatography A 1254, 78–86. https://doi.org/10.1016/j.chroma.2012.07.041
Wu, X., Dodgen, L.K., Conkle, J.L., Gan, J., 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review. Science of The Total Environment 536, 655–666. https://doi.org/10.1016/J.SCITOTENV.2015.07.129
Zhang, D.Q., Hua, T., Gersberg, R.M., Zhu, J., Ng, W.J., Tan, S.K., 2012. Fate of diclofenac in wetland mesocosms planted with Scirpus validus. Ecological Engineering 49, 59–64. https://doi.org/10.1016/j.ecoleng.2012.08.018

Highlights

- Antiretroviral accumulation up to 3463 ng g^{-1}
- Highest accumulation by hydrophobic compounds
- Toxic effects in plants exhibited by reduced biomass