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# Sulfonamides in Tomato from Commercial Greenhouses Irrigated with Reclaimed Wastewater: Uptake, Translocation and Food Safety

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**Abstract:** The presence of antibiotics in crops is mainly caused by their irrigation with reclaimed wastewater and by the use of organic amendments of animal origin. During this work, the fate of sulfonamide antibiotics in tomato crop has been assessed in two commercial greenhouses located in Almería (Spain) irrigated with reclaimed wastewater. Samplings were made annually for two years. Sulfonamides in several parts of the plant (roots, leaves and fruits) as well as reclaimed wastewater, amendments and soils were analyzed by UHPLC-MS/MS. The results showed that sulfonamides accumulated in soils (sulfamethoxazole between 2 and 14 μg $kg^{-1}$; sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine and sulfadimethoxine in concentrations below 1 μg $kg^{-1}$) were in the reclaimed wastewater at concentrations in the ng $L^{-1}$ range. Their distribution in plants depended on the sulfonamide. The sulfonamides detected in tomato were sulfadiazine, sulfapyridine, sulfamethazole, sulfamethoxazole and sulfadimethoxine. Sulfamethoxazole was the antibiotic with highest concentration in tomato fruit, exceeding 30 μg $kg^{-1}$. All sulfonamides were below the Acceptable Daily Intake, however, further studies and legislation are needed to assure food safety.

**Keywords:** antibiotics; water; bioaccumulation; human exposure; crop pollution; soil

## 1. Introduction

Reclaimed wastewater (RWW) is commonly used worldwide for irrigation due to the lack of water, especially in arid zones, reducing the pressure on freshwater sources such as aquifers [1]. In Europe, the irrigation with wastewater is expected to increase in the Mediterranean Region due to the temperature rise provoked by climate change and the population growth [2]. For example, 3222 $mm^3$/year of RWW has been predicted to be reused by the European Union (EU) by 2025. Among the countries of the EU, Spain is the one with the highest rate of RWW reuse, with more than 1200 $mm^3$/year [3]. Approximately 71% of RWW in Spain is used for irrigation of agricultural crops. However, this volume of RWW represents only 17% of the total irrigation water [4].

In addition to become a solution for water scarcity, the use of RWW for agricultural irrigation has other noticeable advantages in the agricultural system such as the supply of nutrients and organic matter to soils and crops or reducing the need of fertilizer application [5]. Several studies have proved their fertilizer effect and their positive impact in the soil enzyme activities involving nutrient cycling and soil microbial biomass [6–8]. Hence, RWW guarantees crop yields due to its nutrients content but mainly to their availability even in drought periods [9].

However, the use of RWW in agriculture also has negative aspects. For example, at a chemical level, RWW could contain high concentrations of salts that could degrade soils over time and become a potential threat to groundwater, especially in dry climates where the irrigated water might evaporate, increasing the presence of salts [10]. RWW can contain multiple pollutants such as heavy metals or contaminants of emerging concern (CEC) in concentrations of $\mu g\ L^{-1}$ and $ng\ L^{-1}$. These CEC could be pesticides, industrial compounds, pharmaceuticals and personal care products (PPCPs), steroids or hormones that wastewater treatment plants (WWTP) are ineffective at removing completely [11,12]. Uncontrolled long-term irrigation with RWW could change the physical and chemical properties of soil and increase the contaminants accumulation in soil [13]. The exposure of crops to CECs could have a negative effect on crop growth and their uptake and accumulation in the edible parts could become a risk due to the entry of toxics in the food-chain [8,14].

Antibiotics generate the most concern in their spread through the environment, due to: (i) the potential generation of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) and (ii) the possible effect on allergic people because of their uptake by crops [15–18]. Their impact on plants includes inhibition of growth, tissues deformation, reduction of photosynthetic rate and chlorophyll content and other phytotoxic effects [19]. The presence of antibiotics, ARB and ARGs in soils and their uptake by crops could be a high risk to human health due to their potential transfer to other bacteria including human commensals or pathogens of clinical relevance [20].

Other paths for antibiotics to reach agro-ecosystems are the fertilization with animal urine or manure and sewage sludge. Animals only partially metabolize antibiotics (between 40–90%), and the main excretion routes are urine and feces [21]. Only in the EU 6431.4 tons of antibiotics were consumed for veterinary use in 2018 [22], in the United States 36,982 tons in 2016 [23] and 105,000 tons in China in 2015 [24]. Animal residues contain the parent antibiotic compounds and metabolites such as glutathione complexes that can also have antibiotic activity. When they interact with soil microbiota and their enzymes, antibiotic complexes can suffer microbial transformation and release the parental compound structure [25].

It has already been proved that antibiotics in soils irrigated with RWW or fertilized with manure are taken up by crops [17], and soil amendments could even increase the half-life of antibiotics and their adsorption [26]. Although their presence in wastewater and organic amendments are low, ng—$\mu g\ L^{-1}$ and $\mu g$—$mg\ kg^{-1}$ respectively, their accumulation over time and the constant uptake by plants could be a potential risk [17,18,21,27]. Several papers reported the antibiotic uptake by crops and its distribution in the different parts of the plant in hydroponic conditions [28–31] or using spiked soils at small scale [32–35]. In all of them, the antibiotics reached different organs of the plants, including their edible tissues.

However, studies of antibiotics uptake on a real scale are scarce. Hence, there is a necessity to study the fate of antibiotics in real scenarios where antibiotics could have accumulated over time by recurrent application of manure and irrigation with RWW. The location chosen of this work was the province of Almería (Spain). This region is located in the southeast of Spain (36°50′ N 02°23′ W). It is also known as “the plastic sea” because it has more than 35,000 ha of intensive horticulture greenhouses that produced 3,286,385 t of fruits and vegetables in the 2016–2017 campaign [36]. According to the Köppen classification, Almería is a tropical and subtropical steppe climate, with an average temperature of 19 °C, 2965 h of sun per year, an average relative humidity of 70% and an annual rainfall between 200 and 250 $L\ m^{-2}$ [37]. Due to this climate conditions and their high productivity, Almería has become the main exporter of vegetables in the EU, and about 70% of the production is exported. Its agricultural area represents 0.02% of the EU. However, it produces 0.6% of vegetables of the EU, a productivity 30 times higher than the EU average [38]. In the Almería region, 80% of the water resources come from underground aquifers, while the other 20% come from transfers, desalination plants, reservoirs and RWW [39]. However, the over-exploitation of aquifers and their quality decrease due to the salinization and presence of pollutant substances such as fertilizers and pesticides [37,40]. Hence, the Spanish Government promotes the re-use of RWW for irrigation, that nowadays this represents 5.26% of the irrigation water used in Almería [37]. The main crops grown in Almería are tomato, pepper, cucumber, melon, watermelon, eggplant and green beans. Many of them are often consumed raw, which makes this study particularly relevant because when crops are cooked before consumption, some antibiotic structures can be altered and in consequence their level in food reduced [41].

The objective of this work was to determine the fate of sulfonamides (SAs) in a real soil-water-plant system of tomato crop from two different commercial greenhouses irrigated with RWW in the region of Almería (Spain) over 2 years. To this aim, SAs were monitored in soil, agricultural inputs such as irrigation water and amendments, and different tissues of the tomato plants (roots, leaves and fruit), to assess the source and uptake of these antibiotics. Finally, the potential risk derived from the SAs in the tomato fruit was assessed.

## 2. Materials and Methods

### *2.1. Sampling*

Samplings of tomato plants (*Solanum lycopersicum* L.), irrigation water, soil and amendments were taken once a year from 2018 to 2019 between the last week of March and the first two weeks of April. Those dates were chosen to obtain mature plants, since they are sown in August and the campaign finalizes in May. The samples were taken from two different commercial greenhouses located in Almería, Spain. The two greenhouses were part of the same irrigator community cooperative, that used RWW, desalinated water and groundwater for irrigation. The origin of RWW was the WWTP of Almería and had an additional filter treatment before the irrigation. During the first year, both greenhouses used desalinated water for irrigation, meanwhile during the second year the first greenhouse (GH1) used a mixture of RWW and groundwater (50/50 $v/v$) and the second greenhouse (GH2) only used RWW for irrigation. Water samples were taken from the reservoir tanks where the water was stored prior to utilization. The concentration of SAs in the irrigation water used in GH1 and GH in 2018 and 2019 seasons were determined.

In the region of Almería, it is common to use the "sandy mulch" technique, that consists in protecting the natural soil with manure and a layer of sand. The layer of sand is useful to reduce the water consumption and the condensation of atmospheric humidity [42]. Every 3 years manure is applied, mixed with the soil and protected with sand. The soil samples were taken with a 15 cm depth, in a radius between 30 and 40 cm from the plant and using a zig-zag distribution all over the greenhouses. Before sampling, the sand of the surface was set aside. During the first year of monitoring (2018) manure samples, coconut fiber and sand were also analyzed, as they were already applied, the three of them were separated for their individual analysis.

Greenhouses are located in the same production area, hence the soils are relatively similar. However, the management of soil, including the use of organic amendments, could produce significant differences. such as the content of organic matter. The soil main properties for GH1 and GH2 were: pH 8.90 $\pm$ 0.05/8.67 $\pm$ 0.05, $pH_{KCl}$ 8.03 $\pm$ 0.02/7.99 $\pm$ 0.02, organic matter 3.37 $\pm$ 0.07/0.99 $\pm$ 0.05, texture composition: sand—72%/64%; silt—15%/22%; clay: 13%/14%. According to the US textural classification, both soils were sandy loam.

Tomato plants samples were randomly taken, choosing three full tomato plants from each greenhouse every year. They were divided into roots, leaves and tomato fruit for their later SAs extraction and analysis, roots were washed carefully with tap water to avoid possible soil interferences. Samples were processed individually. The plant samples were immediately refrigerated at 4 °C previous extraction and analysis of SAs, which were made during the next 48 h. If the extraction was performed afterwards, samples were stored at −72 °C.

### *2.2. Sulfonamides Analysis*

#### 2.2.1. Chemicals and Reagents

SAs are one of the families of antibiotics most present in the environment, mainly because they represent the 9.6% of the most prescribed antibiotics for veterinary use in the EU [22]. The antibiotics analyzed were 13 SAs: sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SP), sulfamerazine (SMR), sulfamethizole (SMT), sulfamethazine (SMZ), sulfamethoxypyridazine (SMP), sulfamonomethoxine (SMM), sulfachloropyridazine (SClP), sulfamethoxazole (SMX), sulfadoxine (SDX), sulfisoxazole (SIX) and sulfadimethoxine (SDM). All standards were obtained from Sigma-Aldrich (St. Louis, MO, USA).

For the quantification, two internal standards (IS) were used, namely sulfathiazole-$^{13}C_6$ and sulfamethoxypyridazine-$D_3$. Both IS were obtained from Sigma-Aldrich. Solid Phase Extraction (SPE) was performed on cartridge Oasis HLB cartridges (Waters Corporation, Milford, MA, USA) for the extraction and purification of the antibiotics. All the organic solvents used were HPLC grade and were purchased from Merck Corporation (Darmstadt, Germany).

#### 2.2.2. Sulfonamides Extraction and Quantification by UHPLC-MS/MS

Tomato fruit samples were homogenized using an Ultra—Turrax (IKA-Werke GmbH & CO, Staufen, Germany). The other vegetable samples (root and leaves) as well as soil, sand, manure and coconut samples were cut and crushed with a chopper mixer. Then, 3 g of sample was taken, spiked with the two IS and extracted with 20 mL of a mixture of EDTA-$Na_2$ 0.1 M and trichloroacetic acid (TCA) 0.1% (1:1) for 10 min with orbital agitation. Then the suspension was centrifuged at 5000 rpm for 10 min. For the extraction of SAs from water, 20 mL of water sample was mixed with 5 mL of EDTA-TCA 0.1% (1:1). After the extraction step, 20 mL of extract was concentrated and purified by SPE Oasis HLB cartridges. SAs were eluted with 8 mL of ethyl acetate, then evaporated to dryness under 12 psi $N_2$ flow at 42 °C. Finally, the residue was reconstituted in 0.25 mL of methanol/water (15/85) [43]. The analysis was based on other methods of detection of antibiotics in milk [44,45].

SAs were analyzed using a UHPLC-MS/MS system consisted of an Acquity UPLC module (Waters) coupled with a Waters TQD triple quadrupole detector (Waters). The instrument was operated using an electrospray source in positive mode with the following parameters: 3.50 kV capillary voltage, 30.0 V cone voltage, 400 °C desolvation temperature, and 600 L $h^{-1}$ desolvation gas flow. Data acquisition was performed using MassLynx V 4.1 software. The column was a UPLC BEH C18 (100 mm × 2.1 mm; particle size 1.7 µM), the temperature was fixed at 45 °C and the injection volume was 10 µL. The gradient program is shown in Table 1. The quantification was made using an external calibration. Stock standards of 1000 µg $mL^{-1}$ of each SAs were prepared in acetonitrile and stored at −4 °C in dark glass bottles during the three-month validity period. IS stock dilutions of 1.5 µg $mL^{-1}$ were prepared in methanol:water (60:40). Dilutions for working standards were made in methanol:water (60:40). The parent ion ($m/z$) and daughter ions ($m/z$) were: SDZ (251.0/91.9, 155.8), STZ (255.9/155.8, 91.8), SP (250.0/155.9, 107.9), SMR (265.0/91.8, 155.8), SMT (271.0/156.0, 92.1), SMZ (279.1/91.9, 185.9), SMP (280.9/91.9, 155.9), SMM (281.0/92.1, 156.0) SClP (284.9/155.8, 91.8), SMX (254.0/156.0, 92.1), SDX (311.0/155.9, 91.9), SIX (268.0/155.8, 91.8) and SDM (311.0/155.8, 91.8) For the IS: sulfathiazole-$^{13}C_6$ (311.0/162.1, 98.1) and sulfamethoxypyridazine-$D_3$ (283.9/92.1, 156.1).

To assess the accuracy of the analytical procedure, the recovery percentage of the 13 SAs was determined in spiked tomato samples at two different concentrations, 5 and 40 ng $kg^{-1}$. The recovery percentages of the 13 sulfonamides were in the range of 77–140% (RSD 7–38%) for the low concentration and 72–130 (RSD 2–11%) for the high concentration. Mean recoveries were 107% for low concentration and 112% for high concentration.

**Table 1.** Gradient elution program of mobile phases for the separation of sulfonamides by UHPLC-MS/MS.

| Time (min) | Flow Rate (mL min$^{-1}$) | Solvent A (%) | Solvent B (%) |
|---|---|---|---|
| 0.00 | 0.50 | 8.00 | 92.00 |
| 5.00 | 0.50 | 15.00 | 85.00 |
| 9.00 | 0.50 | 55.00 | 45.00 |
| 12.00 | 0.50 | 8.00 | 92.00 |

Solvent A: Acetonitrile 0.1% Formic acid; Solvent B: 0.2% oxalic acid + 0.2% Formic acid in Milli-Q water.

2.2.3. Estimation of Bioconcentration, Translocation Factor and Dietary Intake

The bioconcentration factor (BCF), translocation factor (TF) and human exposure (HE) were calculated according to Pan and Chu [17]. The bioconcentration factor gives -information of the SAs accumulation from the soil in the different parts of the plant roots, leaves and fruit:

$$\mathrm{BCF} = \frac{\text{Concentration in crop tissue } (\mu\text{g/kg})}{\text{Concentration in soil } (\mu\text{g/kg})}, \quad (1)$$

The TF is the parameter that represent the translocation of the SAs from the roots to the leaves.

$$\mathrm{TF} = \frac{\text{Concentration in leaf } (\mu\text{g/kg})}{\text{Concentration in root } (\mu\text{g/kg})} \quad (2)$$

The HE represents the potential risk of the consumption of the edible parts of the tomato plant by presence of SAs:

$$\mathrm{HE} = \mathrm{C} \times \mathrm{D} \times \mathrm{W} \times \mathrm{T} \quad (3)$$

where C is the concentration in the tomato fruit, the edible part, in µg kg$^{-1}$ wet weight; D is the average daily consumption of edible crops, 0.72 g weight kg$^{-1}$ body weight day for tomato [46]; W is the body weight of the person consuming the crops, 70 kg; T is the exposure time in days [47].

## 3. Results & Discussion

### *3.1. Sources of Sulfonamides and Their Accumulation in Soil*

The main potential sources of SAs to the tomato crop in both commercial greenhouses (GH1 and GH2) were the water of irrigation and soil amendments, manure, coconut fiber and sand (Tables 2 and 3).

**Table 2.** Irrigation water source over two years and the concentration of sulfonamides (ng L$^{-1}$) (sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SP), sulfamethoxazole (SMX) and sulfadimethoxine (SDM)). Data are represented as the mean ± standard deviation, $n$ = 3. <DL indicates below the detection limit.

| | | GH1 | GH2 |
|---|---|---|---|
| 2018 | | Desalinated water | Desalinated water |
| | SDZ | <DL | <DL |
| | STZ | <DL | <DL |
| | SP | <DL | <DL |
| | SMX | <DL | <DL |
| | SDM | <DL | <DL |
| 2019 | | RWW and groundwater (1:1) | RWW |
| | SDZ | 301 ± 94 | 620 ± 123 |
| | STZ | 10 ± 3 | 9 ± 2 |
| | SP | 395 ± 73 | 365 ± 101 |
| | SMX | 444 ± 16 | 647 ± 133 |
| | SDM | 8 ± 3 | 6 ± 2 |

**Table 3.** Concentration of sulfonamides (sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SP), sulfamethoxazole (SMX) and sulfadimethoxine (SDM)) in manure, sand and coconut fiber (ng $kg^{-1}$).

| | SDZ | STZ | SP | SMT | SMX |
|---|---|---|---|---|---|
| Manure | 321 | <DL | 150 | <DL | 8017 |
| Sand | 538 | 257 | <DL | 941 | 7915 |
| Coconut fiber | 1439 | 3687 | <DL | 4716 | 45,432 |

The detected and quantified SAs in the irrigation waters used in both greenhouses are shown in Table 2. Clearly, RWW contained higher concentrations of SAs than desalinated water, where no antibiotics were detected. The analysis of SAs in the RWW showed that SMX was the most abundant antibiotic, surpassing 647 ng $L^{-1}$. Other antibiotics detected were SDZ, STZ, SP and SDM. These range of SAs concentrations was similar to the ones detected in previous works of wastewater effluents, where SAs were from undetected to reaching concentrations up to 3600 ng $L^{-1}$ in wastewater worldwide [48].

During the first year of analysis (2018) both greenhouses changed their usual irrigation water (RWW) to desalinated water. The concentration of SAs in desalinated water was below the detection limit for all the SAs monitored. However, in 2019 both greenhouses changed their water sources again. Although the water used in GH1 was a mixture of RWW and groundwater (1:1) in 2019, its concentration of SAs was comparable to the irrigation water of GH2 (100% RWW). In some cases, it even had lightly higher concentration of SAs than the values achieved in the RWW from GH2. The presence of SAs in different aquatic environments has been reported in several studies [49]. SAs and other antibiotics were frequently detected in groundwater influenced by agricultural soils fertilized with manure due to antibiotics leaching [50] which could be the reason why SP and SDM in the mixture of RWW and groundwater in GH1 during 2019 was higher than in wastewater.

Manure of animal origin and coconut fiber were applied in 2019, during the second year of monitoring and covered with sand to rebuild the "sandy mulch" system. The sand was recycled from previous crop cycles. Sand, coconut fiber and manure had already been applied when the sampling was done. Hence, the presence of SAs in the samples (Table 3) was because of their adsorption from irrigation water and the possible inherent presence of them in these amendments. These agricultural inputs were shown to be an important source or adsorbent of SAs. The SAs identified in manure, coconut fiber and sand were SDZ, STZ, SP, SMT and SMX. Once again, as happened in the case of water, SMD was the sulfonamide with the highest concentration of the three amendments. Hence, the use of any of these amendments was a clear reservoir of SAs to the soil-plant system. However, the presence of each antibiotic and its concentration was different for each amendment. For example, manure was the only amendment containing SP. Hence, this fact could indicate that manure was a source of SAs. The presence of antibiotics in manure is common because of the extensive use of antibiotics in veterinary medicine, including SAs that is one of the most used family of antibiotics in Spain and Europe [22]. The long-term application of amendments of animal origin to agricultural soils leads to the introduction and dissemination of antibiotics into the environment [51]. SAs, along with other antibiotic families, have been detected in both raw and treated manure, in raw swine manure (including bedding manure, flushing material, liquid manure, slurry and solid manure). SAs had been found in concentrations between 0.5 and 104 ng $g^{-1}$ with high variability in all the manures, in treated manures like composted bedding manure reaching concentrations of 103 ng $g^{-1}$, surpassing the High Risk Quotas in some cases [52]. Different works have shown that antibiotic presence in manure leads to their uptake by plants and in some cases produced negative effects in the plant development, such as inhibition of root and stem growth or biomass reduction [21].

The unexpected presence of SAs in sand was because this material was re-used in several crop seasons. Despite the low ability of sand to adsorb SAs or other pollutants, the continuous application of SAs inputs was finally reflected. The content of SAs in coconut fiber was probably because of the ability of vegetable wastes to adsorb pollutants from water [53], in this case SAs. The detection of SMT in coconut fiber could be because the mobilization of this antibiotic from sand and further adsorption on this matrix because SMT was not detected in the irrigation water. Other possible explanation could be the uptake of SAs during the coconut crop derived from the indirect contamination of soil with SAs by the use of contaminated agricultural inputs.

The number of detected and quantitated SAs in soils from GH1 and GH2 were the same in both greenhouses (Table 4). And in line with the SAs found in RWW, the only difference between the SAs identified in soil with respect to the irrigation waters, was the presence of SMT instead of SDM. Hence, the source of SMT was not RWW. According to the SAs concentration of amendments (Table 3), the sources of SMT were coconut fiber and sand. In addition, the antibiotic SDX was not accumulated into the soil of GH1, probably because this antibiotic was only detected in the year 2019 at a very low concentration.

**Table 4.** Concentration of sulfonamides (sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SP), sulfamethizole (SMT), sulfamethoxazole (SMX) and sulfadimethoxine (SDM)) in soil (ng kg$^{-1}$) over two years. Data are represented as the mean ± standard deviation, $n$ = 3. <DL indicates below the detection limit.

| | | GH1 | GH2 |
|---|---|---|---|
| SDZ | 2018 | 330 ± 84 | 614 ± 58 |
| | 2019 | 1743 ± 111 | 8523 ± 15 |
| STZ | 2018 | 288 ± 79 | 381± 63 |
| | 2019 | 78 ± 35 | 218 ± 43 |
| SP | 2018 | 51 ± 37 | <DL |
| | 2019 | 126 ± 9 | 3250 ± 419 |
| SMT | 2018 | 741 ± 141 | 871 ± 57 |
| | 2019 | 1282 ± 318 | 7690 ± 280 |
| SMX | 2018 | 10,703 ± 331 | 10,291 ± 186 |
| | 2019 | 2394 ± 546 | 13,431 ± 274 |
| SDM | 2018 | <DL | <DL |
| | 2019 | 31 ± 55 | 301 ± 9 |

SAs in soil were in a wide range of concentrations from 31 ng kg$^{-1}$ to 13.4 µg kg$^{-1}$, reaching levels in the range of other studies regarding SAs in soil [54]. The most abundant sulfonamide in the two soils was SMX during the two years of monitoring, as happened with the irrigation waters and amendments. SMX was in a range of concentration between 2.39 and 13.4 µg kg$^{-1}$. SDZ, SP, SMT and SMX increased their levels in soil in 2019, after the application of amendments and the use of RWW as irrigation water.

Soil is a matrix with high ability to adsorb and immobilize pollutants of different nature and origin such as heavy metals, pesticides, polycyclic aromatic hydrocarbons, or pharmaceuticals, including antibiotics. The soil organic matter content is a key factor to adsorb and minimize the leaching of SAs between other pollutants. The adsorption of SAs is higher as the organic matter increases and simultaneously, the desorption is lower denoting a phenomenon of hysteresis [55]. Several works have reported that hysteresis is a common phenomenon of strongly organic pollutants adsorbed in the soil and therefore, a way to increase the pollutant content of the soil [56,57]. Therefore, the use of organic amendments favored the adsorption and immobilization of SAs from RWW and the own organic amendments. For example, Albero et al. [26] reported an increment of SAs concentration because of adsorption when the soil was amended with manure. And these results were in line with Wang et al. [58] who found an increase in SAs adsorption in soil amended with pig manure.

However, not only the organic fraction of the soil is responsible of the SAs adsorption. The mineral fraction such as metal oxides and clay minerals and less specifically, fine particles are other key factor in the SAs adsorption in soil. SAs are immobilized on mineral surfaces by different types of molecular forces such as hydrogen bound, electrostatic interactions and π-π interactions [56]. An important mechanism of SAs adsorption on clay minerals is through cation bridging in the presence of divalent or trivalent metal ions such as $Ca^{2+}$, $Mg^{2+}$ or $Fe^{3+}$ to form SAs-metal-surface ternary complexes due to the anionic nature of SAs in a wide range of pH [56,59].

### *3.2. Antibiotic Uptake by Tomato Plants*

The distribution of SAs in the tomato plants varied with each sulfonamide (Table 5). During 2018, when the irrigation source was desalinated water, SMX was the antibiotic with the highest concentration in the tomato plant, reaching almost 35 µg kg$^{-1}$ in fruit in GH1 and 17.6 µg kg$^{-1}$ in GH2, and almost 1 µg kg$^{-1}$ in in roots for both greenhouses. The other antibiotic with more presence was SDM, with a higher concentration in tomato in the GH1 (2.8 µg kg$^{-1}$) than in the GH2 (1.5 µg kg$^{-1}$) as it happened with SMX. The other antibiotics found were SDZ, STZ, SP, SMT, only SP and SMT were detected in the tomato fruit at concentrations between 236 and 746 ng kg$^{-1}$, with lower concentration in GH2.

**Table 5.** Concentration of sulfonamides (sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SP), sulfamerazine (SMR), sulfamethizole (SMT), sulfamethoxazole (SMX) and sulfadimethoxine (SDM)) in tomato plants (ng kg$^{-1}$) over two years. Data are represented as the mean ± standard deviation. <DL indicates below the detection limit.

| | | ROOTS | | LEAVES | | FRUIT | |
|---|---|---|---|---|---|---|---|
| | | GH1 | GH2 | GH1 | GH2 | GH1 | GH2 |
| 2018 | SDZ | 235 ± 95 | 286 ± 57 | <DL | 2718 ± 653 | <DL | <DL |
| | STZ | 247 ± 102 | 322 ± 90 | <DL | <DL | <DL | <DL |
| | SP | 146 ± 58 | 76 ± 18 | <DL | <DL | 408 ± 253 | 236 ± 91 |
| | SMR | <DL | <DL | <DL | <DL | <DL | <DL |
| | SMT | 249 ± 120 | 356 ± 168 | <DL | <DL | 746 ± 146 | 314 ± 116 |
| | SMX | 9869 ± 851 | 9917 ± 3041 | 748 ± 188 | 1077 ± 190 | 34,884 ± 4948 | 17,577 ± 2126 |
| | SDM | 560 ± 133 | 620 ± 359 | 22,088 ± 8577 | 16,709 ± 12,103 | 2884 ± 1218 | 1497 ± 409 |
| 2019 | SDZ | 331 ± 70 | 202 ± 53 | 346 ± 98 | 3238 ± 846 | 51 ± 18 | 485 ± 206 |
| | STZ | 236 ± 72 | 192 ± 35 | 88 ± 77 | <DL | <DL | <DL |
| | SP | 85 ± 17 | 193 ± 82 | 36 ± 17 | <DL | 14 ± 8 | 33 ± 18 |
| | SMR | <DL | <DL | <DL | <DL | <DL | <DL |
| | SMT | 3667 ± 1281 | 3123 ± 363 | 1316 ± 318 | 2372 ± 1093 | 1452 ± 632 | 2638 ± 1690 |
| | SMX | 2206 ± 997 | 2819 ± 830 | 2825 ± 986 | 3694 ± 1741 | 1927 ± 531 | 735 ± 396 |
| | SDM | 261 ± 84 | 160 ± 59 | 263 ± 128 | <DL | 123 ± 42 | 366 ± 116 |

SAs detected in 2019 were the same as in 2018, the year after the application of organic manure of animal origin and coconut fiber (Table 3), and when the irrigation water was RWW and RWW:groundwater mixture (Table 2). The antibiotics with the highest concentration were SMX and SMT, with concentrations between 6 and 8 µg kg$^{-1}$. SMT was not detected in the irrigation water but on coconut fiber as well as on the sand (Table 3) and could be the main reason of this increase in plants with respect to 2018.

However, SAs concentration in 2019 was lower than in 2018. The introduction of new amendments, as well as the irrigation with RWW, increased the antibiotic presence in soils. Manure-amended soils present a higher ability for antibiotic adsorption [60]. Studies with SMZ in amended-soils reported that SMZ in plant tissue was higher than in soils without amendment [61]. It has been proved that although manures increase the sorption capacity of SAs in soils, they also reduce their mobility [26]. The lower presence of SAs in plants in 2019, could be because after their introduction in soils, SAs underwent a sorption/desorption phenomenon that could form non-exchangeable or bound residues that reduce their bioavailability to the tomato plants [62]. For example, SP has been reported to be adsorbed strongly in soils through its organic matter [4], supporting the fact that in 2019 the SAs were less available to the plant due to the input of new organic amendments. The freely dissolved and exchangeable fractions of PPCP in soils are not only taken up by plants. PPCP could also migrate or be absorbed by microorganism. Some SAs like SMX have been frequently detected in leachate of lands irrigated with RWW, which shows the high mobility of SAs in soil [62]. So the presence of SAs in soil would probably decrease but at the same time be more available for plant uptake. This theory is supported by the results of 2018, when soils had less concentration of SAs than in 2019, as it had been two years since the application of organic amendments, but the SAs uptake by tomato was higher (SP, SMX, SDM) than in 2019 with higher SAs concentration in soils.

The other quantified SAs during 2019 were under 1 μg $kg^{-1}$, except for SDZ in the GH2 that also reached the fruits being the only SAs with higher presence in plant in 2019. The concentrations of SDM were too high in comparation with the quantity detected in soil and RWW. It could be due to a problem of quantification in the soil and RWW matrixes.

In both years, neither SMR nor STZ were translocated to tomato fruit. SMR was not detected in any vegetable sample, root, leaves or fruit. This result was in line with the fact that this sulfonamide was not detected in any agricultural matrix analyzed, water, amendment or soil. STZ was present in roots but did not translocate to the tomato fruit. In fact, the translocation of STZ from root to leaves seem impaired. The same results reported Liu, et al. [63]. In their work, STZ was not detected in cucumber, eggplant, long bean and wheat irrigated with RWW. STZ has a low log Kow (0.002) in comparation with other SAs like SMX (0.890) or SMZ (0.440), which could be the main reason for its low uptake by crops [63].

The other SAs had different distribution in plant depending on the sulfonamide, greenhouse and year. In general, the concentration of SAs in roots or leaves was higher than in fruits. The concentrations of SAs were in the range of ng $kg^{-1}$, but SMT, SMX and SDM surpassed 1 μg $kg^{-1}$ in all the plant organs denoting higher availability and uptake than the other SAs analyzed.

In previous published works that monitored antibiotics uptake after manure application, the concentration of antibiotics in plants was in the same range of concentration. For example, turkey and hog manure spiked with five antibiotics, including SMZ, at range of mg $kg^{-1}$ and mg $L^{-1}$, respectively, produced accumulation of antibiotics in shoot of 11 different vegetables at rates lower than 10 μg $kg^{-1}$ [33].

The behavior of SMR on a similar experiment [17], with spiked wastewater and application of animal manure to different crops, was root > leaves > fruit, that is similar to the results of some of the SAs in this work with low translocation to aerial parts. SAs could be accumulated on roots due to their low cell membrane permeability [30]. The tomato crop of our study was 8 months old while the experiment of Pan and Chu [17] was for 4 months. Hence, the higher time of exposure could favor the translocation to other parts of the tomato in the present work and could be the reason why many SAs were in leaves and fruits more than in roots. In other work with higher concentrations of SAs, between 5 and 20 mg $kg^{-1}$, most of the SAs were accumulated in roots [64]. However, that work was performed at short term. The analysis of antibiotics in real conditions in the north of China showed that their accumulation was higher in leaves than in roots [65], that agree the results of some SAs in this work. In real scenarios with a higher exposure time, the translocation to aerial parts of the plant increases. Another important factor that could enhance the SAs translocation is the high plant transpiration in greenhouses. The high transpiration of crops could play a significant role in the uptake and translocation of PPCP, increasing them in arid and hot climates [66] such as is the case of Almería (southeast of Spain).

During the first growing season (2018), STZ and SDZ were not located in fruit (Figure 1). These SAs were mainly found in root, except SDZ in GH2 which was mainly present in leaves. SP, STM and SMX were found mainly in the tomato fruit (48–78%) followed by root. Their presence in leaves was low or negligible. In contrast, SDM was mainly presented in leaves (85–90%) with an appreciable presence in fruit (8–11%). However, in the following crop cycle, the distribution of SAs in tomato was different in the two greenhouses. The STZ remained undetectable in fruit. This sulfonamide was mainly found in root again.

However, in 2019, SDZ was in fruit in appreciable percentages (7–12%). In this way, SDZ, SP, STM, SMX and SDM showed appreciable presence in fruit. Nevertheless, the percentage of SP, STM and SMX in fruit was clearly lower than in 2018. In 2019, the distribution of SP, STM and SMX was favored towards leaves and root with respect to 2018. In contrast, SDM increased the percentage of distribution towards root and fruit. It is noticeable the same pattern found in both greenhouses between 2018 and 2019. Hence, the application of new organic amendments and RWW as irrigation water in 2019, not only modified the concentration of SAs in tomato tissues (Table 5), also produced modification in the distribution of SAs in the tomato plant. Therefore, the source of SAs and the agricultural practices seem important factors that govern the distribution of SAs in the tomato plant. This redistribution of SAs between tomato tissues is reflected in the translocation factors showed in Table 6. In this table, for example, the TF of SMX increased more than one-fold between 2018 and 2019 whereas the TF of SDM decreased clearly in the same period. The TFs suggested that some of the SAs (STZ, SP, SMT and SMX) in 2018 had a restricted translocation with TFs values <1. Similar results were reported in different studies [41,60]. However, SDM showed TF higher than 26, indicating very high translocation from root to leaves in 2018 but a strong decrease in 2019. In contrast, SDZ and SMX increased their TFs in 2019 with respect to 2018.

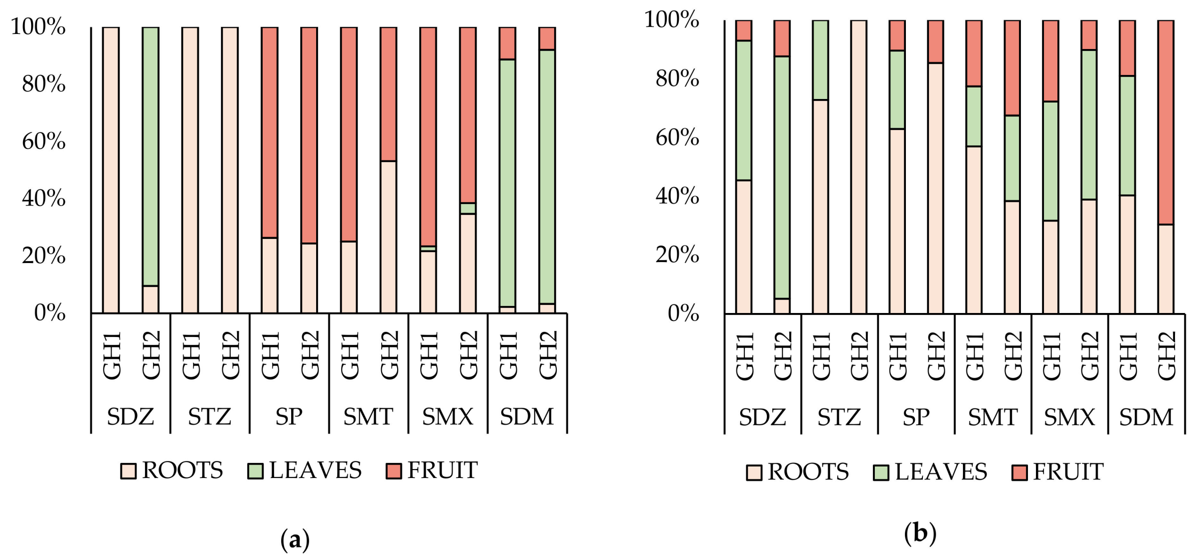


**Figure 1.** Sulfonamides (sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SP), sulfamethizole (SMT), sulfamethoxazole (SMX) and sulfadimethoxine (SDM)) distribution in roots, leaves and fruits of the tomato plant during (**a**) 2018 and (**b**) 2019.

The BCF (Table 7) ranged between negligible values to 8.42, 8.48 and 3.97 for SDM in root, leaves and fruit, respectively, in 2019. This factor was clearly influenced by the agricultural practices of 2018 and 2019. SP and SMX showed BCFs for fruit higher than 1 in 2018, but these values decreased below 1 in 2019. In contrast, the BCF of SDM increased form negligible values in 2018 to 3.97 in 2019 or in the case of SMT, the BCFs between 2018 and 2019 were similar. In contrast to the BCF of fruit, the BFC of leaves for SMT and SMX were clearly higher in 2019 with respect to 2018. Similar values of BFC were reported for SMX by Christou et al. [67] who irrigated tomato with RWW for 3 years. Attending to the BFCs, SAs are translocated within the tomato plant vascular system at moderate rates [60]. In other studies of PPCPs, the accumulation in root for anionic compounds was higher than in leaves, meanwhile in neutral or cation compounds the accumulation was similar [66], which could explain the slightly higher $BCF_{root}$ in comparation $BCF_{leaves}$ on most SAs.

**Table 6.** Translocation factors of sulfonamides (sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SP), sulfamethizole (SMT), sulfamethoxazole (SMX) and sulfadimethoxine (SDM)) in the tomato plants from two commercial greenhouses in 2018 and 2019.

| | | GH1 | GH2 |
|---|---|---|---|
| SDZ | 2018 | - | 9.50 |
| | 2019 | 1.05 | 16.03 |
| STZ | 2018 | - | - |
| | 2019 | 0.37 | - |
| SP | 2018 | - | - |
| | 2019 | 0.42 | - |
| SMT | 2018 | - | - |
| | 2019 | 0.36 | 0.76 |
| SMX | 2018 | 0.08 | 0.11 |
| | 2019 | 1.28 | 1.31 |
| SDM | 2018 | 39.44 | 26.95 |
| | 2019 | 1.01 | - |

**Table 7.** Bioconcentration factor (BCF) of sulfonamides (sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SP), sulfamethizole (SMT), sulfamethoxazole (SMX) and sulfadimethoxine (SDM)) in the tomato plants from two commercial greenhouses in 2018 and 2019.

| | | ROOT | | LEAVES | | FRUIT | |
|---|---|---|---|---|---|---|---|
| | | GH1 | GH2 | GH1 | GH2 | GH1 | GH2 |
| SDZ | 2018 | 0.59 | 0.25 | - | 4.43 | - | - |
| | 2019 | 1.00 | 0.33 | 0.20 | 0.38 | 0.03 | 0.06 |
| STZ | 2018 | 1.24 | 0.85 | - | - | - | - |
| | 2019 | 3.03 | 0.88 | 0.37 | - | - | - |
| SP | 2018 | 0.20 | 0.09 | - | - | 2.79 | 3.11 |
| | 2019 | 0.07 | 0.03 | 0.29 | - | 0.16 | 0.17 |
| SMT | 2018 | 0.34 | 0.41 | - | - | 1.01 | 0.36 |
| | 2019 | 2.86 | 0.41 | 1.03 | 0.31 | 1.13 | 0.34 |
| SMX | 2018 | 0.92 | 0.96 | 0.07 | 0.10 | 3.26 | 1.71 |
| | 2019 | 0.92 | 0.21 | 1.18 | 0.28 | 0.80 | 0.05 |
| SDM | 2018 | - | - | - | - | - | - |
| | 2019 | 8.42 | 0.53 | 8.48 | - | 3.97 | 1.22 |

SAs have octanol-water partition coefficient values that range between −1.5 and +1, they are relatively hydrophilic at pH values commonly encountered in the soil environment [41]. Studies on organic pollutants such as insecticides or PAHs indicated that hydrophilic compounds were brought into the plant by the xylem and the distribution in the plant depended on their hydrophilicity, meanwhile hydrophobic compounds were not translocated easily [68]. Log TF values were plotted against log D for all the SAs in both antibiotics for both years (Figure 2), in the graph a negative correlation could be appreciated meaning that SAs with more hydrophobicity tended to be retained in the roots and their distribution to other organs of the plants was limited ($p = 0.057$). The same results were observed by Wu et al. [69] with different PPCPs (including SMX) in lettuce, spinach, cucumber and pepper. Log BCF values were plotted against log D for all the SAs in both greenhouses for both years (Figure 3), the graph showed a negative correlation for the accumulation on roots ($r_p = -0.595$, $p = 0.03$). For tomato fruit and leaves there was not a clear relation.

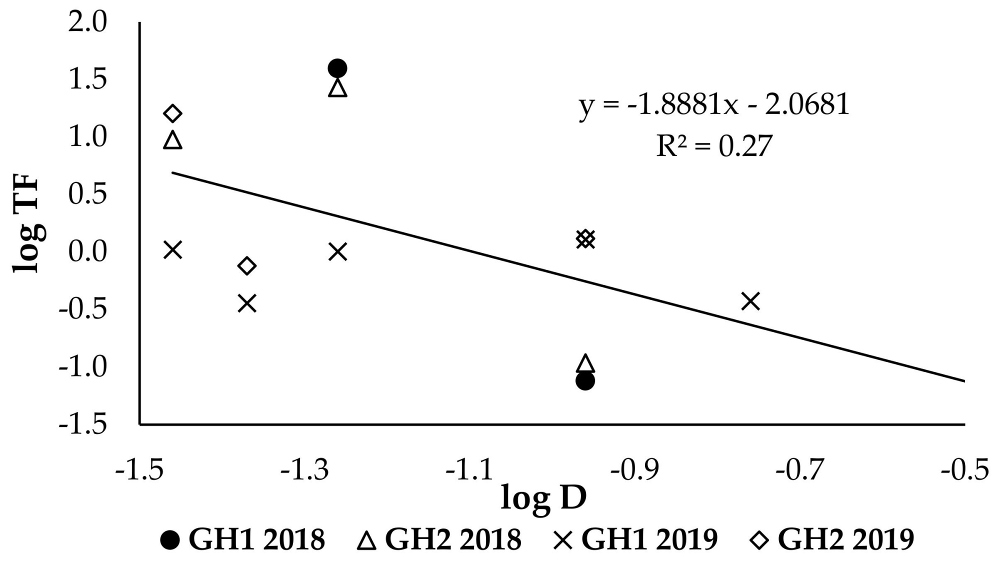


**Figure 2.** Correlation between log TF and log D of target sulfonamides in soils and RWW.

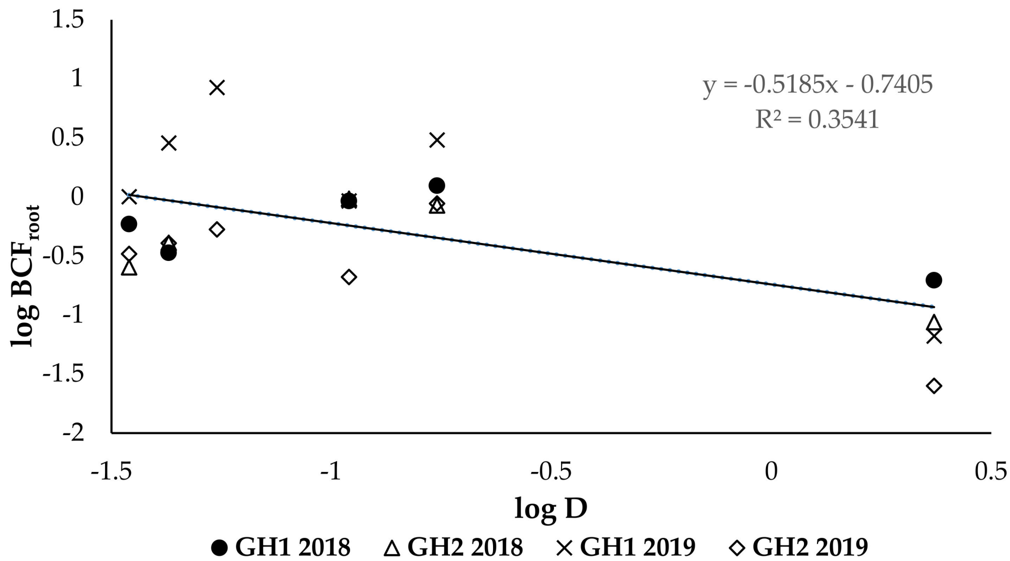


**Figure 3.** Correlation between log BCF and logD of target sulfonamides in soils and RWW in roots.

*3.3. Human Exposure to SAs*

It has been proved that the presence of antibiotics in tomato fruit could decrease their quality attributes such as soluble solids and carbohydrates (fructose, glucose, saccharose, total sugars) [70]. In addition to the environmental health and the loss of food quality, the main concern is the impact on human health.

The levels of human exposure (HE) to five antibiotics (Table 8) were in a range between 0 and 150 ng $kg^{-1}$ body weight $day^{-1}$, with the exception of SMX in 2018 that reached 1.75 μg $kg^{-1}$ body weight $day^{-1}$, under the acceptable daily intake (ADI) according to the guidelines of Joint FAO/WHO Expert Committee on Food Additives (JECFA) for SAs, that only have two SAs included in their list of contaminants and food additives, SMZ and STZ, with ADIs of 0–50 μg $kg^{-1}$ bw $day^{-1}$ for both SAs, and their latest evaluation is from 1994 (SMZ) and 1989 (STZ) [71].

**Table 8.** Human exposure to sulfonamides (sulfadiazine (SDZ), sulfapyridine (SP), sulfamethizole (SMT), sulfamethoxazole (SMX) and sulfadimethoxine (SDM)) in in edible parts of tomato (fruit). The results are expressed in ng kg$^{-1}$ body weight day$^{-1}$ for the daily exposure and µg kg$^{-1}$ body weight year$^{-1}$ for annual exposure.

| | | GH1 | | GH2 | |
|---|---|---|---|---|---|
| | | **Daily (ng kg$^{-1}$ bw day$^{-1}$)** | **Annual (µg kg$^{-1}$ bw year$^{-1}$)** | **Daily (ng kg$^{-1}$ bw day$^{-1}$)** | **Annual (µg kg$^{-1}$ bw year$^{-1}$)** |
| SDZ | 2018 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 2019 | 2.57 | 0.94 | 24 | 8.92 |
| SP | 2018 | 20.56 | 0.76 | 12 | 4.34 |
| | 2019 | 0.71 | 0.26 | 1.66 | 0.61 |
| SMT | 2018 | 37.60 | 13.72 | 15.83 | 5.78 |
| | 2019 | 73.18 | 26.71 | 132.96 | 48.53 |
| SMX | 2018 | 1758.15 | 641.73 | 885.88 | 323.35 |
| | 2019 | 97.12 | 35.45 | 37.04 | 13.52 |
| SDM | 2018 | 145.35 | 53.05 | 75.45 | 27.54 |
| | 2019 | 6.20 | 2.26 | 18.45 | 6.73 |

The results were expected to be under the ADI, as other studies with plants in presence of higher SAs concentrations did not reach the ADI levels. Soils spiked with SMR and SMX with concentrations of 5, 10 and 20 mg kg$^{-1}$ [64], or tomatoes irrigated for 3 years with two different effluent had a high accumulation of SMX in tomato with 5.26 µg kg$^{-1}$ [67], reported daily exposure levels far below the ADI.

Besides the behavior of SAs, the daily HE of different families of antibiotics on different crops had been assessed previously and in general, the results did not exceed ADIs, such as the case of lettuce and carrot irrigated with tetracycline at concentrations of 0.1 and 0.15 µg L$^{-1}$ [72] or a real irrigation of lettuce with wastewater in Ghana [73].

The only works with high antibiotic uptake by crops occurred when they were spiked at high concentrations. Rice crop where tetracycline was added in concentrations of 50 mg L$^{-1}$, and after 15 days the results in roots were higher than 1000 mg kg$^{-1}$ [74]. Another work of 13 antibiotics in the south of China with concentrations in soils between 100 and 1500 µg kg$^{-1}$ resulting concentration of antibiotics in plants higher than 100 µg kg$^{-1}$ [27].

It is very unlikely to find SAs in concentration that suppose a risk to human health in edible parts of the crops in a real scenario if they are compared to the ADI. There is a necessity to review the ADI levels of antibiotics, SAs specifically, as they are only 2 compounds classified, both reviewed but without a modification for more than 25 years as it was previously exposed.

Maximum Residue Limits (MRLs) are established for SAs and other antibiotics on meat with values of 100 µg kg$^{-1}$ [75]. However, while the total amount of vegetables consumed surpasses the meat consumption, there is no established MRLs for vegetables [76]. Setting MRLs for antibiotics in plants should be considered, as the exposure to humans due to the presence in the edible part, and in consequence the consumption is similar to the exposure induced by other kinds of food. In addition, this MLRs should consider the total amount of antibiotics, not only each antibiotic individually, to improve the risk assessments and in consequence, strategies to deal with them.

Although the presence of antibiotics at low doses could not have an immediate impact in human health, it could be related to the apparition of ARGs. Several studies have been made about the ARGs in wastewater [14,59,77] or soil [15,78], but there is not much information about the possible implication in crops. Further studies are necessary and more specifically with sensitive groups of the population such as elderly people, infants, chronic sufferers and pregnant women [5,67].

## 4. Conclusions

This work assesses the fate of sulfonamide antibiotics in two commercial greenhouses irrigated with reclaimed wastewater at real scale. Sulfonamides are recalcitrant antibiotics able to persist in soil during more than one crop cycle and susceptible to be taken up by tomato plant and translocated to fruits. The irrigation water from reclaimed wastewater and agricultural amendments are a clear source of sulfonamides to tomato crop. However, the use of organic amendments decreases the uptake of sulfonamides by tomato and modifies the distribution of these antibiotics in plant tissues. Sulfamethoxazole was the most abundant antibiotic in tomato fruit. Attending to the available values of acceptable daily intake (ADI) for sulfonamides, their concentration in tomato fruit reached admissible values. However, it is clear the necessity of extend the ADIs for all the sulfonamides present in agricultural environments and the maximum residue levels in vegetables.

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