Human health risk estimation of antibiotics transferred from wastewater and soil to crops

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Abstract

Antibiotics enter into agricultural land, via manure application or wastewater irrigation. The practices of using untreated wastewater in the agricultural system help in the bioaccumulation of antibiotics in vegetables and other crops. Exposure to the bio accumulated antibiotics poses serious health risks to ecosystem and human. In this study, the prevalence of two fluoroquinolones (levofloxacin and ciprofloxacin), their bioaccumulation in five crops (*Daucus carota* L., *Pisum sativum* L., *Raphanus raphanistrum* L., *Lactuca sativa* L., *Spinacia oleracea* L.) and associated human health risks were investigated. Lettuce showed highest bioaccumulation of levofloxacin (LEV) (12.66 µg kg^{-1}) and carrot showed high bioaccumulation of ciprofloxacin (CIP) (13.01 µg kg^{-1}). In roots, Bioconcentration factor (BCF_{root}) was observed to be relatively high in radish (LEV 0.24-0.43, CIP 0.32-0.49) and observed to be lower in spinach (LEV 0.05-0.13, CIP 0.12-0.19). The translocation factor (TF) for LEV and CIP was generally >1 for all five crops under all treatment. The final transfer and distribution of LEV and CIP in the edible parts of the crops were as follows: leaves > shoots > roots for both antibiotics. Risk quotient of both LEV and CIP in current study is found to be in

between 0.018-0.557 and shows a medium risk (0.1 to 1) to human health due the discharge of untreated wastewater into the fields. However, our study reports that both antibiotics do accumulate in the edible plant parts, therefore it poses potential risks to human health.

Keywords:

Antibiotics; Fluoroquinolones; Levofloxacin; Ciprofloxacin; Antibiotic accumulations; Human health risks

1. Introduction

Antibiotics are consumed for the treatment and prevention of bacterial infections, but their intense usage has led to the rapid development and dissemination of antibiotic resistance. This decreases the efficacy of treating infectious diseases in humans and animals leading to higher morbidity, mortality and costs (Wei et al. 2016; Zhao et al. 2018). In recent years, antibiotics have appeared as environmental contaminants causing toxic effects even to the non-targeted organisms and extensively discharged from different sources; agricultural, hospital, pharmaceutical, industrial and municipal effluents (Segura-Egea et al., 2018; Kumari and Kumar, 2020). Their persistence nature is a serious concern for environment and humans (Kumari and Kumar 2020).

Due to fresh water shortages, untreated wastewater is often used for irrigation purposes in arid and semi-arid areas (Pan and Chu 2017c). Other input pathways of antibiotics into the agricultural land include: i) pharmaceutical companies' wastewater, ii) drug manufacturing process, and iii) by grazing animals urine/feces (Zhang and Li, 2011; Wu et al. 2015; Riaz et al., 2018; Rahman et al., 2018), iv) sewage sludge application, and v) bio-solids/manure used as soil alterations and compost (Carvalho and Santos, 2016; Massé et al., 2014). In developing countries like Pakistan, therefore hospitals wastewater is not managed properly causing antiobiotics accumulation in the agricultural lands (Tudor et al., 2005). There is no appropriate facility available for treating wastewater in the hospitals of Pakistan, regardless of the hospital sizes. Hospitals discharge their wastewater directly into the wastewater drains/streams, which enters into rivers and canals from where farmers use this wastewater for irrigation purposes without any treatment (Rehman et al., 2015; Ashfaq et al., 2016)

Third-generation Fluoroquinolone (FQ) antibiotics are effective against a broad spectrum bacterial infections. They are widely used in hospitals, veterinary applications, households (Grave et al., 2012) and growth promoters in fish and animal farming (USEPA, 2011; Van Doorslaer et al., 2014). Levofloxacin (LEV) and Ciprofloxacin (CIP) belonging to the FQ family of antibiotics, they are known as emerging contaminants. They have been found in wastewater, groundwater, surface water as well as in drinking water worldwide (Van Doorslaer et al., 2014; Mohapatra et al., 2016). Monitoring studies in Pakistan has reported CIP in wastewater (2.2 µg L^{-1}) and in soil (28 µg kg^{-1}) (Ashfaq et al. 2016; Christou et al. 2017). A study in Pakistan reported CIP, LEV and enrofloxacin in wastewater (from hospitals, households and pharmaceutical industries) at concentrations of 58, 48.9 and 42.1 g L^{-1}, respectively (Riaz et al., 2017; Riaz et al. 2018). These concentrations exert eco-toxicological effects on aquatic animals, bacteria, algae and invertebrates (Xiong et al., 2017a; Xiong et al. 2017). In Pakistan, the *Salmonella paratyphi* and *Salmonella typhi* strains are reported to be resistant against fluoroquinolones (Hasan et al. 2008; Qamar et al. 2014) .

Behavior and fate of the antibiotics into the environment are determined by their physiochemical characteristics (chemical structure, water solubility, sorption capacity, volatility, and lipophilicity), soil properties (pH, cation exchange, ionic strength, and organic matter content) and weather conditions. Antibiotics can persist in the environment from less than a day to many months (Albero et al. 2018). In recent years, researchers have given special consideration to the bioaccumulation of antibiotics, pharmaceuticals and personal care products and their risks to the human health (Wu et al. 2014; Wu et al. 2015; Prosser and Sibley 2015; Hurtado et al. 2016). Antibiotics becomes persistent in the environment once they are released from any source. Because of their pseudo-persistent contaminant characteristics, these compounds show their constant presence and contribution in the environment, even if their half-lives are shorter (Sarmah et al., 2006; Van Boeckel et al., 2015). Groundwater and farm soils are considered as two primary reservoirs of antibiotic residues (Pan and Chu 2017).

The residues of antibiotics make their own way into the soil and in aquatic systems once they are released from manure or other source and then taken up by plants consequently. Depending on the concentration and exposure time of antibiotics, different plants and their organs/tissues respond differently (Hu et al., 2010). Migliore et al. (2010) conducted a study on *Lythrum salicaria* and reported that sulfadimethoxine phyto-toxicity varied from organ to organ. Some parts of the plant, such as the roots, cotyledons and petioles, are more prone to toxic effects. Whereas, at low concentrations, leaf length and internodes show an increase in growth (Michelini et al. 2012; Tasho and Cho 2016). Many studies on antibiotic uptake and toxicity have been conducted on lettuce, maize, spinach, onion, and tomato (Azanu et al. 2016; Qiu et al. 2019). Accumulation of veterinary antibiotics or pharmaceutical antibiotics in plants is complex and it fluctuates with species and tissues (Hillis et al. 2011; Zhang et al. 2017). The previous studies on the antibiotic's contaminations have demonstrated that dissipation of antibiotics depends on the environmental factors, like pH, temperature or physiochemical characteristics of soil and also intrinsic properties of antibiotics (Zhao et al. 2018). Antibiotics are low volatile compounds which results in more absorption by the roots, thus their toxic effects are more prominent at the root tip (Kumar et al. 2005; Xie et al. 2011; Li et al. 2013; Zhang et al. 2017). Antibiotic dissipation can also be influenced by the antibiotic concentrations in the different matrices (Srinivasan and Sarmah 2014). Interaction of antibiotics with organic matter and soil minerals is directly dependent on their chemical properties (Wegst-Uhrich et al. 2014). Studies have been designed and conducted to investigate antibiotics fate over time in ecosystem (Pan and Chu 2016a; Chen et al. 2018). From the previous studies there is a limited understanding regarding of antibiotic's uptake, transfer and bioaccumulation in the crops when they are irrigated with wastewater (Li et al. 2015; Song et al. 2017). Corresponding entry of antibiotic contaminants into the environment causes the health risks to humans

(Yang et al. 2017; Yan et al. 2020). In Pakistan, there is no baseline data on the monitoring and uncontrolled antibiotic discharge of hospitals wastewater into the agricultural lands resulting in high antibiotics contaminants, causing risks to human health and accumulation of antibiotics in crops.

The main objectives of this study were to: (i) examine the dispersal of two different antibiotics from wastewater to soil and crops; (ii) evaluate antibiotic uptake, transfer and translocation in five selected crops; (iii) compare the bioaccumulation of the two antibiotics at different concentrations in plant tissue; and (iv) assess the human risk of exposure to antibiotics.

2. Materials and methodology

2.1. Chemicals and antibiotics

LEV (500mg/100ml) and CIP (200mg/100ml) were purchased in the form of infusion from a pharmacy in Lahore. Stock and standard solutions were prepared by dissolving in the methanol at 100 mg L^{-1} and stored at -20^{0}C in the amber glass vials. Their molecular characteristics are shown in Table S1.

2.2. Experimental design

D. carota (carrot), *P. sativum* (peas), *R. raphanistrum* (radish), *L. sativa* (lettuce) and *S. oleracea* (spinach) were selected as objective crops because they have distinctive edible parts, economic value and frequent utilization as raw vegetable. Seeds of the selected crops were taken from the "Punjab Seed Certification Department", 4 Lytton Road, Lahore. Soil for seed growth was collected from botanic garden (Government College University, Lahore), sieved through a 2mm sieve. The nature of soil was "clay-loam" with an organic carbon content of 0.80% and pH between 6.24-6.42.

Pot experiments were conducted in the green house at Botanic Garden of GCU Lahore. For antibiotic accumulation, three treatments were prepared; low dose at 6 µg L^{-1} (LD6), medium dose at 20 µg L^{-1} (MD20) and high dose of 40 µg L^{-1} (HD40). Each plastic pot (15cm x 48cm) was filled with 2.3 kg of soil. Each crop was irrigated using 200 mL water mixture of LEV and 200 mL water mixture of CIP was spiked separately in the pots every other day (FAO, 2010), with three levels of doses. Control set up was not spiked with antibiotics, all the set ups were in triplicates. All experimental setups were performed at 25±2^{o}C, 60% relative humidity and 70% water holding capacity. Harvesting was done at crop maturity (carrot 120d; lettuce and peas 60d; spinach 45d and radish 20d).

2.3. Chemical extraction and Analysis

The plants were harvested and rinsed with deionized water to remove excess soil. Roots, shoots and leaves were separated from the plants and their biomass was measured. Before grinding, dry weight of every edible part of the

plant was measured. Extraction of antibiotics was done as described by Pan and Chu (2017c). For the analysis of soil samples, 1g dried and frozen soil was crushed, which were spiked with 100ml for each internal standard of 1.0 µg L^{-1}. Extraction buffer (30 mL) consisting of acetonitrile and citric acid (0.2M, v: v = 1:1, pH 4.4) was mixed with the soil samples. Samples were then mixed using a vortex mixer for 60 seconds, kept in an ultrasonic bath (40-60 kHz) for 15 mins and then centrifuged for 10 mins at 1370 x g. The supernatant was collected, and the above extraction procedure was repeated twice. Next, 10 ml Na_2EDTA was added to make dilution of 200mL with the Milli-Q water and filtered with GF/F filters (0.7 µm) (Zhao et al. 2019). Oasis HLB cartridges were used for solid phase extraction (SPE). Preconditioning of the SPE cartridges were done with methanol (10 mL) and Milli-Q water (10 mL), at a flow rate of 10 mL min^{-1}. Ethanol (10 mL) from the cartridge was used to rinse the analytes, concentrated using a gentle N_2-stream and re-dissolved in methanol (1 mL). Nylon syringe filters of 0.2 mm were used to filter the final extract into a 2 mL amber glass vial and stored at 18°C before analysis.

For plant tissue extraction, distilled water was used to rinse the outside of the fresh crops. The plants were then freeze-dried at -20°C. Subsequently, 1g tissue (root, stem and leaf) of each edible part of crop was crushed into a powder form and spiked with 100mL of each internal standard (1.0 µg L^{-1}). The samples of each crop were extracted thrice with 30mL of a mixed solution of acetone (v:v =1:1) and acidified acetonitrile (pH 3) by vortex mixing for 60 seconds , ultra-sonication for 15mins, followed by centrifugation in an air cooled condition for 15min at 12000xg. The extracts were combined and evaporated to remove organic solvents. After that 10 ml Na_2EDTA was added to make dilution of 200 mL with the Milli-Q water and filtered with GF/F filters preceding to SPE in duplicate as described above for the extraction of soil samples. Analysis of target antibiotics from the extracts of crops and soil samples was done by HPLC-MS Agilent Liquid Chromatography 1100 series (Pan and Chu, 2017c). The limit of quantification for LEV and CIP were 0.2 and 0.13 µg g^{-1}, respectively. To find out how much percentage of antibiotic dissipated in the soil we use percentage conversion method. Antibiotic dissipation in percentage was also calculated by ratio of total antibiotic accumulation to antibiotic dose injected as given in eq 1:

$$Antibiotic\ dissipation\ (\%) = \frac{\text{Total antibiotic in soil}}{\text{Dose injected}} \quad \ldots\ldots (1)$$

2.4. Measuring bioconcentration factor, translocation factor and human exposure

The estimation of antibiotic accumulation from the soil in the plant was measured through the formula for bioconcentration factor (BCF) as given in eq 2:

$$BCF = \frac{\text{Concentration in crop tissue (µg/kg)}}{\text{Concentration in soil (µg/kg)}} \quad \ldots.. (2)$$

153 The antibiotic translocation from roots to the shoots, or potentially into the leaves, was measured by a formula
154 known as translocation factor (TF) as given in eq 3. (Pan and Chu, 2017c).

155 $$TF = \frac{\text{Concentration in leaf/shoot (µg/kg)}}{\text{Concentration in root (µg/kg)}} \quad \text{..... (3)}$$

156

157 The human exposure level was measured through the formula given in eq 4 (Pan and Chu, 2017a):

158 $$\text{Human exposure} = C \times D \times W \times T \quad \text{.... (4)}$$

159 Where C = antibiotic concentration in the edible parts (ug kg^{-1} wet weight); D = daily average consumption of
160 edible parts of crops (wet weight/kg body weight day); W = person's weight (kg); T = exposure time in days.
161 The antibiotic concentration in crops was obtained by converting them into fresh weight data by using the average
162 water content of each crop; carrot (88.3%), lettuce (94.6%), peas (88.89%), spinach (91.4%) and radish (95.2%).
163 The annual exposure of an average 70 kg human was assessed by using average daily intake of edible crops (0.30,
164 0.35, 0.23, 0.5 and 0.5 g wet weight per kg body weight per day for carrot, peas, lettuce, spinach, and radish,
165 respectively).

166 **2.5. Human health risk estimation**

167 The evaluation of human health risk was assessed by following the guidelines of medicine products recommended
168 by European agency (Grung et al. 2008). The risk quotient (RQ) is the ratio of predicted environmental
169 concentrations (PEC) or measured environmental concentrations (MEC) to the PNEC (predicted no environmental
170 concentration) and can be calculated using the formula given in eq 5:

171 $$RQ = \frac{MEC}{PNEC} \quad \text{.... (5)}$$

172 For the risk estimation, criteria for RQ have been set under which risk will be categorized as low, medium or high.
173 Values between 0.01-0.1 were classified as low risk; between 0.1-1 as medium risk; and values greater than 1 as
174 high risk. MEC was calculated from the measured antibiotic concentration and PNEC was taken from reported
175 values (Ashfaq et al. 2017; Riaz et al. 2017).

176 **2.6. Statistical analysis**

177 Data was analyzed using a two-way analysis of variance (ANOVA) using SPSS version 16, including factors of
178 two types of antibiotics and five types of crop species under three different antibiotic dose treatments. ANOVA
179 was used to check the homogeneity of all variances at the significance level of 5%.

3. Results and discussion

3.1. Crop Biomass

Crop biomass was calculated to assess negative impacts of wastewater polluted with antibiotics on the different crops. The fresh weight of each crop (crop tissue) is presented in Fig 1, Table S2, Table S6d and S6e. No increase or decrease in biomass was observed of each crop between the antibiotic contaminated water and control, which suggests that no phytotoxicity was caused by the antibiotics at any concentration. Significant differences in the biomass of all crops were found ($p < 0.05$) which showed low phytotoxicity among crops (Pan and Chu 2017c).

3.2. Antibiotic uptake by plants

The antibiotic concentration trend in the tissues of the five crops is shown in Fig 2. LEV and CIP were detected in all above and underground edible parts of the crops in variable concentration (Table S3, S6a, S6b & S6c). The concentration of LEV was detected in carrot (1.22-12.9 μg kg^{-1}), peas (0.77-14.33 μg kg^{-1}), radish (0.73-13.81 μg kg^{-1}), lettuce (0.73-12.66 μg kg^{-1}) and spinach (0.93-11.45 μg kg^{-1}). For CIP accumulated concentration was different, carrot (0.73- 13.10 μg kg^{-1}), peas (0.79-14.05 μg kg^{-1}), radish (0.79-12.68 μg kg^{-1}), lettuce (0.72-12.27 μg kg^{-1}) and spinach (0.63-15.57 μg kg^{-1}). In roots, both targeted antibiotics were detected in carrot, peas, radish, lettuce and spinach, and at different concentration ranges for LEV, accumulation ranges from 1.22-12.9, 0.89-3.53, 1.43-13.81, 0.73-5.59 and 0.93-5.22 μg kg^{-1}, respectively. While for CIP, the accumulation ranges from 2.66-13.10, 1.13-3.12, 2.91-12.68, 0.72-6.49, 1.11-4.86 μg kg^{-1}, respectively. In the HD treatments (40 μg L^{-1}), both antibiotics were detected in all five crop tissues and in a higher concentration than other two treatments. LEV was found with high concentration in tissues of peas, radish and spinach ranged from 3.67-28.23, 2.95-26.69 and 4.08-23.53 μg kg^{-1} whereas, CIP ranged from 3.96-29.22, 3.97-25.85 and 4.34-25.67 μg kg^{-1} in peas, spinach and radish, respectively. The concentration of CIP in other two crops was found slightly higher than LEV in peas ranged from 3.96-29.22 μg kg^{-1}. The distribution of LEV and CIP in edible parts of crops under these three treatments of wastewater were as follows: leaves > roots > shoots for lettuce and spinach. Whereas, in carrot and radish it was roots > leaves > shoots. In peas the bioaccumulation was found to be high in leaves than roots as they have different edible parts. Similarly, high accumulation of tetracycline and sulfamethoxazole is reported in cherry tomato, lettuce and cucumber (Ahmed et al. 2015). Pan and Chu (2017b) reported that wastewater (contaminated with antibiotic) and animal manure used in agriculture cause high uptake and accumulation of antibiotics in crops. Similar to this study, number of other studies has also reported CIP in vegetable farming soil at 0.11-0.52 μg g^{-1} (Li et al. 2014; Wu et al. 2014; Liu et al. 2018), ciprofloxacin and ofloxacin in carrot (0.51-0.85 μg kg^{-1}) (Hussain et al. 2016). High concentration of antibitics in soil may cause high accumulation in the

edible crops, due to their different molecular structure, resistance, adsorption and half-lives in soils (Sun et al. 2021).

3.3. Antibiotic dissipation

Antibiotics were analyzed in the pot soil, after the harvesting of crops (Fig. 3). The crops, antibiotic types, antibiotic concentration and interaction between crops and antibiotic are all statistically significant (crops $p < 0.05$; antibiotic $p < 0.05$; crops x antibiotic $p < 0.05$, Table S6l). The concentration of LEV dissipated was 11.67-26.67%, 13.01-22.03% and 12.75-21.50% in LD6, MD20 and HD40, respectively. Whereas dissipation of CIP showed 15.02-21.67%, 15.03-20.08% and 14.75-22.05% in LD6, MD20 and HD40, respectively. Both antibiotics had shown a high rate of dissipation under low dose treatments. The response in all crops are different, for LEV the dissipation rate was as follows radish = Carrot > lettuce = pea = spinach. The principal mechanism of fluoroquinolone dissipation in the soils would be the degradation by microbes (Liao et al. 2016). In previous studies, dissipation rate of sulfonamides or fluoroquinolones are faster (Pan and Chu 2016b). In a pot study, fluoroquinolones were found to be more persistent (237-336 μg kg^{-1}) than other antibiotics, as compared to other study done for rice-wheat system (Yang et al. 2018). This demonstrates that crops can boost the absorption of antibiotics from contaminated soils. According to the study conducted by Yang et al. (2012) the antibiotics may be retained in the soil during crop growth. Their concentration in soil increases with increasing dose. Studies reported that dissipation rate depends on the concentration of antibiotics in the soil, its bio-availability (Pan and Chu, 2017), uptake by plants and microbial degradation (Li et al., 2016). Similarly, other factors that contribute in dissipation rate include hydrolysis (Yang et al., 2018), photolysis, biodegradation and fluidity (Xu et al., 2021). Electro-philicity of CIP hydroxyl group is also reported to enhance dissipation (Liu et al. 2019).

3.4. Bioconcentration factor

Bioconcentration factor (BCF) for antibiotic accumulation in different parts of five crops are shown in (Fig. 4). $BCF_{(leaf)}$ was maximum by lettuce (0.5) and least by raddish (0.1) in CIP and in LEV the least was observed in carrot. This is very alarming as lettuce leaves are edible. All crops differ significantly ($p < 0.05$) in their $BCF_{(leaf)}$ (Table S6j). The bioaccumulation of CIP is higher than LEV in the roots of the five crops, under the low dose treatment experiment. $BCF_{(root)}$ of crops under high dose treatment showed lowest accumulation than other two treatments. The lowest $BCF_{(root)}$ was observed in pea. strangely the crop whose root is edible showed higher $BCF_{(root)}$ like radish (CIP, 0.5) and carrot (CIP, 0.45). This phenomenon of bio-accumulating more antibiotic in edible roots is alarming, as they raise health concerns in humans. Statistical analysis (ANOVA) reveled that all the 5 crops and 3 treatments differ significantly ($p < 0.05$) for $BCF_{(root)}$ (Table S6h).

In crop shoots (BCF_{shoot}), highest values were found in carrot (LEV 0.42, CIP 0.39) and low values are observed in peas (LEV 0.042, CIP 0.044). BCF_{shoot} shows statistical significance among crops ($p < 0.05$) and antibiotic ($p < 0.05$) (Table S6i). Comparable to the previous studies (Boonsaner and Hawker, 2012; Azanu et al. 2016), it is observed that antibiotic bioaccumulation increases when exposure concentration increases in the soil. Overall, bioaccumulation of CIP was found higher than LEV in the different crops. The antibiotic uptake and translocation in all five crops varied significantly ($p < 0.05$). The BCF of pharmaceutical and personal care products tends to be higher in plants producing cereal as compared to vegetables or fruits (Pullagurala et al. 2018a). Mikes et al. (2009) reported that BCF values were high in plants and soils when exposed to low levels of antibiotics, and the value decreased with the increase of antibiotics. Azanu et al. (2016) concluded that a higher BCF value means plant has accumulated more antibiotics and will pose more risk of human health. Different studies have studied various factors like biotic and abiotic factors, which can effect uptake and absorption of antibiotics by plants. Uptake and translocation of the antibiotic was shown to be higher in both barley and carrot (Eggen and Lillo 2012; Pullagurala et al. 2018b)

3.5. Translocation factor

LEV and CIP distribution in five crops i.e carrot, peas, radish, spinach, and lettuce found to be in a different pattern as according to their edible parts (Fig. 5). The LEV showed high translocation in spinach and lettuce whereas CIP showed the high TF in spinach. This suggests that transport of antibiotics is dependent on the transpiration stream of plant vascular tissue. The uptake by plants is considered to occur by passive transport. This process will then distribute the antibiotic, or other chemical, to other parts of the plant. The root to leaf concentration ratio depends on the amount of water transpired and the rate of plant growth (Pan et al. 2014). The translocation factor (TF) for LEV and CIP was generally >1 for all five crops under LD treatment. All the crops and treatments differed significantly from one another (species $p < 0.05$; dose $p < 0.05$; species x dose $p < 0.05$). A translocation factor larger than 1 was reported for chloramphenicol in corn, rice and water spinach (Pan et al. 2014). The overall TF values for LEV and CIP ranged from 0.86-9.06 and 0.98-8.61, respectively. The results specify that the higher the antibiotic concentration, the greater the translocation in different parts of crops (Table S6k). Antibiotic translocation in crops depends on two major parameters; physical and chemical properties of crops and antibiotics as well as the antibiotic concentrations in the soil (Pan et al. 2014). Antibiotic accumulation and distribution in the crops can be affected by the type of species and growth stage (Wu et al. 2014). Hillis et al. (2011) reported ten different antibiotic effects on the growth of plants and he suggested that no significant effects

has been observed on the germination process even at high dose of antibiotic (10000 µg L^{-1}). Similarly, our study concluded that plants did not show growth inhibition even at highest dose (40 µg L^{-1}).

Pan and Chu, (2017c) studied the TFs for different antibiotic accumulation under antibiotic contaminated water treatments and observed relatively high accumulation. Values for TFs for wastewater and manure treatment ranged from 0.1-4.8 and 0.1-2.1, respectively. The overall results of our study propose that transpiration is the vital factor for translocation of antibiotics after uptake by roots. Wu et al. (2013) reported that high translocation is possibly due to the hydrophilic characteristic of compounds, which are mobile in the xylem. It is noted that the process of antibiotic uptake and translocation varies from species to species. For example, the uptake of norfloxacin and sulfamethoxazole, and their translocation were observed to be higher in radish as compared to Chinese cabbage at the same dosage (Wang et al. 2016; Jia et al. 2018). The crop type, concentration of antibiotics and physio-chemical properties of antibiotics (e.g K_{ow}) of LEV (-0.39) and CIP (1.32) can also effects the uptake and accumulation in the crops (Sitovs et al., 2021).

3.6. Human health risk estimation

The average daily human exposure of LEV and CIP by consuming edible parts of five different crops ranged between 1.11 - 46.87 ng (Fig. 6 and Table S4 and S6m). Both antibiotics were found in all crops, however CIP was detected at relatively high concentrations in the crops compared to LEV ranging from 2.25-46.87 and 1.11-43.89 ng (daily human exposure), respectively. Taking into consideration the daily human consumption of the five edible crops, annual human exposure was estimated in the range of 0.40-17.1 µg. The annual exposure values for both antibiotics in this study were found to be relatively high in the edible parts of spinach and carrot (2.41-17.1 and 1.59-17.2 µg, respectively). The risk quotient for both LEV and CIP (Fig. 7 and Table S5) showed low to medium risk to human health because the range of the effect falls between the 0.1-1. From risk quotient values, it can be concluded that both antibiotics can cause low to medium risk to human health. Still there is a lack of a complete study on the assessment of risks to human health related to exposure of antibiotics (Malchi et al. 2015).

4. Conclusion:

This study concludes that, when crops are irrigated/exposed to wastewater (contaminated with CIP and LEV), the crops are capable of accumulating the antibiotics. Different antibiotics showed different uptake and translocation rate/phenomenon in different crops because of the difference in their physio-chemical properties (e.g log Kow) . Both antibiotics were accumulated by all crops at different concentrations and highly absorbed by the leaves and shoots. The results show that antibiotic residues in the edible tissues of crops depend upon the concentration in the irrigation water. Moreover, crops irrigated with antibiotic polluted water can cause high risk, if not treated

properly before using for irrigation purpose. Both targeted antibiotics LEV and CIP showed low to medium risk to human health. In addition, they show that there is little evidence of hidden risk and that information about their effects on the health of humans is restricted. However, daily treatment with antibiotics should be taken in consideration to measure the risk of their accumulation in the body. The results of this study contribute to a better understanding of the fate of antibiotic contamination in irrigation water and the factors which play role in the antibiotics' accumulation in crop tissues and pose risk to humans.

Ethical Approval

The research did not involve human participants and animals. No ethical approval applicable.

Consent to Participate

"Consent to participate" not applicable. The research did not involve human participants.

Consent to Publish

All authors agreed with the content and that all gave consent to submit and we have obtained consent from the responsible authorities at the University where the work has been carried out, **before** the work is submitted.

Authors Contributions

M.F, F.S and A.W planned this research. M.F and NMD. conducted this research. M.F, A.W, L.C, F.S and NMD analyze the data. All authors contributed in research paper writing

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Competing Interests

Dr. Muhammad Farhan has received research grants from Government College University Lahore Pakistan. Dr. Muhammad Farhan has received 'Professor J.H. Orbison Medal' from Forman Christian College (A Chartered University) Lahore. Dr. Muhammad Farhan has received 'Best Teacher Award' from Forman Christian College (A Chartered University) Lahore. Dr. Abdul Wahid has received 'Best Teacher Award' from Higher Education Commission Islamabad Pakistan. Dr. Abdul Wahid has received 'Out Standing Researcher Award' from Pakistan Science Foundation

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

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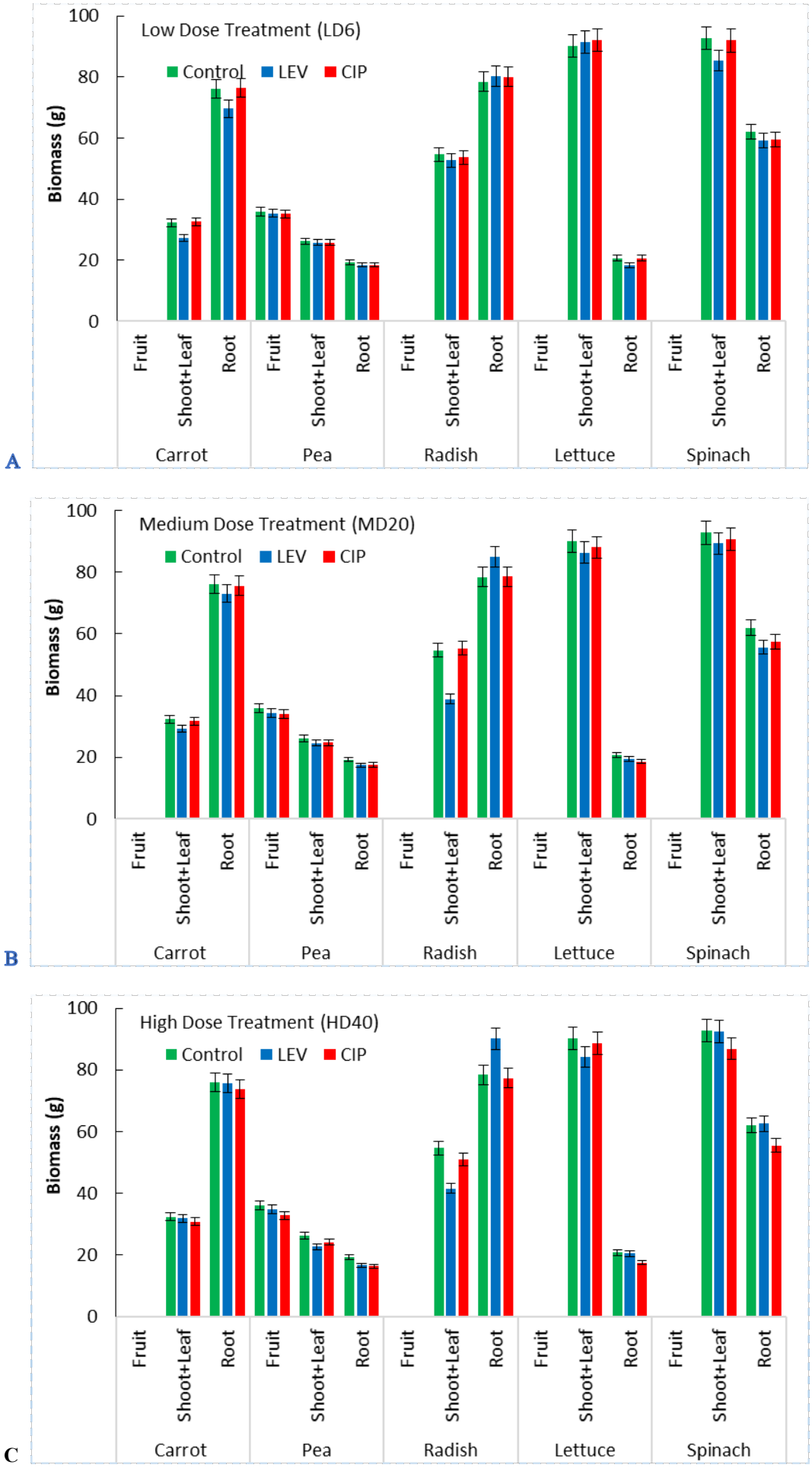


Fig 1. Biomass of crops edible parts (g/pot fresh weight) A) Low dose treatment: 6 μgL^{-1} antibiotic concentration B) Medium dose treatment: 20μgL^{-1} antibiotic concentration C) High dose treatment: 40μgL^{-1} antibiotic concentration.

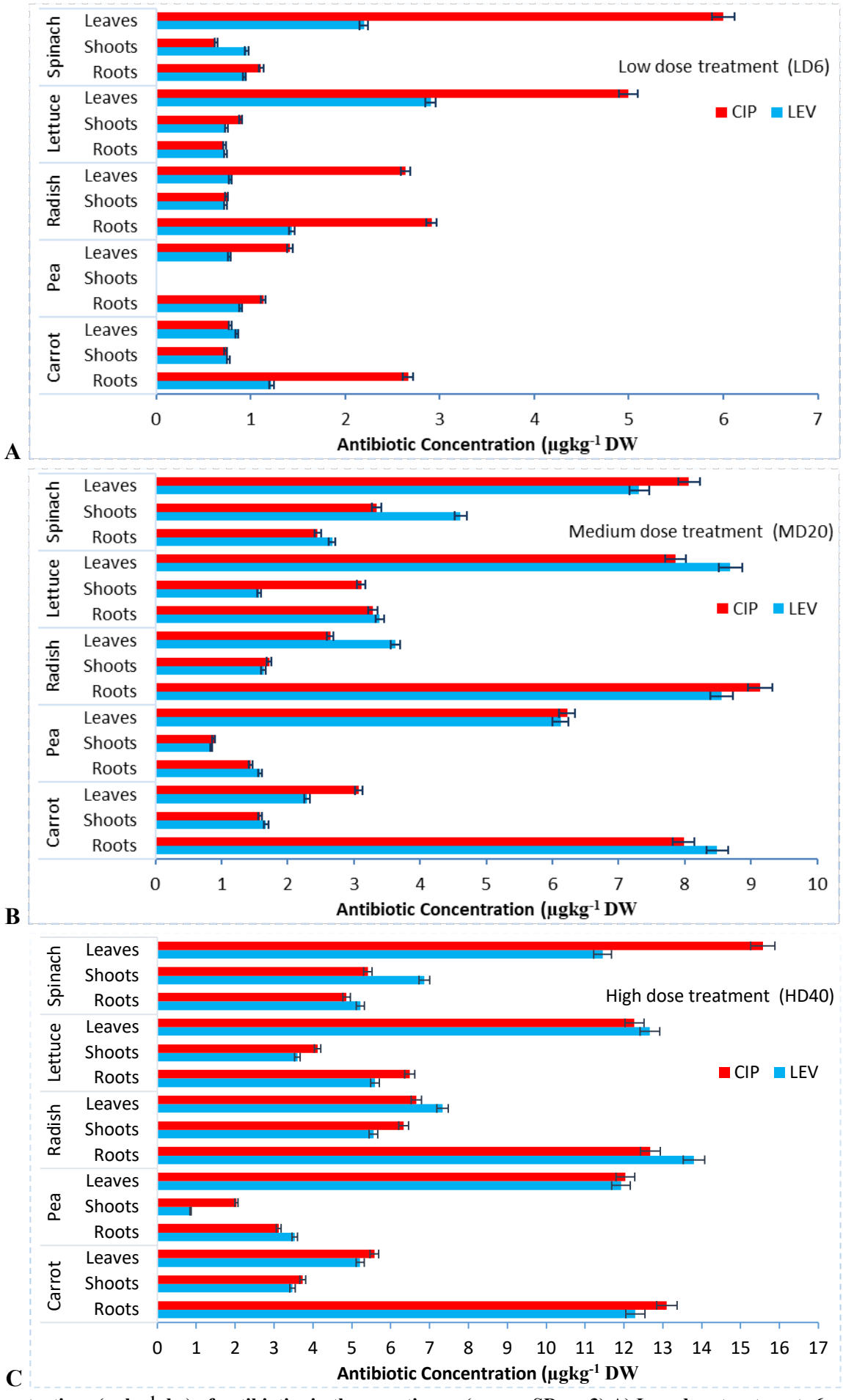


Fig 2. Concentrations (µgkg^{-1} dw) of antibiotics in the crop tissues (mean ±SD, n=3) A) Low dose treatment: 6 µg L^{-1} initial antibiotic concentration as wastewater treatment B) Medium dose treatment: 20 µg L^{-1} antibiotic concentration as wastewater treatment C) High dose treatment: 40 µg L^{-1} antibiotic concentration as wastewater treatment

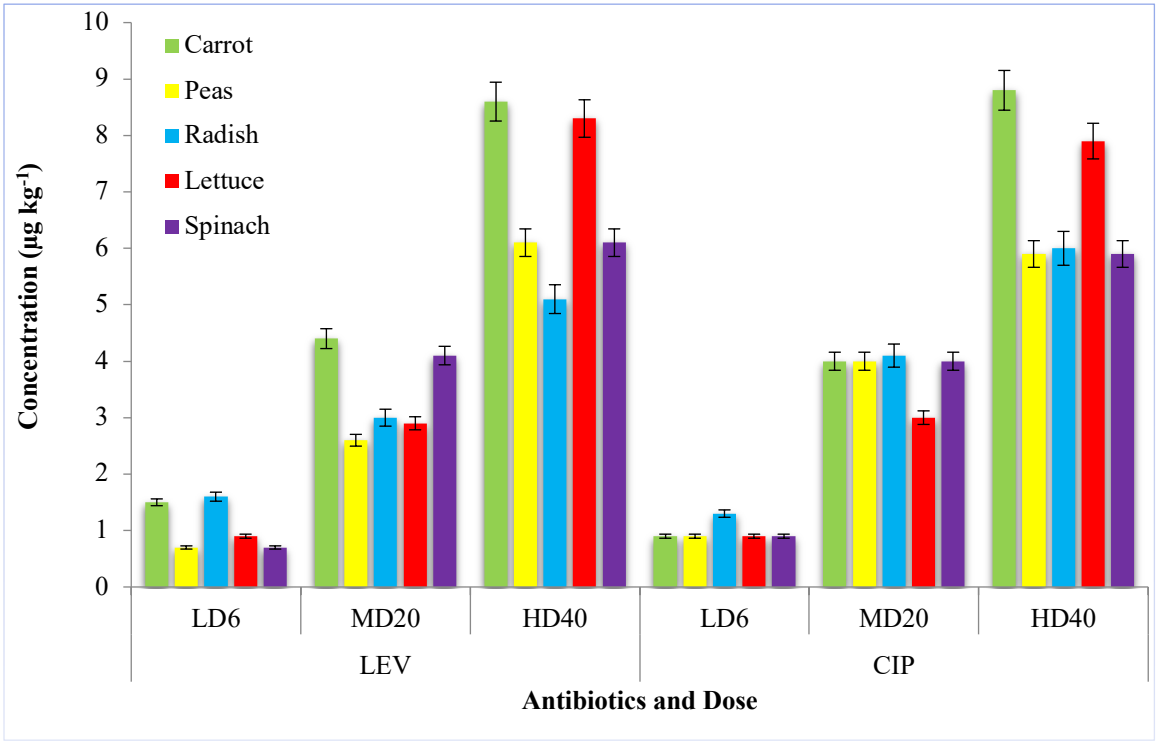
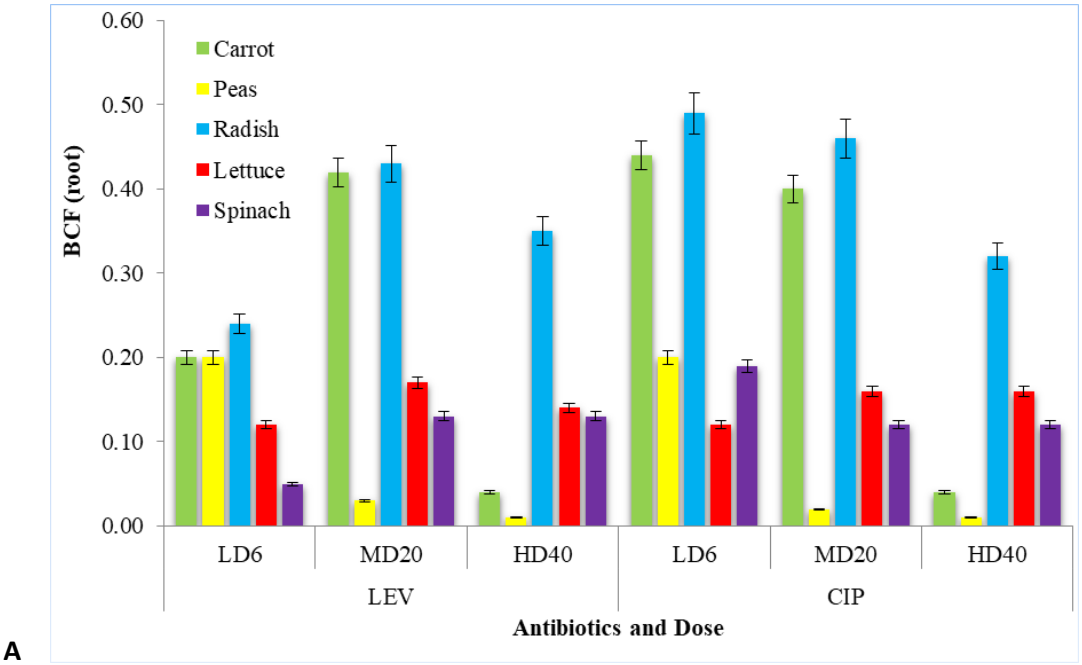
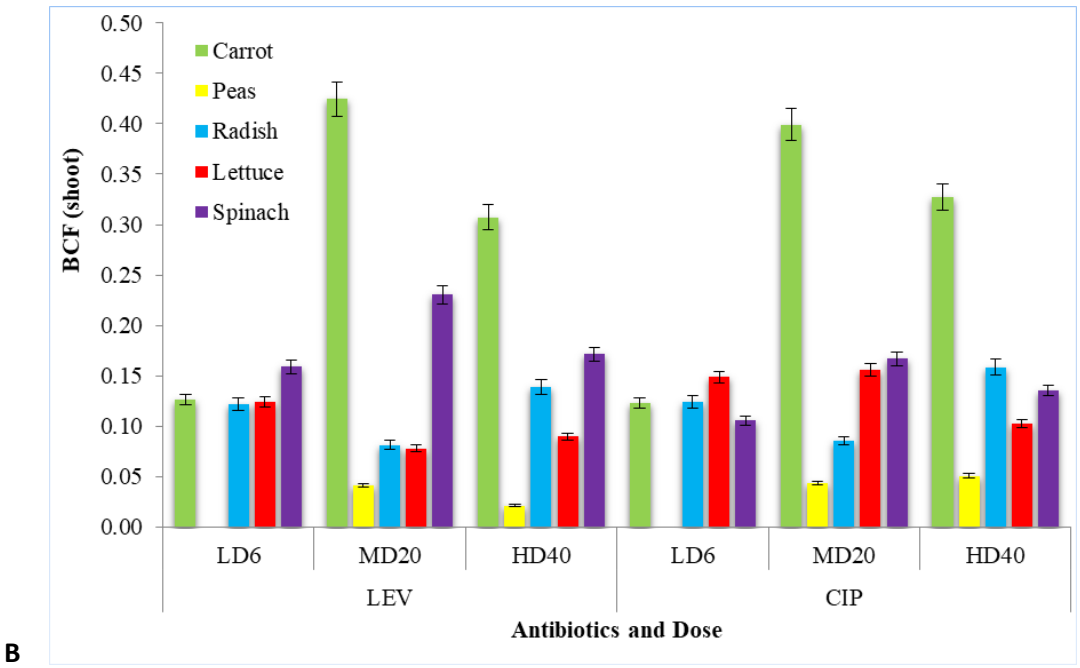


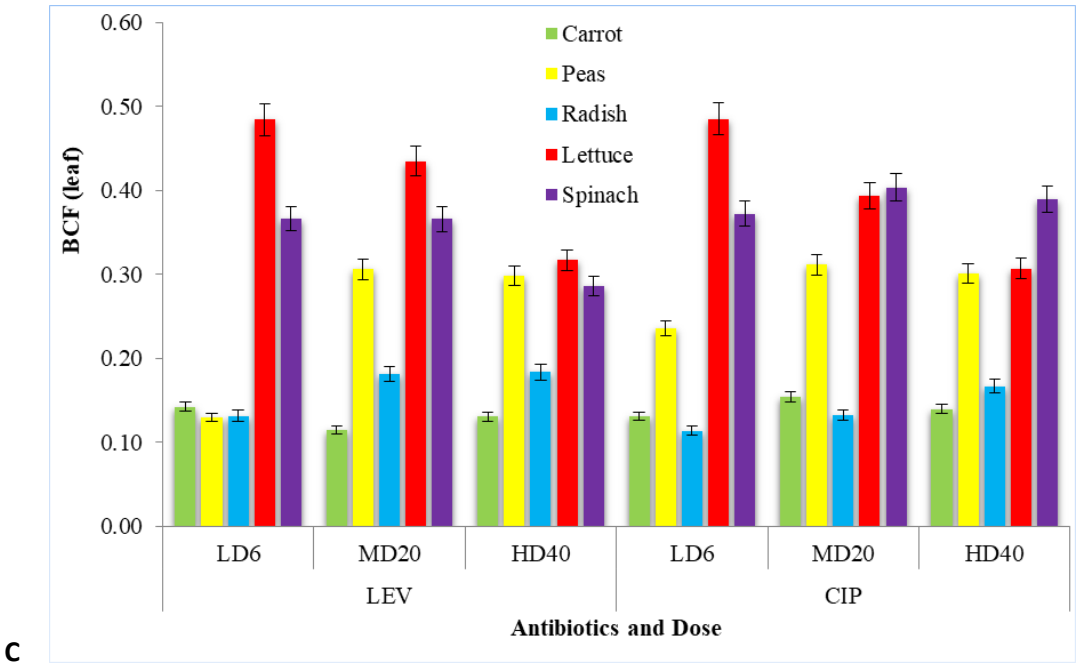
Fig 3. Antibiotic concentration in the soil treated by target antibiotics under three different treatments after harvesting of the crops (LD, MD and HD: 6ugL^{-1}, 20ugL^{-1} and 40ugL^{-1} respectively)



A



B



C

Fig 4. Bio concentration factor (BCF) values for the target antibiotics in different parts of crops A) BCF (root) B) BCF (shoot) C) BCF (leaf)

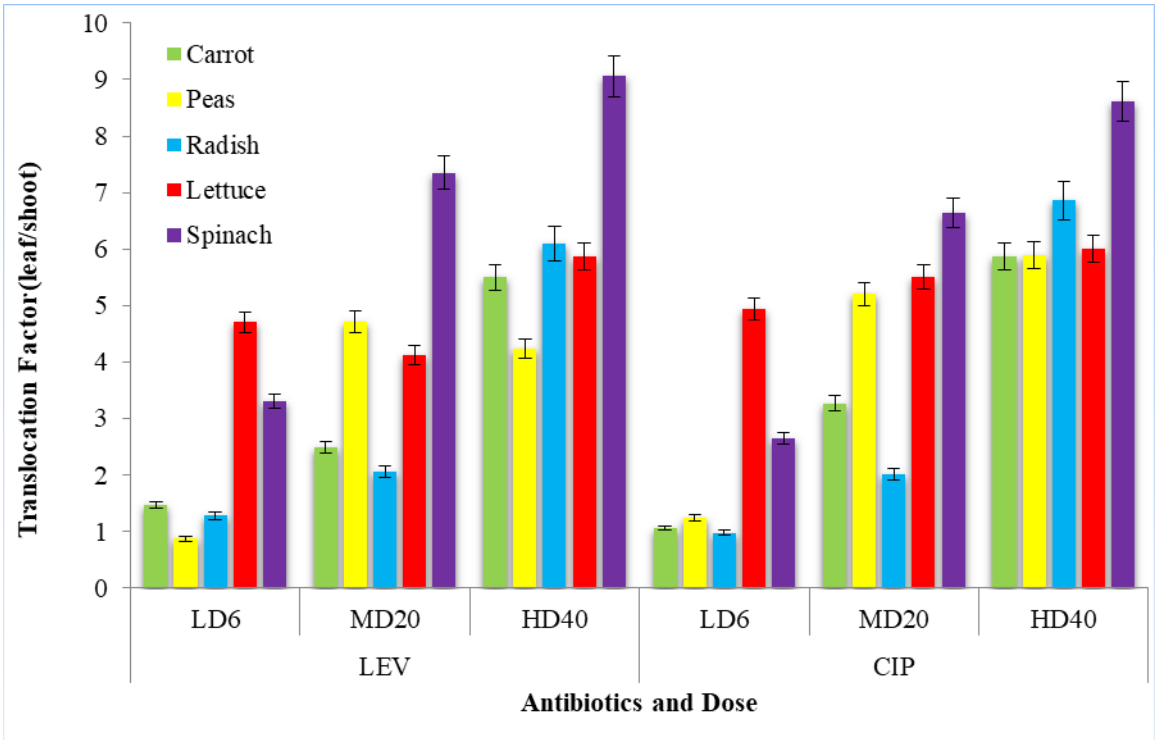
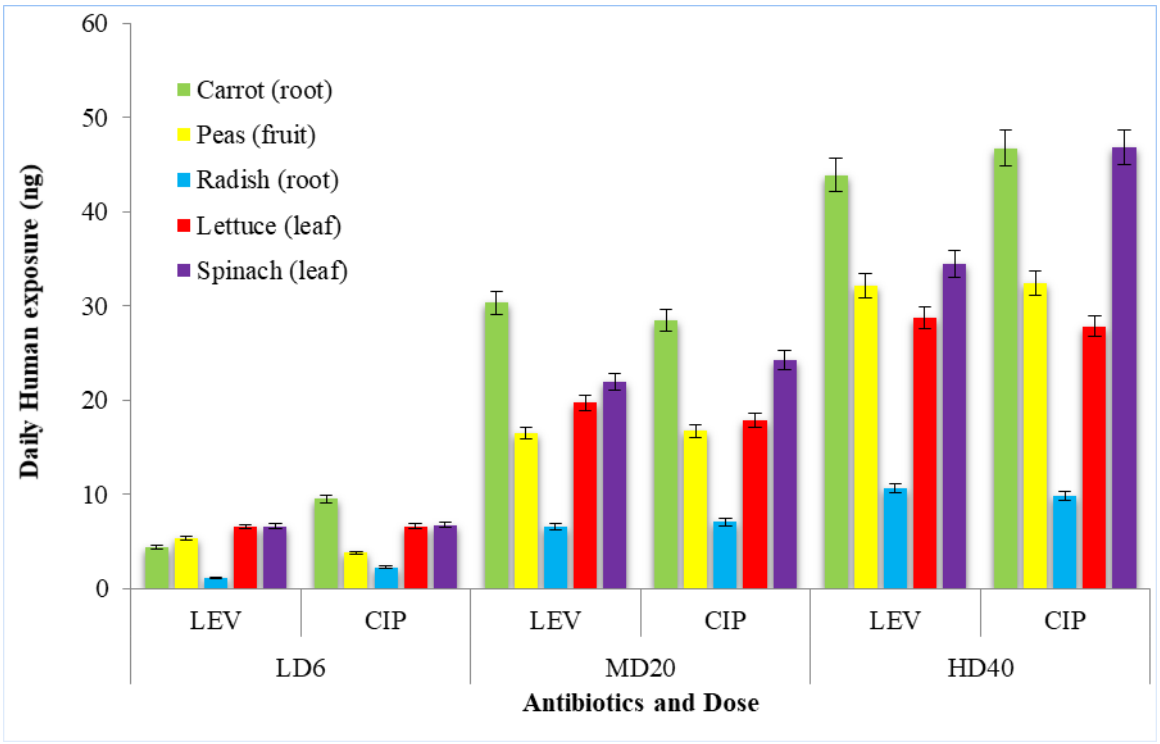


Fig. 5. Translocation factor of antibiotics in five crops (species $p < 0.05$; antibiotic $p < 0.05$; species x antibiotic $p < 0.05$)

A



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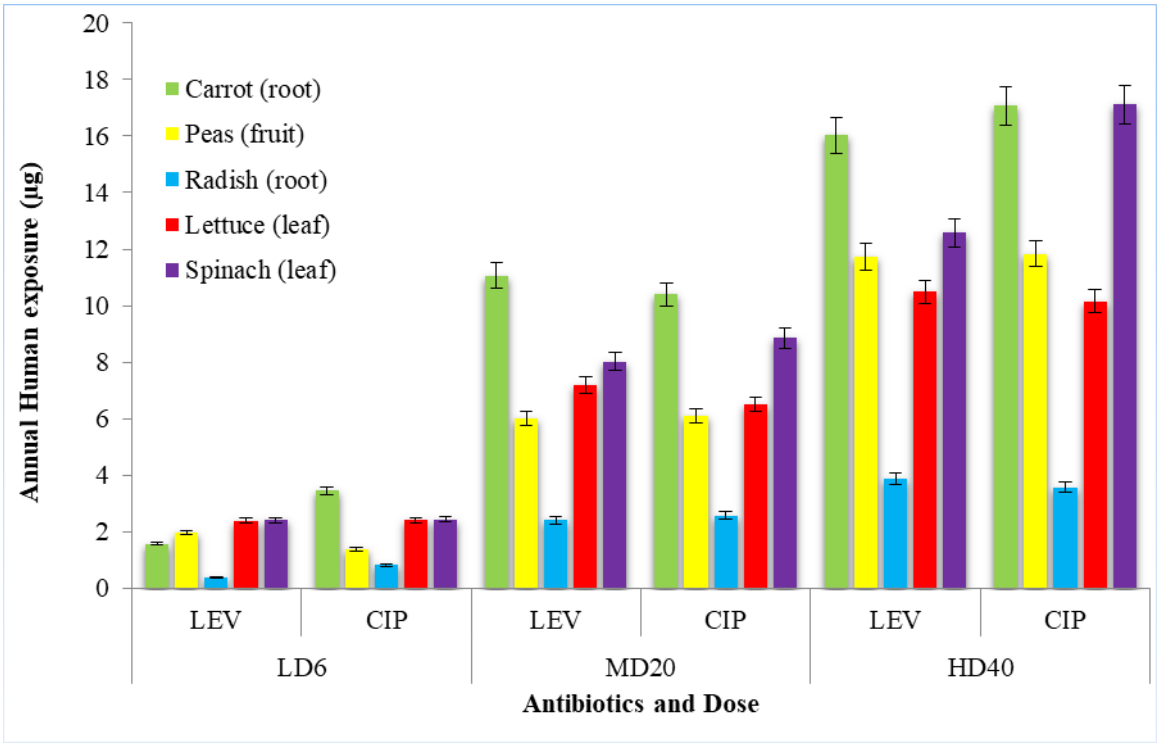
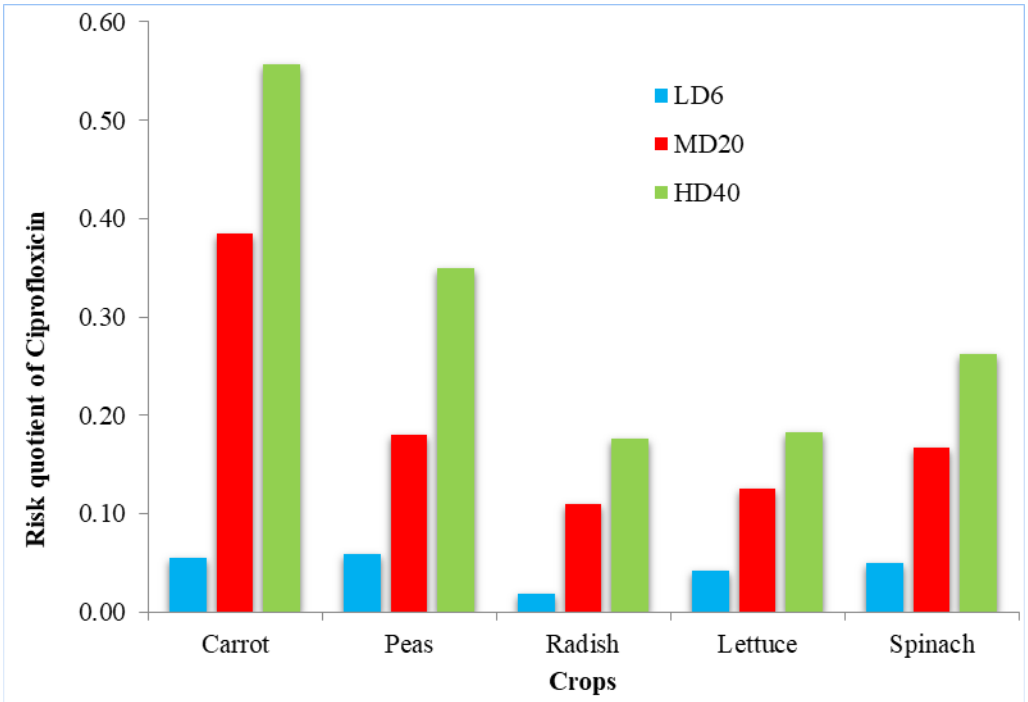


Fig 6. Human exposure to antibiotics via five crops on daily and annual consumption basis. LD6 = 6µg L^{-1} , MD20 = 20µg L^{-1}, HD40 = 40µg L^{-1} A) Daily human exposure (µg) B) Annual human exposure (µg)

A



B

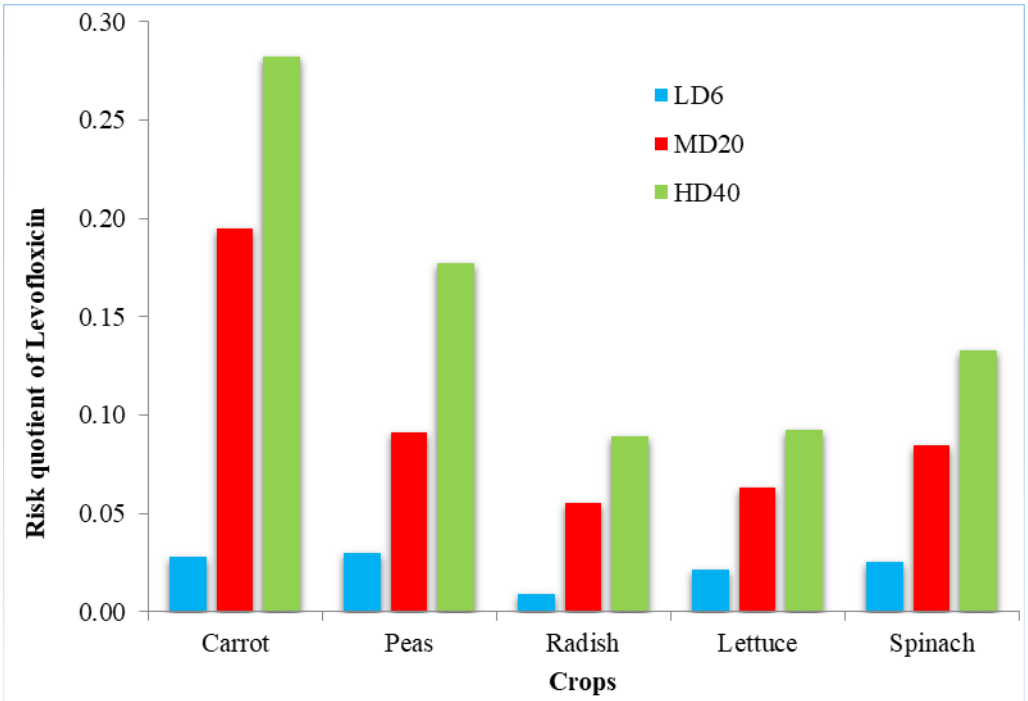


Fig 7. Risk quotient of Ciprofloxacin (CIP) and Levofloxacin (LEV) on five crops. LD6 = 6µg L^{-1} , MD20 = 20µg L^{-1}, HD40 = 40µg L^{-1} of antibiotics A) Risk quotient of ciprofloxacin B) Risk quotient of levofloxacin

Table S1. Physicochemical properties of the antibiotics investigated in this study.

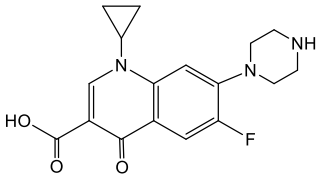
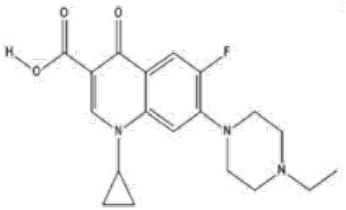
Compound	Molecular formula	Molecular weight	pK_a	$logK_{ow}$	Structure	References
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331.34	6.09 8.74	1.32		(Jia et al., 2018)
Levofloxacin	$C_{18}H_{20}FN_3O_4$	361.37	2.1	-0.39		(Sitovs et al., 2021)

Table S2: Biomass of crops edible parts (g/pot fresh weight).

	LEVOFLOXACIN				CIPROFLOXACIN		
Crop	**Treatments**	**FRUIT**	**SHOOT/ LEAF**	**ROOT**	**FRUIT**	**SHOOT/ LEAF**	**ROOT**
CARROT	Control (0)	NA	32.33±1.07	76.07±0.13	NA	32.63±0.25	76.07±0.13
	6 µg/L (LD)	NA	27.16±0.80	69.6±0.43	NA	32.63±0.25	76.46±0.37
	20 µg/L (MD)	NA	29.23±0.30	73.06±0.47	NA	31.76±0.15	75.63±0.25
	40 µg/L (HD)	NA	31.83±0.20	75.76±0.76	NA	30.8±0.2	73.66±0.20
PEAS	Control (0)	35.46±0.51	25.6±0.3	18.83±0.20	36.16±0.25	27.63±0.25	19.16±0.51
	6 µg/L (LD)	35.4±0.34	25.8±0.1	18.46±0.37	35.1±0.75	25.8±0.1	18.46±0.37
	20 µg/L (MD)	34.4±0.43	24.56±0.25	17.43±0.2	33.96±0.70	24.73±0.37	17.56±0.35
	40 µg/L (HD)	34.76±0.15	22.7±0.26	16.6±0.36	32.83±0.58	24.13±1.00	16.3±0.43
RADISH	Control (0)	NA	90.6±0.3	16.2±0.3	NA	89.43±0.15	21.16±0.80
	6 µg/L (LD)	NA	91.33±1.12	18.33±0.45	NA	92.06±0.68	20.7±0.79
	20 µg/L (MD)	NA	86.36±0.86	19.46±0.35	NA	88±1.58	18.63±0.25
	40 µg/L (HD)	NA	84.2±0.86	20.4±0.75	NA	17.53±1.00	17.53±1.00
LETTUCE	Control (0)	NA	90.6±0.3	59.5±0.26	NA	128.53±0.50	55.56±0.35
	6 µg/L (LD)	NA	85.43±0.41	59.3±0.32	NA	127.46±0.55	54.61±0.3
	20 µg/L (MD)	NA	89.3±026	55.6±0.43	NA	129.73±0.80	55.23±0.35
	40 µg/L (HD)	NA	92.36±0.30	62.6±0.26	NA	127.96±0.65	51.03±0.66
SPINACH	Control (0)	NA	54.56±0.35	78.06±0.20	NA	55.56±0.35	79.1±0.62
	6 µg/L (LD)	NA	52.1±0.2	80.26±0.65	NA	54.6±0.3	80.03±0.5
	20 µg/L (MD)	NA	38.9±0.81	84.93±0.65	NA	55.23±0.35	78.6±0.4
	40 µg/L (HD)	NA	41.53±0.40	90.06±0.55	NA	51.03±0.66	77.36±0.97

C: Control treatment; NA: not available; LD: 6 µg/L initial antibiotic concentration as wastewater treatment; MD: 20 µg/L antibiotic concentration as wastewater treatment; HD: 40 µg/L antibiotic concentration as wastewater treatment.

Table S3. Concentrations (μg/kg dw) of antibiotics in the crop tissues (mean ±SD, n=3). LD6, MD20 and HD40: 6, 20 and 40 μg/L initial antibiotic concentrations as wastewater treatment.

	Crops	Organ	LEV	CIP
LD6	**Carrot**	Roots	1.22±0.09	2.66±0.40
		Shoots	0.76±0.12	0.73±0.15
		Leaves	0.85±0.06	0.78±0.11
		Soil	1.48±0.30	0.86±0.28
	Pea	Roots	0.89±0.02	1.13±0.49
		Shoots	0.77±0.10	0.79±0.12
		Leaves	2.01±0.10	1.92±0.02
		Soil	0.73±0.15	0.93±0.151
	Radish	Roots	1.43±0.27	2.91±0.02
		Shoots	0.73±0.19	0.74±0.08
		Leaves	0.78±0.12	0.68±0.16
		Soil	1.60±0.36	1.33±0.25
	Lettuce	Roots	0.73±0.11	0.72±0.13
		Shoots	0.74±0.06	0.89±0.01
		Leaves	2.90±0.26	2.91±0.2
		Soil	0.91±0.06	0.86±0.25
	Spinach	Roots	0.93±0.15	1.11±0.26
		Shoots	0.95±0.07	0.63±0.30
		Leaves	2.19±0.12	2.23±0.22
		Soil	0.73±0.15	0.93±0.15
MD20	**Carrot**	Roots	8.49±0.56	7.98±0.13
		Shoots	1.67±0.11	1.58±0.39
		Leaves	2.29±0.16	3.07±0.16
		Soil	4.41±0.13	4.01±0.50
	Pea	Roots	1.58±0.11	1.63±0.25
		Shoots	2.22±0.26	2.40±0.45
		Leaves	9.21±0.29	8.77±0.55
		Soil	2.62±0.32	4.01±0.45
	Radish	Roots	8.55±0.36	9.13±0.08
		Shoots	1.63±0.22	1.71±0.15
		Leaves	3.62±0.13	2.64±0.42
		Soil	3.01±0.10	4.07±0.14
	Lettuce	Roots	3.39±0.22	3.28±0.41
		Shoots	1.56±0.31	3.11±0.02
		Leaves	8.69±0.40	7.86±0.25
		Soil	2.88±0.11	2.98±0.11
	Spinach	Roots	2.67±0.11	2.45±0.39
		Shoots	4.61±0.29	3.34±0.48
		Leaves	7.31±0.26	8.06±0.06
		Soil	4.13±0.20	4.01±0.45
HD40	**Carrot**	Roots	12.9±0.43	13.10±0.35
		Shoots	3.47±0.27	3.74 ±0.18
		Leaves	5.21±0.11	5.58 ±0.36
		Soil	8.60±0.14	8.82±0.34
	Pea	Roots	3.53±0.20	3.12±0.20
		Shoots	4.56±0.25	3.91±0.20
		Leaves	14.33±0.98	14.05±0.16
		Soil	6.11±0.20	5.86±0.15
	Radish	Roots	13.81±0.09	12.68±0.16
		Shoots	5.56±0.30	6.33±0.15
		Leaves	7.33±0.25	6.66±0.25
		Soil	5.10±0.04	6.01±0.03
	Lettuce	Roots	5.59±0.30	6.49±0.46
		Shoots	3.06±0.03	4.12±0.22
		Leaves	12.66±0.20	12.27±0.39
		Soil	8.33±0.30	7.87±0.26
	Spinach	Roots	5.22±0.09	4.86±0.25
		Shoots	6.86±0.25	5.41±0.26
		Leaves	11.45±0.39	15.57±0.46
		Soil	6.11±0.20	5.86±0.15

Table S4. Human exposure to antibiotics via five different crops;carrot, peas, radish, lettuce and spinach on average daily (ng) and annual (ug) consumption basis. LD: 6µg/L initial concentration as low dose treatment; MD: 20µg/L antibiotic concentration as medium dose treatment; HD: 40µg/L antibiotic concentration as high dose treatment.

Human exposure	Crop	LEV		CIP	
		Daily (ng)	Annual (ug)	Daily (ng)	Annual (ug)
LD6	Carrot (root)	4.36	1.59	9.52	3.47
	Peas (fruit)	5.40	1.97	3.81	1.39
	Radish (root)	1.11	0.40	2.25	0.82
	Lettuce (leaf)	6.58	2.40	6.60	2.41
	Spinach (leaf)	6.61	2.41	6.72	2.45
MD20	Carrot (root)	30.32	11.07	28.50	10.40
	Peas (fruit)	16.50	6.02	16.77	6.12
	Radish (root)	6.61	2.41	7.06	2.58
	Lettuce (leaf)	19.71	7.19	17.84	6.51
	Spinach (leaf)	22.01	8.03	24.27	8.86
HD40	Carrot (root)	43.89	16.02	46.78	17.07
	Peas (fruit)	32.13	11.73	32.44	11.84
	Radish (root)	10.66	3.89	9.80	3.58
	Lettuce (leaf)	28.73	10.49	27.83	10.16
	Spinach (leaf)	34.46	12.58	46.87	17.11

Table S5. Risk quotient of LEV and CIP on all five crop. LD6, MD20 and HD40: 6, 20 and 40 µg/L initial antibiotic concentrations as wastewater treatment.

	Crop species	LEV			CIP		
		LD6	MD20	HD40	LD6	MD20	HD40
RQ	Carrot	0.028	0.195	0.282	0.055	0.385	0.557
	Peas	0.030	0.091	0.177	0.059	0.180	0.350
	Radish	0.009	0.055	0.090	0.018	0.109	0.177
	Lettuce	0.021	0.063	0.092	0.042	0.125	0.182
	Spinach	0.026	0.085	0.133	0.050	0.168	0.263

Table S6. Pair-wise comparison of ANOVA to analyze different concentration treatments and different crops

Table S6a. Variable: Root Accumulation of LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	487.584[a]	14	34.827	129.810	.000
Intercept	685.165	1	685.165	2553.760	.000
CROP	195.887	4	48.972	182.528	.000
TREATMENTS	224.320	2	112.160	418.046	.000
CROP * TREATMENTS	67.377	8	8.422	31.391	.000
Error	4.024	15	.268		
Total	1176.774	30			
Corrected Total	491.609	29			

a. R Squared = .992 (Adjusted R Squared = .984)

Table S6b. Variable: Shoot Accumulation of LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	104.214[a]	14	7.444	29.326	.000
Intercept	156.089	1	156.089	614.935	.000
CROP	25.683	4	6.421	25.296	.000
TREATMENTS	63.836	2	31.918	125.746	.000
CROP * TREATMENTS	14.695	8	1.837	7.237	.001
Error	3.807	15	.254		
Total	264.111	30			
Corrected Total	108.022	29			

a. R Squared = .965 (Adjusted R Squared = .932)

Table S6c. Variable: Leaf Accumulation of LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	528.427[a]	14	37.745	53.971	.000
Intercept	986.821	1	986.821	1411.035	.000
CROP	128.997	4	32.249	46.113	.000
TREATMENTS	363.107	2	181.554	259.600	.000
CROP * TREATMENTS	36.323	8	4.540	6.492	.001
Error	10.490	15	.699		
Total	1525.739	30			
Corrected Total	538.918	29			

a. R Squared = .981 (Adjusted R Squared = .962)

Table S6d. Variable: Root Biomass of LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	21882.406[a]	14	1563.029	108.665	.000
Intercept	74375.306	1	74375.306	5170.747	.000
CROP	21859.438	4	5464.860	379.930	.000
TREATMENTS	1.722	2	.861	.060	.942
CROP * TREATMENTS	21.246	8	2.656	.185	.989
Error	215.758	15	14.384		
Total	96473.470	30			
Corrected Total	22098.164	29			

a. R Squared = .990 (Adjusted R Squared = .981)

Table S6e. Variable: Shoot/Leaf Biomass of LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	31306.690[a]	14	2236.192	7.034	.000
Intercept	100510.935	1	100510.935	316.143	.000
CROP	29218.580	4	7304.645	22.976	.000
TREATMENTS	463.039	2	231.519	.728	.499
CROP * TREATMENTS	1625.072	8	203.134	.639	.734
Error	4768.927	15	317.928		
Total	136586.552	30			
Corrected Total	36075.617	29			

a. R Squared = .868 (Adjusted R Squared = .744)

Table S6f. Variable: Daily exposure of LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5474.333[a]	14	391.024	56.762	.000
Intercept	10309.723	1	10309.723	1496.592	.000
CROP	1511.975	4	377.994	54.871	.000
TREATMENTS	3399.058	2	1699.529	246.709	.000
CROP * TREATMENTS	563.300	8	70.412	10.221	.000
Error	103.332	15	6.889		
Total	15887.388	30			
Corrected Total	5577.665	29			

a. R Squared = .981 (Adjusted R Squared = .964)

Table S6g. Variable: Annual Exposure LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	729.382[a]	14	52.099	57.172	.000
Intercept	1373.633	1	1373.633	1507.389	.000
CROP	201.238	4	50.309	55.208	.000
TREATMENTS	453.405	2	226.702	248.777	.000
CROP * TREATMENTS	74.739	8	9.342	10.252	.000
Error	13.669	15	.911		
Total	2116.684	30			
Corrected Total	743.051	29			

a. R Squared = .982 (Adjusted R Squared = .964)

Table S6h. Variable: Root BCF of LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.554[a]	14	.040	8.259	.000
Intercept	1.113	1	1.113	232.417	.000
CROP	.339	4	.085	17.716	.000
TREATMENTS	.065	2	.032	6.781	.008
CROP * TREATMENTS	.149	8	.019	3.901	.011
Error	.072	15	.005		
Total	1.738	30			
Corrected Total	.625	29			

a. R Squared = .885 (Adjusted R Squared = .778)

Table S6i. Variable: Shoot BCF of LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.300[a]	14	.021	34.699	.000
Intercept	.565	1	.565	915.556	.000
CROP	.203	4	.051	82.404	.000
TREATMENTS	.024	2	.012	19.464	.000
CROP * TREATMENTS	.072	8	.009	14.655	.000
Error	.009	15	.001		
Total	.874	30			
Corrected Total	.309	29			

a. R Squared = .970 (Adjusted R Squared = .942)

Table S6j. Variable: Leaf BCF of LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.404[a]	14	.029	28.180	.000
Intercept	2.012	1	2.012	1966.544	.000
CROP	.346	4	.087	84.638	.000
TREATMENTS	.005	2	.002	2.316	.133
CROP * TREATMENTS	.053	8	.007	6.416	.001
Error	.015	15	.001		
Total	2.431	30			
Corrected Total	.419	29			

a. R Squared = .963 (Adjusted R Squared = .929)

Table S6k. Variable: Shoot/Leaf TF of LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	154.249[a]	14	11.018	37.761	.000
Intercept	555.991	1	555.991	1905.535	.000
CROP	44.389	4	11.097	38.034	.000
TREATMENTS	86.041	2	43.021	147.444	.000
CROP * TREATMENTS	23.819	8	2.977	10.204	.000
Error	4.377	15	.292		
Total	714.617	30			
Corrected Total	158.626	29			

a. R Squared = .972 (Adjusted R Squared = .947)

Table S6l. Variable: In Soil dissipation of LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	333.422[a]	14	23.816	2.993	.022
Intercept	204076.965	1	204076.965	25649.184	.000
CROP	118.567	4	29.642	3.725	.027
TREATMENTS	4.647	2	2.323	.292	.751
CROP * TREATMENTS	210.209	8	26.276	3.302	.022
Error	119.347	15	7.956		
Total	204529.734	30			
Corrected Total	452.769	29			

a. R Squared = .736 (Adjusted R Squared = .490)

Table S6m. Variable: RQ of LEV and CIP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.365[a]	14	.026	4.104	.005
Intercept	.533	1	.533	83.843	.000
CROP	.114	4	.028	4.479	.014
TREATMENTS	.196	2	.098	15.430	.000
CROP * TREATMENTS	.055	8	.007	1.086	.423
Error	.095	15	.006		
Total	.993	30			
Corrected Total	.460	29			

a. R Squared = .793 (Adjusted R Squared = .600)