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Uptake, accumulation and impact of antiretroviral and antiviral pharmaceutical compounds in lettuce

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1	THIS IS AN ACCEPTED PAPER FOR SCIENCE OF THE TOTAL ENVIRONMENT THAT
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5	Uptake, Accumulation and Impact of Antiretroviral and Antiviral Pharmaceutical
6	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

43 **1** Introduction

Plant uptake of active pharmaceutical ingredient (API) molecules has been reported in food 44 and non-food crops. Exposure to APIs can either be under a controlled environment 45 (hydroponic and pot trial experiments) (e.g. Goldstein et al., 2014; Ahmed et al., 2015; Hurtado 46 47 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 48 biosolids (e.g. Prosser et al., 2014; Paz et al., 2016; Ben et al., 2018; Carter et al., 2018; 49 Bagheri et al., 2019). Uptake studies, however, have primarily focussed on antibiotics, 50 51 antidepressants, analgesics, anti-epileptics, hormones and non-steroidal anti-inflammatory 52 therapeutic based drugs. Past studies and reviews (Navarro et al., 2014; Wegst-Uhrich., 2014; 53 Wu et al., 2015; Bártíková et al., 2016a; Zhang et al., 2017; Madikizela et al., 2018; Christou 54 et al., 2019) reveal that there is limited information on an equally significant therapeutic class 55 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 56 deficiency, recommending plant ARVD uptake studies. 57

The environmental occurrence of ARVDs is ostensibly less frequent and manifests at trace 58 59 concentration levels in high and upper-middle-income countries and hence of minimal concern 60 (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 61 of uptake studies are undertaken in these countries. The consumption of ARVDs and the 62 63 extent of their contamination in the Sub-Saharan Africa region (particularly Kenya and South 64 Africa) compared with Europe is detailed by Fekadu et al., (2019) and Nannou et al., (2020) 65 in fresh surface waters and wastewaters respectively. Recent antiretroviral data from Lusaka, Zambia, report on the occurrence of three antiretrovirals, lamivudine nevirapine and 66 zidovudine in the aquatic environment at concentrations in the range of 60 - 118,970 ng L⁻¹ 67 (Ngumba et al., 2020). The three compounds were also detected in source-separated urine 68 at concentrations in the range $7740 - 12800 \ \mu g \ L^{-1}$, which is several magnitudes higher than 69 70 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 71 72 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

78 ARVDs were highly potent. In a class of 50 therapeutic APIs, ARVDs were predicted to be 79 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 80 development processes (Hillis et al., 2011; Liu et al., 2013; Christou et al., 2018; Sun et al., 81 2018; Rede et al., 2019). For example, Sun et al., (2018) exposed cucumber to a mixture of 82 17 APIs at concentrations of up to 1000 μ g L⁻¹ and found that the leaves exhibited burn-like 83 features. At present, there is a limited understanding of potential ARVD effects on plants. A 84 review of the impact of APIs on plants presented by Bártíková et al. (2016). It outlines the toxic 85 effects brought by antibiotics, anti-parasitic drugs, hormones, growth promoters and 86 antifungals on plants but contains hardly any information on the impact of ARVDs on plants. 87

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 95 experimental approach is necessary to elucidate plant root-ARVD interactions 96 comprehensively. In this hydroponic study, the uptake of three antiretroviral APIs (nevirapine, 97 lamivudine and efavirenz) and one anti-influenza antiviral API (oseltamivir) into lettuce 98 (Lactuca sativa) was investigated. Selection of the APIs was based on their frequency of 99 100 detection from previous (Ngumba et al., 2016; K'oreje et al., 2016) studies. The advantage of 101 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 102 studies. The major drawback, however, is that hydroponic studies do not provide the 103 complexity of a real agroecosystem environment (Wu et al., 2015). Leafy vegetables 104 accumulate organic contaminants greatest in comparison to root vegetables, cereals and fruit 105 106 vegetables (Christou et al., 2019), hence the selection of lettuce. Uptake can be described 107 either as a passive or active process (Kumar and Gupta, 2016). This study focussed on active 108 processes (solute specific), i.e. it solely concentrated on the characteristic of the molecule 109 rather than the existing environmental conditions.

110 2 Materials and methods

111 2.1 Reagents

112 Methanol (MeOH), acetonitrile (MeCN), HPLC water and 0.1 % formic acid in water were 113 obtained from Fisher Scientific, UK, and ammonium hydroxide (NH₄OH) solution (25% v/v) 114 and formic acid from Sigma Aldrich, UK. Nevirapine (NVP) and lamivudine (LVD) standards 115 were obtained from Sigma Aldrich, UK, efavirenz (EFV) from Tokyo Chemical Industries, and 116 oseltamivir (OSV) from Fisher Scientific, UK. All antiviral API standards had a purity grade of 117 > 99 %.

118 2.2 Standards and stock solutions

119 Preparation and storage of stock solutions and standards followed the European Commission,

120 SANTE/11813/2017 (2018) guidelines. LVD and OSV stock solutions were prepared in 50 %

121 (v/v) MeOH: HPLC water solution. NVP and EFV stock solutions were made in 100 % MeOH.

122 Dilutions of the four ARVD mix were prepared at concentrations of 0.1 to 100 μ g L⁻¹.

123 2.3 Liquid Chromatography

124 Analyte separation was performed by liquid chromatography (Dionex Ultimate 3000, Thermo 125 Scientific) on a PhenylHexyl (*Ace ultracore*) (2.5 μ m × 100 mm) stationary phase. The column 126 temperature was isocratic at 50 °C. The analytical run time was 9.8 min, starting from a mobile 127 phase composition of 100 % 0.1% ammonia (in HPLC water) and 0.1 % formic acid solution 128 (1:1, v/v), adjusted to pH 8.4, before transitioning to 100 % MeOH. The equilibration time was 129 2 minutes and at a flow rate of 500 μ L min⁻¹.

130 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 $^{\circ}$ 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 $^{\circ}$ 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.

137 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

141 (Error! Reference source not found.), it was necessary to determine the optimal extraction-142 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).

146 **2.5.1 Extraction pH and recoveries**

Fresh lettuce was obtained from a local grocer. The leaves were washed with high purity water 147 (HPW), chopped and freeze-dried. The freeze-dried sample was then ground to powder. 148 149 Recovery experiment was performed at two spiking levels, 10 ng g⁻¹ and 50 ng g⁻¹. Ground 150 lettuce (0.2 g) was placed in a 50 mL polypropylene centrifuge tube and spiked with the four 151 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, 152 v/v) was added to the sample, and the tube placed in an ultrasonic bath operated at 50 Hz for 153 15 min. The resulting supernatant was collected and centrifuged at 4000 rpm for 5 min. The 154 sample residue was re-extracted and the supernatants combined before solvent reduction to 155 ca 500 µL under a gentle stream of nitrogen gas. The reduced extract was diluted with 20 mL 156 HPW water and then filtered (GF/F, 0.7 µm). The optimal extraction pH was obtained by 157 158 extracting set of samples (n = 3) at four pH values: pH 2.4 (adjusted using 1 % (v/v) formic 159 acid), the native sample pH 5.4; pH 7.4 and 9.4 (1 M NH₄OH solution adjusted).

160 **2.5.2 Solid-phase extraction**

161 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 (2.5 162 mL aliquots). The 20 mL sample extract was loaded onto the cartridge at a rate of 2 mL min⁻ 163 ¹. After loading, the sorbent was dried and washed with 1 mL of HPLC water. Aliquots (2 x 2.5 164 mL) of the extracting solvent mix were used for sample elution. The 5 mL extract was 165 166 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°C in the 167 dark before analysis. 168

169 **2.5.3** Linear range, MLoD, MLoQ and Matrix effect

The LC-HRMS was calibrated at the $0.1 - 100 \ \mu g \ L^{-1}$ concentration range using matrixmatched (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).

178

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

1

179

180 **2.6 Hydroponic experiments**

The hydroponic system was housed in a greenhouse. The experimental approach was 181 182 adapted from the OCSPPC 850.4800 Plant Uptake and Translocation Test guidelines (EPA, 183 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-184 fertilizer solution for seven days to nurture and acclimatize the roots to a water-only 185 environment. Individual seedlings were each exposed to 400 mL of the four ARVD mix 186 standard nutrient solution, composed of water and commercial fertilizer (Flora Gro, NPK 3:1:6 187 at a concentration of 0.5 mL L⁻¹). The set up consisted of 6 replicates per exposure 188 189 concentration. In total, there were 24 lettuce plant samples. The sample containers (glass) 190 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. 191 192 The experiment was run for 21 days with the exposure solution renewed on days 7 and 14. 193 Loss of water due to evaporation and evapotranspiration during the growing period did not 194 exceed 20 % of the initial volume of the nutrient solution placed at the beginning of each 195 experiment.

196 **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.

208	$BCF(mL/g) = C_{plant}/C_{exposure solution}$	
209	· · · · · · · · · · · · · · · · · · ·	(2)
210	$RCF(mL/g) = C_{root}/C_{exposure solution}$	
211		(3)
212	$LCF(mL/g) = C_{leaf}/C_{exposure solution}$	
213		(4)

214
$$TF(L/g) = C_{leaf}/C_{root}$$
(5)

(5)

216 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., 217 218 2018).

219 2.8 **Data analyses**

Data were analyzed using Ms Excel 2016 and IBM SPSS Statistic v 24 software. One-way 220 221 analysis of variance (ANOVA) and Dunnett's T3 test was used to measure statistical differences between means at the 95 % confidence interval. 222

223 3 **Results and discussion**

224 3.1 Method optimization

3.1.1 225 Recoveries and effect of pH

The objective of this experiment was to select the optimal extraction pH for the target analytes. 226 227 Since the recovery of an analyte is concentration-dependent; while performing recovery 228 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 229 nanogram level in the environment, hence 10 ng g⁻¹ and 50 ng g⁻¹ level were selected as they 230 231 were considered critical spike levels for the recovery test. The extraction recoveries were 232 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 233 cumulatively evaluated across the two spike levels. Mean ARVD recoveries at the two spike 234 levels did not vary significantly (p=0.55), being 68 ±22 % (± SD) and 65±19 % for the 10 ng g⁻ 235 ¹ and 50 ng g⁻¹ level respectively. Individually, NVP exhibited the highest recoveries (mean 88 236 237 %). 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 238 EFV recoveries. OSV recoveries were relatively consistent across three (pHs 2.0-7.4) with 239 only a minimal drop at highest pH. Contrastingly, LVD recoveries varied widely, which 240 241 ultimately contributed to its low mean recoveries.

243 Cumulatively, the highest ARVD extraction recoveries were obtained at pH 5.4 (mean of 72 244 %). 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 245 environment exhibited recoveries of < 65 %. Referring to EU SANTE/11813/2017 guidelines, 246 it states that satisfactory extraction recoveries may vary between 70-120 %. Nonetheless, it 247 clarifies that reproducible recoveries of 30-70 % are also acceptable. For this reason, pH 5.4 248 was selected as the optimal pH for subsequent extraction procedures. It is noteworthy to 249 mention that pH 5.4 was also the natural extraction pH, so working at this pH meant that 250 subsequent buffer additions were unnecessary. 251

252

253 3.1.2 Linearity, MLoD and MLoQ

254 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 255 256 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 257 reason, linearity was assessed using analyte in the matrix. Error! Reference source not 258 **found.** shows that analyte in matrix linearity was satisfactory at $r^2 > 0.990$. Likewise, the limit 259 of detection is also matrix-dependent (Kruve et al., 2015b); accordingly, matrix-spiked 260 samples were used in the determination of MLoD and MLoQ. The MLoD of the ARVD analytes 261 varied from 1.51 to 5.61 ng g⁻¹ and the MLoQ from 5.13 to 18.7 ng g⁻¹ (Error! Reference 262 source not found.). 263

264 3.1.3 Matrix effects (ME)

Ultrasonic extraction mechanically breaks down the sample matrix allowing the release of the 265 target analytes (Schantz, 2006; Tadić et al., 2019). However, a drawback is its lack of 266 selectivity necessitating intensive subsequent clean-up processes (Ros et al., 2016). For this 267 268 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 269 exhibited the lowest ion suppression at \leq 30 %. NVP, OSV, and EFV, on the other hand, 270 showed ion suppression ranging from 46–50 %. Classification of the ME was made according 271 272 to Barreales-Suárez et al., (2018), i.e. ME of < 20 % considered as low, ME 20 - 40 % as a 273 medium, ME 40-60 % as high and ME as 60 % as very high. In general, ME lay in the medium 274 to high-level region, indicating significant signal suppression. Varying levels of ME following 275 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 276 of 30 to 60 % was reported in the analyses of 7 antibiotics in lettuce (Albero et al., 2019). 277

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.

282 **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 μ g 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 μ g 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 μ g L⁻ ¹ exposed samples.

296 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 297 observation is consistent with Al-Farsi et al. (2017) and González García et al. (2018). They, 298 in a separate hydroponic API exposure experiments, reported that the magnitude of 299 accumulation was directly associated with the concentration of the pharmaceutical in exposure 300 solution. In the current study, the mean plant concentration factor (CF) rise between the 1 µg 301 L¹ and 10 μ g L¹ treatment was 17.7 ± 9.32 (± 1 SD), while the CF between the 10 μ g L¹ and 302 100 μ g L⁻¹ treatment was 11.7 ± 5.7 (± 1 SD). Cumulatively, the mean increased CF in the 303 304 plant matrix between the lowest (1 μ g L⁻¹) and highest exposure solution treatment (100 μ g L⁻¹) 305 ¹) was 189.8 ± 87.5 (± 1 SD).

The RCF, BCF and TF values discussed herein are the means across the 10 μ g 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 μ g L⁻¹ and 100 μ g 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 $(0.044 > 0.025 > 0.016 > 0.005 \text{ mL g}^{-1})$.

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.

323 3.3 pH - adjusted octanol-water partitioning coefficient (Log Dow) influence on 324 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 octanolwater 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.

332 Characterization of the relationship between log Dow and uptake of the APIs was achieved by plotting log Dow against log BCF, RCF, and TF, respectively (5). For BCF, exhibiting an R² 333 334 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). 335 336 The contribution to linearity by the hydrophilic LVD and OSV (log Dow = -1.1 and -1.44, 337 respectively) however could not be decisively defined. A closer inspection revealed that had 338 the log Kow 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 339 observation further shows thatlog Dow of hydrophilic ionizable molecules (in this study OSV 340 341 and LVD), potentially may not accurately describe uptake in plants. Thus, OSV's relatively low log Dow value may not be precise in describing its minimal accumulation in the lettuce. 342

The relationship between the RCF and log Dow, $R^2 = 0.47$, 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 Dow 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 Dow 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 Dow between -3 and 4.

356 **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 357 0.4 µm thick and composed of polysaccharides which naturally reduce the cell wall 358 permeability to solutes. As a result, uptake of anionic molecules is constrained by the 359 electrostatic repulsion by the root hair cells (Trapp, 2000; Miller et al., 2015; Christou et al., 360 361 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 362 is the cytoplasm that is in contact with the root cell wall (Trapp, 2000; Goldstein et al., 2014) 363 364 and transport of molecules is predominantly via the symplastic pathway (through the cell's 365 cytoplasm) rather than the apoplastic pathway (Goldstein et al., 2014; Pan et al., 2014; 366 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 99.8 367 % in the cationic form (Error! Reference source not found.). OSV was thus potentially 368 electrostatically attracted to the negatively charged root hairs. In contrast, LVD, NVP, and EFV 369 were predominantly neutral at the exposure pH. Strong retention due to sorption, however, 370 could have impeded the permeation of the highly cationic OSV further into the roots. One may 371 372 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 Dow of -373 0.43 In Chuang et al. (2019). Lettuce was exposed to 50 µg L⁻¹ of TMP in a hydroponic-374 based experiment. The concentration of TMP in the leaf in was < 25 % of the API's total 375 376 accumulation, which is similar to the OSV leaf accumulation measured (< 20 %) in this study 377 (3B). TMP also exhibited a TF value of 0.1, demonstrating its limited ability to be translocated 378 to the above root tissues. Referring to 4, OSV had a comparable TF of approximately 0.1.

In contrast to OSV, NVP (log Dow 2.48, neutral) had the highest TF of the four ARVDs, being
> 1. NVP lies in the region of moderate hydrophobicity (1 < log Dow < 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 Kow whose maxima lies in the range between log Kow 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.

388 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 389 390 NVP (i.e., log Dow 2.5 and neutral charge), displayed a TF pattern that was analogous to NVP, 391 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 392 lettuce irrigated with CBZ-spiked treated wastewater. Similarly, Shenker et al. (2011) found 393 394 that 76-84 % of CBZ accumulated in the leaf compartment of cucumber plants compared to 395 roots in a hydroponic and pot trial experiment.

396 EFV showed the greatest whole plant bioaccumulation in lettuce in comparison with the other 397 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 398 neutral at the experimental pH. Organic compounds with log Dow > 4.5 hardly experience any 399 400 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 401 root EFV sorption experiments. Sorption tests enable the quantification of the fraction of EFV 402 403 that adsorbed onto the root surface and the actual fraction that permeates into the bulk of the 404 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 405 rather than the core of the plant. For this study, supposing that the bulk of the EFV permeated 406 407 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 408 409 compounds with log Kow (> 4), are primarily retained by the lipid cell components in the 410 endodermis and do not reach the vascular tissues for subsequent transport to above root 411 tissues. As EFV was in its neutral form and its log $Dow = \log Kow = 4.3$ (Error! Reference 412 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 413 the plant root is analogous to diclofenac (DCF). DCF, a non-steroidal anti-inflammatory drug 414 (NSAID) with a log Dow of 4.5 is similar to EFV but differs in terms of pKa. González García 415 et al., (2018) measured and found that 89 % of DCF was retained on lettuce roots. Likewise, 416

417 Zhang et al., (2012) reported higher root accumulation factors (0.40-1.36 mL g⁻¹) of DCF in 418 the roots of the macrophyte, *Scirpus validus,* compared with the shoot accumulation (0.17– 419 0.51 mL g⁻¹). These two studies further affirm that the roots retain highly hydrophobic APIs 420 (log Dow > 4).

421 LVD exhibited the lowest bioaccumulation in lettuce (3B). LVD also had the lowest octanol-422 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 423 permeation into the lipophilic root cell membranes. LVD was primarily uncharged at the test 424 pH. LVD uptake can be compared with caffeine, which is highly soluble in water and has a log 425 426 Kow 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 427 of > 1 for caffeine in lettuce. These two contrasting degrees on uptake highlight the need for 428 more in-depth investigations into the uptake of LVD and other similar hydrophilic molecules 429 430 into vascular plant tissues.

431

432 **3.6** Impact of ARVD exposure on plant physiology

Accumulation of APIs in plants may induce toxicity as (at high concentration) or hormesis (at 433 lower concentration levels) (Christou et al., 2018). Hormesis is a positive, non-distress effect 434 experienced by a plant when exposed to small doses of xenobiotics, characterized by a non-435 436 linear dose-response relationship (Agathokleous et al., 2018). Hormesis may be characterized 437 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 438 439 deformation, reduced length or mass of the root and shoot. Also, reduced reproduction rate, 440 and enzymatic activity (Liu et al., 2009; Hillis et al., 2011; Furtula et al., 2012; Liu et al., 2013; 441 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. 442

7 shows the root and leaf mean wet weight (n=6) across the four concentration exposure levels 443 444 (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. 445 Likewise, the 1 and 10 μ g L⁻¹ treatment contrasted with the 100 μ g L⁻¹ treated sample 446 (Dunnett's T3, p=0.012 and p=0.07 respectively). The mean root and leaf mass of the control 447 was 1.80 ± 0.26 g (± 1 SD) and 3.10 ± 0.34 g respectively, while for the 100 μ g L⁻¹ exposed 448 lettuce was 1.25±0.32 g and 2.02±0.53 g respectively, representing a mean 34 % reduction in 449 450 mass.

452 The significant difference in tissue mass between the control sample and the ARVD exposed 453 samples was likely an indicator of potential physiological effect induced by the ARVDs. Hillis 454 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 μ g L⁻¹. Wei et al., (2009) 455 indicated that tetracycline applied at 1, 10 and 100 mg kg⁻¹ impacted the mass of the root of 456 457 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 458 with this study, in that most significant biomass variation occurred in the shoots (leaves) rather 459 460 than with the roots.

Contrasting the phytotoxic effect observed in the 100 μ g L⁻¹ treatment, a possible hormetic 461 influence was exerted on the 1 μ g L⁻¹ and 10 μ g L⁻¹ treated samples. An inspection of 7 shows 462 that the two sets of treated lettuce samples had a relatively higher mean biomasses (though 463 not significantly different, p=0.70 and p=0.96 respectively) of 18 % and 8 % respectively 464 compared with the control. A comparable response was exhibited by *Phragmites australis* 465 (common wetland plant) when exposed to a mixture of 3 antibiotics, ciprofloxacin, 466 oxytetracycline and sulfamethazine in the 0.1 -1000 µg L⁻¹ concentration range (Liu et al., 467 2013). Hormetic response (on root activity) was evident at the lower exposure level (0.1 - 10 468 469 μ g L⁻¹) and not in the > 100 μ g L⁻¹ treatment range. Root activity in the 0.1 - 10 μ g L⁻¹ exposed 470 plants displayed a negative rate of inhibition. In contrast, toxicity was dominant on the 100 and 471 1000 μ g L⁻¹ exposed plants, depicting a positive inhibition of root activity at 18 % and 36 % compared to the control. 472

473

474 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.

481

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487

488 **Conflict of interest declaration**

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

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491 4 Reference

- 492 Agathokleous, E., Kitao, M., Calabrese, E.J., 2018. Human and veterinary antibiotics induce hormesis 493 in plants: Scientific and regulatory issues and an environmental perspective. Environment 494 International 120, 489-495. https://doi.org/10.1016/j.envint.2018.08.035
- 495 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., 496 2015. Distribution and accumulative pattern of tetracyclines and sulfonamides in edible 497 vegetables of cucumber, tomato, and lettuce. Journal of Agricultural and Food Chemistry 63, 498 398-405. https://doi.org/10.1021/if5034637
- Al-Farsi, R.S., Ahmed, M., Al-Busaidi, A., Choudri, B.S., 2017. Translocation of pharmaceuticals and 499 500 personal care products (PPCPs) into plant tissues: A review. Emerging Contaminants 3, 132-501 137. https://doi.org/10.1016/j.emcon.2018.02.001
- 502 Albero, B., Tadeo, J.L., Del Mar Delgado, M., Miguel, E., Pérez, R.A., 2019. Analysis of multiclass 503 antibiotics in lettuce by liquid chromatography-tandem mass spectrometry to monitor their plant 504 uptake. Molecules 24. https://doi.org/10.3390/molecules24224066
- 505 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 506 507 South Africa: An initial screening. Environmental Forensics 20, 129–138. 508 https://doi.org/10.1080/15275922.2019.1597780
- 509 Bagheri, M., Al-jabery, K., Wunsch, D.C., Burken, J.G., 2019. A deeper look at plant uptake of 510 environmental contaminants using intelligent approaches. Science of the Total Environment 651, 561-569. https://doi.org/10.1016/j.scitotenv.2018.09.048 511
- Barreales-Suárez, S., Callejón-Mochón, M., Azoulay, S., Bello-López, M.Á., Fernández-Torres, R., 512 513 2018. Liquid chromatography quadrupole time-of-flight mass spectrometry determination of six 514 pharmaceuticals in vegetal biota. Uptake study in Lavandula dentata. Science of the Total 515 Environment 622-623, 655-663. https://doi.org/10.1016/j.scitotenv.2017.11.244
- 516 Bártíková, H., Podlipná, R., Skálová, L., 2016a. Veterinary drugs in the environment and their toxicity 517 to plants. Chemosphere 144, 2290-2301. https://doi.org/10.1016/j.chemosphere.2015.10.137
- 518 Bártíková, H., Podlipná, R., Skálová, L., 2016b. Veterinary drugs in the environment and their toxicity 519 to plants. Chemosphere 144, 2290-2301. 520
 - https://doi.org/10.1016/J.CHEMOSPHERE.2015.10.137
- 521 Ben Mordechay, E., Tarchitzky, J., Chen, Y., Shenker, M., Chefetz, B., 2018. Composted biosolids 522 and treated wastewater as sources of pharmaceuticals and personal care products for plant 523 uptake: A case study with carbamazepine. Environmental Pollution 232, 164-172. 524 https://doi.org/10.1016/j.envpol.2017.09.029
- 525 Boxall, A.B.A., Johnson, P., Smith, E.J., Sinclair, C.J., Edward Stutt, A., Levy, L.S., 2006. Uptake of 526 Veterinary Medicines from Soils into Plants. https://doi.org/10.1021/JF053041T
- 527 Briggs, G.G., Bromilow, R.H., Evans, A.A., 1982. Relationships between lipophilicity and root uptake 528 and translocation of non-ionised chemicals by barley. Pesticide Science 13, 495-504. 529 https://doi.org/10.1002/ps.2780130506
- 530 Burken, J.G., Schnoor, J.L., 1998. Predictive relationships for uptake of organic contaminants by 531 hybrid poplar trees. Environmental Science and Technology 32, 3379-3385. 532 https://doi.org/10.1021/es9706817
- 533 Carter, L.J., Williams, M., Martin, S., Kamaludeen, S.P.B., Kookana, R.S., 2018. Sorption, plant 534 uptake and metabolism of benzodiazepines. Science of the Total Environment 628-629, 18-25. 535 https://doi.org/10.1016/j.scitotenv.2018.01.337
- Christou, A., Michael, C., Fatta-Kassinos, D., Fotopoulos, V., 2018. Can the pharmaceutically active 536 537 compounds released in agroecosystems be considered as emerging plant stressors? 538 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 539 540 their potential to uptake and accumulate contaminants of emerging concern 170, 422-432. 541 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. 542 543 Mechanistic study on uptake and transport of pharmaceuticals in lettuce from water. 544 Environment International 131, 104976. https://doi.org/10.1016/j.envint.2019.104976

- 545 Collins, C., Fryer, M., Grosso, A., 2006. Plant uptake of non-ionic organic chemicals. Environmental
 546 Science and Technology 40, 45–52. https://doi.org/10.1021/es0508166
- 547 De Clercq, E., Li, G., 2016. Approved antiviral drugs over the past 50 years. Clinical Microbiology 548 Reviews 29, 695–747. https://doi.org/10.1128/CMR.00102-15
- 549 De Voogt, P., Janex-Habibi, M.L., Sacher, F., Puijker, L., Mons, M., 2009. Development of a common
 550 priority list of pharmaceuticals relevant for the water cycle. Water Science and Technology 59,
 551 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.
- 555 https://doi.org/10.1002/elps.201700438
- 556 EPA, 2012. Plant Uptake and Translocation Test -OCSPP 850:4800, Ecological effects test
 557 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
- 603 Kumar, K., Gupta, S.C., 2016. A framework to predict uptake of trace organic compounds by plants. 604 Journal of Environmental Quality 45, 555–564. https://doi.org/10.2134/jeq2015.06.0261

- 605 Ledezma, P., Kuntke, P., Buisman, C.J.N., Keller, J., Freguia, S., 2015. Source-separated urine 606 opens golden opportunities for microbial electrochemical technologies. Trends in Biotechnology 607 33, 214-220. https://doi.org/10.1016/j.tibtech.2015.01.007
- 608 Li, Y., Sallach, J.B., Zhang, W., Boyd, S.A., Li, H., 2019. Insight into the distribution of 609 pharmaceuticals in soil-water-plant systems. Water Research 152, 38-46. https://doi.org/10.1016/j.watres.2018.12.039 610
- 611 Liu, L., Liu, Y.H., Liu, C.X., Wang, Z., Dong, J., Zhu, G.F., Huang, X., 2013. Potential effect and 612 accumulation of veterinary antibiotics in Phragmites australis under hydroponic conditions. Ecological Engineering 53, 138-143. https://doi.org/10.1016/j.ecoleng.2012.12.033 613
- Madikizela, L.M., Ncube, S., Chimuka, L., 2018. Uptake of pharmaceuticals by plants grown under 614 hydroponic conditions and natural occurring plant species: A review. Science of the Total 615 Environment 636, 477-486. https://doi.org/10.1016/j.scitotenv.2018.04.297 616
- Miller, E.L., Nason, S.L., Karthikevan, K.G., Pedersen, J.A., 2015. Root Uptake of Pharmaceuticals 617 618 and Personal Care Product Ingredients. https://doi.org/10.1021/acs.est.5b01546
- 619 Mlunguza, N.Y., Ncube, S., Mahlambi, P.N., Chimuka, L., Madikizela, L.M., 2020. Determination of 620 selected antiretroviral drugs in wastewater, surface water and aquatic plants using hollow fibre 621 liquid phase microextraction and liquid chromatography - tandem mass spectrometry. Journal of 622 Hazardous Materials 382, 121067. https://doi.org/10.1016/j.jhazmat.2019.121067
- 623 Nannou, C., Ofrydopoulou, A., Evgenidou, E., Heath, D., Heath, E., Lambropoulou, D., 2020. Antiviral 624 drugs in aquatic environment and wastewater treatment plants: A review on occurrence, fate, 625 removal and ecotoxicity. Science of the Total Environment 699, 134322. 626 https://doi.org/10.1016/j.scitotenv.2019.134322
- 627 Ngumba, E., Gachanja, A., Nyirenda, J., Maldonado, J., Tuhkanen, T., 2020. Occurrence of 628 antibiotics and antiretroviral drugs in source-separated urine, groundwater, surface water and 629 wastewater in the peri-urban area of chunga in lusaka, Zambia. Water SA 46, 278-284. 630 https://doi.org/10.17159/wsa/2020.v46.i2.8243
- 631 Ngumba, E., Gachanja, A., Tuhkanen, T., 2016. Occurrence of selected antibiotics and antiretroviral 632 drugs in Nairobi River Basin, Kenya. Science of the Total Environment 539, 206-213. 633 https://doi.org/10.1016/j.scitotenv.2015.08.139
- 634 Pan, M., Wong, C.K.C., Chu, L.M., 2014. Distribution of antibiotics in wastewater-irrigated soils and 635 their accumulation in vegetable crops in the Pearl River Delta, Southern China. Journal of 636 Agricultural and Food Chemistry 62. https://doi.org/10.1021/jf503850v
- 637 Paz, A., Tadmor, G., Malchi, T., Blotevogel, J., Borch, T., Polubesova, T., Chefetz, B., 2016. Fate of 638 carbamazepine, its metabolites, and lamotrigine in soils irrigated with reclaimed wastewater: 639 Sorption, leaching and plant uptake. Chemosphere 160, 22-29. 640 https://doi.org/10.1016/j.chemosphere.2016.06.048
- 641 Prosser, R.S., Lissemore, L., Topp, E., Sibley, P.K., 2014a. Bioaccumulation of triclosan and 642 triclocarban in plants grown in soils amended with municipal dewatered biosolids. Environmental 643 Toxicology and Chemistry 33, 975-984. https://doi.org/10.1002/etc.2505
- 644 Prosser, R.S., Trapp, S., Sibley, P.K., 2014b. Modeling Uptake of Selected Pharmaceuticals and 645 Personal Care Products into Food Crops from Biosolids-Amended Soil. Environmental Science 646 & 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., 647 648 2019. Individual and mixture toxicity evaluation of three pharmaceuticals to the germination and 649 growth of Lactuca sativa seeds. Science of the Total Environment 673, 102-109. 650 https://doi.org/10.1016/j.scitotenv.2019.03.432
- Ros, O., Vallejo, A., Olivares, M., Etxebarria, N., Prieto, A., 2016. Determination of endocrine 651 652 disrupting compounds in fish liver, brain, and muscle using focused ultrasound solid-liquid 653 extraction and dispersive solid phase extraction as clean-up strategy. Analytical and 654 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 655 and prioritization of environmental risks of pharmaceuticals in surface waters. Regulatory 656 Toxicology and Pharmacology 39, 158–183. https://doi.org/10.1016/J.YRTPH.2003.12.006 657 SANTE/11813/2017, 2018. ANALYTICAL QUALITY CONTROL AND METHOD VALIDATION 658
- 659 PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS.
- 660 Schantz, M.M., 2006. Pressurized liquid extraction in environmental analysis. Analytical and Bioanalytical Chemistry 386, 1043-1047. https://doi.org/10.1007/s00216-006-0648-2 661 662 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, 905-663 664

- 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(20009)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
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706 Highlights

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