Is it safe to commercialize ornamental plants irrigated with antimicrobial-contaminated water?

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Research Article

Keywords: Enrofloxacin, fluoroquinolone, quality, Spathiphyllum wallisii, Zantedeschia rehmannii

Posted Date: February 15th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2394016/v1

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on August 17th, 2023. See the published version at https://doi.org/10.1007/s11356-023-29317-8.

Abstract

Enrofloxacin (Enro) has been widely encountered in natural water sources, and that water is often used for irrigation in crop production systems. Due to its phytotoxicity and accumulation in plant tissues, the presence of Enro in water used for crop irrigation may represent economical and toxicological concerns. Here, we irrigated two ornamental plant species (*Zantedeschia rehmannii* and *Spathiphyllum wallisii*) with water artificially contaminated with the antimicrobial enrofloxacin (Enro; 0, 5, 10, 100 and 1000 μg L^{-1}) to evaluate its effects on ornamental plant production, as well as its accumulation and distribution among different plant organs (roots, leaves, bulbs, and flower stems), and examined the economic and environmental safety of commercializing plants produced under conditions of pharmaceutical contamination. The presence of Enro in irrigation water was not found to disrupt plant growth (biomass) or flower production. Both species accumulated Enro, with its internal concentrations distributed as following: roots > leaves > bulbs > flower stems. In addition to plant tolerance, the content of Enro in plant organs indicated that both *Z. rehmannii* and *S. wallisii* could be safety produced under Enro-contaminated conditions and would not significantly contribute to contaminant transfer. The high capacity of those plants to accumulate Enro in their tissues, associated with their tolerance to it, indicates them for use in Enro-phytoremediation programs.

1. introduction

Livestock farming is a leading economic activity in Brazil (Leal et al., 2013), and the animal manure generated serves as an important source of nutrients for agriculture (Camotti Bastos et al., 2018). The intensive use of manure as a fertilizer, however, can serve as a source of contamination of agricultural soils and surface waters by veterinary antimicrobials (VA) (Li et al., 2020). Administered drugs are often not completely absorbed by the treated animals, and are eliminated in their feces and urine (Nunes et al., 2019). As such, when animal excrement is used as a fertilizer on agricultural land, it can be a significant source of active antimicrobial compounds transferred to the soil and surface waters (due to soil erosion or lixiviation) (Marques et al., 2021; Rocha et al., 2021).

Fluoroquinolones (FQs) are among the main classes of VA (Parente et al., 2019) as they are efficient for treating infections caused by both Gram-positive and Gram-negative bacteria – but their chemical structures generally remain unchanged during composting (where temperature variations between 10–55º C are common) or hydrolysis (Kumar et al., 2012). FQ degradation occurs only in the presence of light, through microbial biodegradation, or through costly oxidative processes (He et al., 2015). FQs may persist in surface waters due to the presence of organic constituents and metals that slow their natural degradation (Linke et al., 2010). Enrofloxacin (Enro), for example, is an FQ extensively used in animal production, and has been found at levels up to 800 ng L^{-1} in surface waters (Gomes et al., 2022), 14 mg L^{-1} in effluent of treatment plant and 6.5 mg L^{-1} in lakes (Fick et al., 2009). Considering their environmental persistence and widespread occurrence (Gomes et al., 2022, 2017), it is highly probable that waters contaminated with Enro are used in the production of economically important plants (Marques et al., 2021). In light of the inefficiency of sewage treatments for removing antimicrobial

residues from water (Al-Gheethi et al., 2015), irrigated plants may not be free from pharmaceuticals even in systems that use previously treated water. Enro was not found to disrupt the growth or yields of beans and corn at 10 μg L^{-1}, but negative effects observed with soybeans means that its presence in the water used for irrigating that crop can result in productivity losses (Marques et al., 2021). It will therefore be important to investigate the effects of Enro on other economically important plants, such as ornamental crops.

The market for ornamental plant has shown a consistent upward trend in recent years, with the cut flower segment, with its high added value, being the most important, especially in terms of the use of high quality flowers and foliage at social events (Pereira et al., 2021). The ornamental industry is mainly based on cultivating foliage and flowering plants suitable for bed and pot cultivation. Cut flowers represent the largest segment of the industry, followed by potted flowering plants, trees, and nursery crops (such as flower bulbs) used for plant propagation (Lawson, 1996). In light of the rapid increase in international demands, the commercial production of ornamental plants has been growing worldwide, and economic interest in those plants has increased in many countries (Lawson, 1996). Ornamental plant production generates approximately 70 billion dollars a year globally, and ranks 4th behind petroleum, coffee, and bananas in export earnings (BCMAFF, 2003). The ornamental plant market generated 22 billion U.S. dollars in 2019 in Europe (European Commision, 2020), approximately 1.7 billion U.S. dollars in Brazil (Brainer, 2019), and approximately $4.42 billion U.S. dollars in the United States (USDA, 2020).

An important aspect of floriculture is the quality of the water used for plant production. Rising demands for water for both domestic and industrial uses has created the necessity of reusing wastewater (Gallego et al., 2012). However, due to their stable chemical proprieties and the inefficiency of sewage treatment plants for removing antimicrobials (Al-Gheethi et al., 2015), water contaminated with FQs is commonly used for crop production (Marques et al., 2021). Although the tolerance of some ornamental plants to organic contaminants (and their use for phytoremediation purposes) has been examined (Rocha et al., 2022b), to date, only a recent study focused on the effects of pharmaceuticals on the commercial production of plant parts (such as bulbs, foliage, and flowers) (Rocha et al., 2022a). Authors observed that when grown in soil and/or manure contaminated with Enro, the antimicrobial did not affect aesthetical and commercial value of Calla lily (*Zantedeschia aethiopica*) plants. In addition to production aspects, it will be also important to investigate if watering ornamental plants with Enro-contaminated water will represent a source of contamination for other environments. The possible accumulation of the pharmaceutical in marketable parts of the plants can result in the transport of Enro via plants, which can contribute to the spread of environmental contamination (Rocha et al., 2022b).

Floriculture is an interesting and sustainable option, especially in small rural properties, for remedying contaminated areas while generating stable incomes – especially for women, who make up the majority of the workforce on flower farms (Junqueira and Peetz, 2018). Promoting the economic empowerment of those women in the field serves as a precondition for collective empowerment (Raynolds, 2021), it also encourages rural women to actively participate in entrepreneurship in tourism, and can reduce migration to large urban centers (Xu et al., 2018).

The genus *Zantedeschia* (family Araceae) represents one of the most important groups of ornamental plants. In countries such as New Zealand, calla lilies (*Zantedeschia* genus) account for 20% of the cut flowers exported, with earnings second only to orchids (Benschop et al., 2010). Similarly, *Spathiphyllum*, commonly known as peace lilies, are also economically important throughout the world as ornamental plants (Mounika et al., 2017). We therefore investigated the effects of Enro-contaminated irrigation water on the production of *Z. rehmannii* and *S. wallisii*. Additionally, we evaluated the accumulation and distribution of Enro among the different plant organs, which could help predict the safety of commercializing plants produced under Enro-contaminated conditions. In addition to contributing to toxicological studies involving Enro, we indicate those plants as useful for reclaiming antimicrobials from water in a technology that combines both economic and environmental advantages.

2. Material And Methods

2.1 Experimental conditions

Propagative material of *Z. rehmannii* Engl. Was acquired from a certified commercial producer in Holambra (São Paulo, Brazil). The tubers were visually checked for quality, and those between 12.5 and 16 cm long were used in the experiments. The tubers were sown at a depth of 10 cm in the substrate (one tuber/pot). *S. wallisii* Regel. seedlings were donated by the Floriculture Sector of the Federal University of Paraná, having been obtained by dividing clumps; they were standardized for root size (5 cm) and numbers of leaves (three fully expanded leaves).

The seedlings of *S. wallisii* and *Z. rehmannii* were cultivated in five-liter pots containing four kg of a sand:vermiculite substrate (2:1 v:v). This substrate was chosen to represent a worst-case scenario, as it would allow full plant exposure to the contaminant (Enro). Before the experiments with Enro, plants were grown in this substrate and we have noted that with correct fertilization, plant growth and development (including flowering) were not limited. The pots were randomly arranged in a greenhouse under a temperature regime of between 15/31º C, with a mean luminosity of 625 µmol photons m^{-2} s^{-1}.

A stock solution of Enro (10 mg L^{-1}) was prepared in ultra-pure water using analytical grade enrofloxacin (Vetranal, Sigma-Aldrich, Canada); the stock solution was subsequently used to prepare the required test solutions. The plants were exposed to increasing concentrations of Enro (0, 1, 10, 100 and 1000 µg L^{-1}) based on typical concentrations of that antibiotic found in surface waters (Gomes et al., 2022; Meng et al., 2019; Watkinson et al., 2009). The treatment with 1000 µg Enro L^{-1} represents a worst scenario of water contamination, allowing us to better understand the plant tolerance and antimicrobial accumulation and distribution in plant tissues. The plants were irrigated twice weekly with either 300 ml of distilled water, or with a freshly prepared solution of Enro, to maintain the 70% retention capacity of the substrate. Irrigation was performed using a graduated cylinder at soil level to avoid contaminating the shoots with the antibiotic. Plant nutrition was provided by Hoagland and Arnon nutrient medium (Hoagland and Arnon, 1950) supplied on a weekly basis. A total of five pots/treatment/species were used, totaling 25 experimental units for each species, with each pot constituting a replicate. The plants

were harvested when they reached commercial harvesting standards, with a complete opening of the spatha (Carneiro et al., 2012), approximately six months after planting the tubers.

2.2 Biomass production

The numbers of flower stems per pot were counted when the plants were harvested. The harvested plants were washed thoroughly with distilled water, gently dried with absorbent paper, and separated into roots, bulbs (propagative material), leaves, and flower stems. After determining their fresh weights, those plant materials were dried at 45º C to determine their dry masses.

2.3 Chemical analyses

To study Enro degradation in the substrate during the interval of Enro applications, the concentration of the antibiotic was evaluated in pots without plants immediately after, and for 84 h after the first application of the Enro solution. Enro and ciprofloxacin (Cipro, a metabolite of Enro) concentrations in the substrate of those pots, as well as in pots with plants were also evaluated after plant harvesting. Three samples from each pot were collected at depths up to 5 cm, corresponding to rhizosphere, homogenized (constituting one replicate/pot), and evaluated as described below. The antibiotics were extracted from substrate following (Janusch et al., 2014). Dried samples (6.0 g) were homogenized by sonication (5 min) and shaking (30 min) with 3 ml of sodium phosphate buffer (pH 7.0) and 6 ml of chloroform. The chloroform fraction was then extracted and dried in a SpeedVac machine (RC1010, XXXhermos). For plants, once dried, the plant organs (shoots, roots, bulbs, and flower stems) were ground to obtain homogenates of each organ. The extraction of antimicrobials was performed in three sub-replicates from each homogenate (with a total of three replicates/organs/pot), using 200 mg of plant material and 1.5 ml of acetonitrile containing 1% acetic acid. After extraction, the samples were dried in a SpeedVac machine (RC1010, Thermo), the residues suspended in acidified water (pH 4.5), and filtered through C18 solid phase extraction cartridges previously conditioned with 15 ml of methanol and 5 ml of water (pH 3.0) (Shi et al., 2009). The cartridges containing the samples were then washed with 1.6 ml of methanol:water (60:40, v/v), the eluate dried in a SpeedVac machine, and the residues resuspended in 0.4% aqueous triethylamine pH 3.0, acetonitrile and methanol (75:10:15 v/v/v) (Shi et al., 2009). In addition to Enro, Cipro concentrations were also investigated. The concentrations of those antimicrobials were determined following Shi et al. (2009) (LOD 0.19 ppb, LOQ 0.62 ppb) using high performance liquid chromatography (HLPC) and a fluorescence detector (Agilent 1290 Infinity II LC, Wilmington, DE, USA) after elution on a C18 column (Discovery® HS C18 column 250 x 4.6 mm, particle size 5 µm, Sigma-Aldrich). The FLD excitation/emission detection was 278/445 nm. Each batch of samples included three blanks, one standard, and one fortified sample (as a quality control). To determine recovery rates, substrate (85%) and plant samples (without Enro treatment) (94.5%) were spiked with known concentrations of Enro and Cipro, and then subsequently extracted and evaluated.

To evaluate how Enro was distributed among the different plant organs (Translocation factor, TF), the Enro content (Enro concentration x organ DW) of the aboveground organs (shoots + flower stem) was divided by the Enro content of the subterranean organs (roots + bulbs) as follows:

$$Content = Enroconcentrationorgan * organDW$$

$$Translocationfactor = \frac{(Shootcontent + Flowercontent)}{(Rootcontent + propagativeorgancontent)}$$

Total content Enro was calculated as the sum of the contents of each organ.

2.4 Statistical analysis

The results are expressed here as the averages of five replicates for plant evaluations and three replicates for substrate evaluations. Statistical analyses were performing using JMP software 10.0 (SAS Institute Inc.). The results were submitted to tests of normality (Shapiro-Wilk) and homogeneity (Bartlett) and were then statistically evaluated. The effects of Enro on plant biomass and flower production were evaluated separately for each species using ANOVA, and the means compared using the Tukey test ($P < 0.05$). Enro distributions among the different organs of both species were evaluated using Three-Way ANOVA. Interactions between species (*S. wallisii* and *Z. rehmannii*), Enro concentrations (0, 1, 10, 100 and 1000 µg L^{-1}), and plant organs (root, leaves, bulbs, and flower stem) were included in the model. To evaluate the differences in Enro contents in the different organs between the species, the data were evaluated using Two-Way ANOVA, including the interactions between Enro concentrations and species in the model. When differences were detected by ANOVA, the means were compared using the Contrast test at a 0.05% level of significance. The data of antibiotic concentrations in substrate was compared for each Enro concentration, individually, between the species and pots without (- plants) by One-Way ANOVA, and when differences were detected by ANOVA, the means were compared using the Contrast test at a 0.05% level of significance.

3. Results

3.1 Flower and biomass production

Neither the numbers of flower stems produced ($P > 0.05$) nor the times until flowering were affected by Enro in either of the species (Fig. 1). Moreover, regardless the treatment, the flower stems were in accordance with commercial quality standards, without injuries, mechanical damage, physiological disorders, or symptoms of nutritional deficiencies (Fig. 1). The addition of fertilizer ensured that the plants completed their cycles without presenting symptoms of phytotoxic effects of the antibiotic or any nutritional deficiencies. Similarly, with the exception of the shoot dry weight of *S. wallisii*, the fresh and dry weights of the different plant organs were not affected by Enro in either of the two species (Tables 1 and 2). Dry weight production by *S. wallisii* was reduced, however, in plants exposed to 1000 µg L^{-1} Enro in relation to the control (Table 2).

Table 1
Biomass production by different organs of *Z. rehmannii* plants exposed to increasing concentrations of enrofloxacin through irrigation with enrofloxacin-contaminated water.

	Fresh weight				**Dry weight**			
Enro	**Leaves**	**Roots**	**Tubers**	**Total**	**Leaves**	**Roots**	**Tubers**	**Total**
µg L^{-1}	g.$plant^{-1}$							
0	12.40 ± 5.41	18.40 ± 1.67	61.8 ± 13.00	92.60 ± 19.00	1.24 ± 0.37	0.98 ± 0.31	22.63 ± 5.93	24.85 ± 6.26
5	10.50 ± 4.09	17.50 ± 2.87	61.4 ± 19.08	89.40 ± 20.69	1.16 ± 0.43	0.84 ± 0.27	24.30 ± 7.74	26.30 ± 7.67
10	12.76 ± 2.27	16.60 ± 3.20	64.0 ± 8,.5	93.36 ± 11.57	1.79 ± 0.20	1.25 ± 0.66	26.56 ± 3.37	29.60 ± 3.71
100	12.20 ± 3.34	18.60 ± 4.56	65.4 ± 9.23	96.20 ± 16.22	1.28 ± 0.45	0.86 ± 0.31	21.85 ± 3.47	24.01 ± 4.17
1000	16.24 ± 4.94	21.40 ± 6.10	73.0 ± 13.94	110.64 ± 20.87	1.53 ± 0.45	1.03 ± 0.38	25.63 ± 1.73	28.19 ± 2.28
Prob < W	0.36	0.17	0.79	0.68	0.74	0.11	0.31	0.25
Bartlett	0.55	0.18	0.50	0.82	0.64	0.38	0.08	0.08
F	1.26	1.02	0.62	1.06	2.10	0.80	0.80	0.99
Pr > Fc	0.31	0.41	0.64	0.40	0.11	0.54	0.53	0.43

Values represent means ± SD of five replicates.

Table 2
Biomass production by different organs of *S. wallisii* plants exposed to increasing concentrations of enrofloxacin through irrigation with enrofloxacin-contaminated water.

	Fresh weight				Dry weight			
Enro	**Leaves**	**Roots**	**Rhizomes**	**Total**	**Leaves**	**Roots**	**Rhizomes**	**Total**
μg L^{-1}	g $plant^{-1}$							
0	11.50 ± 3.53	5.49 ± 2.29	23.00 ± 7.07	39.99 ± 12.90	1.46 ± 0.01 a	1.48 ± 0.20	1.79 ± 0.72	4.72 ± 0.95
5	8.56 ± 2.56	4.26 ± 0.48	12.84 ± 3.52	25.66 ± 6.35	1.36 ± 0.14 a	0.78 ± 0.25	1.27 ± 0.09	3.42 ± 0.44
10	9.50 ± 3.20	4.25 ± 0.87	14.60 ± 4.15	28.35 ± 7.01	1.23 ± 0.35ab	0.69 ± 0.27	1.26 ± 0.21	3.18 ± 0.80
100	7.25 ± 3.50	5.68 ± 0.53	14.25 ± 6.02	27.18 ± 9.65	1.07 ± 0.46ab	0.67 ± 0.50	1.52 ± 0.17	3.27 ± 0.94
1000	6.00 ± 1.00	3.92 ± 1.19	13.00 ± 2.00	22.92 ± 3.81	0.93 ± 0.04b	0.88 ± 0.50	1.08 ± 0.26	2.90 ± 0.48
Prob < W	0.41	0.05	0.38	0,38	0.40	0.82	0,38	0.41
Bartlett	0.63	0.20	0.72	0.91	0.06	0.60	0.07	0.70
F	1.41	2.36	1.61	1.77	3.21	1.99	2.63	2.14
Prob > Fc	0.28	0.10	0.20	0.17	0.034*	0.15	0.07	0.13
Values represent means ± SD of five replicates								

3.2 Antimicrobial degradation and concentration in substrates

Regardless of the time of evaluation, no Enro or Cipro was observed in substrate of pots without Enro treatment (data not shown). After 84 h of the first irrigation with Enro, up to 43%, 33%, 38% and 30% of the antibiotic was degraded in pots without plants irrigated with 5, 10, 100 and 1000 μg Enro L^{-1} (Fig. 2A). Some Cipro residues were detected over the time in the substrate of pots without plants irrigated with Enro concentrations ≥ 10 μg L^{-1} (Fig. 2B). At the harvesting, no Cipro was observed in the substrate of pots with or without plants (data not shown). Regardless of the species and Enro treatment, greater Enro concentration was observed in pots without plants in relation to those planted receiving the

same Enro concentration (Fig. 2C). Moreover, under irrigation with 100 and 1000 µg Enro L^{-1}, the final Enro substrate concentration was greater in pots of *Z. rehmannii* in relation to those of *S. wallisii* (Fig. 2C).

3.3 Distribution of antimicrobial compounds in different plant organs

Significant interactions between Enro treatments, species, and organs were observed for Enro and ciprofloxacin (Cipro) concentrations in plants (Table 1S). The greatest concentrations of Enro in both plant species were encountered in the roots (Fig. 3A and C). Ciprofloxacin (Cipro), in addition to Enro, was detected in the roots of *Z. rehmannii* plants that had been submitted to irrigation with Enro (Fig. 3B); in leaves, however, Cipro was detected only in plants submitted to 100 and 1000 µg Enro L^{-1} (Fig. 3B). The stem flowers of *Z. rehmannii* were the only organ of that plant in which Enro was detected (and then only in plants submitted to 100 and 1000 µg Enro L^{-1}; Fig. 3A). With exception of plants irrigated with water contaminated with 10 µg Enro L^{-1}, the concentrations of Enro were greater in the roots and leaves of *S. wallisii* than in *Z. rehmannii* (Fig. 3A and C).

Significant interactions between Enro treatments and species were observed for Enro contents in plant organs, as well as for total Enro content, and the translocation factor (TF) (Table 2S). The Enro contents of roots (Fig. 4A) and leaves (Fig. 4C) increased in both species when treated with Enro concentration ≥ 10 µg L^{-1} in relation to those treated with 5 µg L^{-1}. Enro contents of the roots and leaves of *S. wallisii* were highest in plants treated with 1000 µg Enro L^{-1}, while root (Fig. 4A) and leaf (Fig. 4C) contents of Enro in *Z. rehmannii* did not differ significantly between plants treated with 10, 100 or 1000 µg Enro L^{-1}. Regardless of the Enro treatment, the Enro contents in the roots of *S. wallisii* were greater than those of *Z. rehmannii* (Fig. 4A). The Enro contents of the leaves differed between the species only in plants treated with 1000 µg Enro L^{-1}, being higher in *S. wallisii* (Fig. 4C). The Enro contents of bulbs did not significantly differ among Enro treatments, regardless of the plant species, although concentrations were greater in *S. wallisii* than in *Z. rehmannii* plants treated with 1000 µg Enro L^{-1} (Fig. 4B). Regardless of the Enro treatment, the total content of Enro was greater in *S. wallisii* plants than in *Z. rehmannii* (Fig. 4D). In contrast, the TF only differ in plants treated with 5 µg Enro L^{-1} (being greater in *Z. rehmannii*) (Fig. 4E).

4. Discussion

Shoot biomass production is critical for predicting the commercial value of plants used in landscaping projects or in the floriculture market. Shoot biomass is related to leaf area and, consequently, photosynthetic rates (Liu et al., 2018). Biomass is therefore a good indicator for characterizing the performance of ornamental plants exposed to contaminant stress (Huang et al., 2020). The biomass productions of both species tested here were not significantly affected by the presence of Enro in the irrigation water (Tables 1 and 2), indicating their tolerance to that pharmaceutical. Moreover, flower stem production and quality were not affected by the antimicrobial agent, and the plants were able to produce

flower stems with the required commercial quality (i.e., stem strength, color, good condition of the foliage, with no chlorotic or necrotic points, free from physical defects). This is a very important commercial aspect, as flower stems are the main target for commercial production in both species (Franco-Hermida et al., 2020; Junqueira and Peetz, 2018; Noman et al., 2017). Similarly, *S. wallisii* flower stem production and quality were not affected by contaminants in the wastewater of a rural community (Zamora-Castro et al., 2019). Those plants have also been investigated for their ability to remove contaminants in wetland systems (Zamora-Castro et al., 2019), although to the best of our knowledge, this is the first time that the effects of irrigation water contaminated with a pharmaceutical has been investigated in terms of flower quality and production for both species.

Although Enro residues are expected even in irrigation systems using treated water, our results show that *S. wallisii* and *Z. rehmannii* are tolerant of Enro, and therefore environmentally representative concentrations found in water systems throughout the world should have no economic impacts on their production. It is necessary to consider, however, that not only the economic aspects of that production system should be taken in count, for it will also be important to investigate the safety of those marketable products – in our case, flower stems, potted plants, and bulbs. Although some plants have the ability to metabolize fluoroquinolones (Aristilde et al., 2010; Gomes et al., 2019, 2017; Hoang et al., 2013; Lillenberg et al., 2010) the persistence times of those antimicrobial agents in plant tissues are not well known. It is therefore important to investigate how much Enro is accumulated in the marketable parts of decorative plants to assure the safety of their commercialization.

Some plant species appear to metabolize Enro to Cipro (the main Enro metabolite) (Gomes et al., 2019). Here, Cipro was only found in roots and leaves of *Z. rehmannii* (Fig. 3A and B). It is not possible to affirm, however, that *Z. rehmannii* can metabolize Enro to Cipro, as that transformation can occur in the growth substrate and/or through microbial degradation, after which Cipro could be taken up by the plants (Marques et al., 2021). Indeed, we observed the formation of Cipro in the substrates of pots receiving the Enro-contaminated water along the interval of the first two Enro applications (Fig. 2B). Once in the plant roots, Cipro can reach the shoots through the transpiration pathway (Rocha et al., 2021). Nonetheless, the Cipro contents in the organs of *Z. rehmannii* were minimal (up to 12 ng in roots and 9 ng in leaves), and therefore only Enro accumulation was considered in evaluating the safety of plant organs. While Enro was not detected in the flower stems of *S. wallisii*, some residues of that pharmaceutical were observed in the flower stems of *Z. rehmannii* (Fig. 1). The highest Enro accumulations were observed in plants irrigated with 1000 µg Enro L^{-1}, with Enro contents in the flower stems of up to 0.68 ± 0.24 ng. Under those conditions, a small bouquet of flowers (with approximately 15 flower stems) would hold up to 10.2 ng of Enro. At large events, which can demand (for example) 10,000 flower stems, only 6.8 µg of Enro would be held by all of those flower stems considered together. That minimal concentration could not significantly contribute to environmental contamination with the eventual disposal of those flower stem biomasses. Moreover, Enro concentrations found in *S. wallissi and Z. rehmannii* plants are much lower than that observed to cause ecological risk for aquatic and terrestrial organisms, which varies from 0.031 to 100 mg L^{-1} (Table 3). For instance, to achieve 0.031 mg, it will be necessary more than 45,000 flowers

from plants irrigated with 1000 µg Enro L^{-1}. Furthermore, all the residues from these flowers must be diluted in a volume of only one litter, which is a not real situation. Possible residues of Enro released by plants during their decomposition will be incorporated by soil particles, with part of them being adsorbed and, thus, not bioavailable (Rocha et al., 2021); Enro can also runoff to bodies of water but in that case will be diluted in large amount of water. Therefore, the use of water contaminated by Enro to irrigate *S. wallisii* and *Z. rehmannii* plants does not represent a significant threat when flower stems are sold..

Table 3
Ecological risk (ecotoxicity analyzes, EC_{50}) of enrofloxacin

Taxonomic group	Species	Time of exposure	EC_{50}	References
Bacteria	*Bacillus amyloliquefaciens*	16 hours	8.89 mg L^{-1}	Menz et al. (2018)
Bacteria	*Pseudomonas putida*	16 hours	5.53 mg L^{-1}	Menz et al. (2018)
Microalgae	*Scenedesmus obliqus*	72 hours	52.4 mg L^{-1}	Rico et al. (2018)
Microalgae	*Pseudokirchneriella subcapitata*	72 hours	3.33 mg L^{-1}	Lee et al. (2021)
Cyanobacteria	*Microcystis aeruginosa*	72 hours	0.031 mg L^{-1}	Rico et al. (2018)
Plants	*Sinapsis alba*	120 hours	265 mg L^{-1}	Timmerer et al. (2020)
Plants	*Solanum lycopersicum* (seeds)	N.I	0.95 mg L^{-1}	Gros et al. (2019)
Earthworms	*Lumbricus terrestris*	N.I	71.12 mg L^{-1}	Zhou et al. (2020)
Earthworms	*Lumbricus terrestris*	N.I	100 mg L^{-1}	Gros et al. (2019)
Earthworms	*Eisenia fetida*	3 days	500 mg L^{-1}	Li et al. (2015)
Crustaceans	*Moina macrocopa*	48 hours	85.2 mg L^{-1}	Lee et al. (2021)
Crustaceans	*Daphnia magna*	21 days	6.5 mg L^{-1}	Dalla Bona et al. (2015)
Fish	*Oryzias latipes* (juvenile)	48 and 96 hours	> 100 mg L^{-1}	Lee et al. (2021)
N.I: not informed				

Based on their respective Enro contents (Fig. 2), the leaves of *S. wallisii* carry up to 291.56 ng of Enro each, while a leaf of *Z. rehmannii* can *c*arry up to 103.79 ng. Considering a pot with 20 leaves and five flower stems, up to 5958 ng and 2493 ng of Enro (including the highest root and bulb contents) would be

present per pot of *S. wallisii* and *Z. rehmannii* respectively. The amounts of Enro held by flower stems and leaves are therefore minimal in comparison to those that would be required to significantly contribute to environmental contamination (Table 3). In addition to cut flowers and potted plants, the commercialization of bulbs from these species is quite common, as they are used for propagation purposes. Also called ornamental geophytes, their bulbs significantly contribute to the global ornamental industry (Benschop et al., 2010). The bulbs of both species evidenced very low accumulations of Enro, which is relevant information for producers who use that material for propagation and in genetic breeding programs. Based on our results, we conclude that no restrictions are necessary in terms of producing *S. wallisii* and *Z. rehmannii* plants using water contamination by Enro. Similarly, we have recently showed the safety of the commercialization of flowers of calla lily grown under Enro-contaminated soil and manure, indicating the use of ornamental plants for phytoremediation purposes (Rocha et al., 2022a).

In addition to certifying the safety of commercializing plants produced under conditions of Enro contamination by irrigation, we also indicate the usefulness of both plant species for the phytoremediation of water contaminated by that antibiotic. At harvesting, the concentration of Enro in the substrate of pots without plants was greater than those observed in pots planted with *S. wallisii* and *Z. rehmannii* (Fig. 2C). In addition to the observed Enro-concentrations in plant tissues, these results indicate that the Enro-uptake by plants contributes to the removal of the antimicrobial from substrate. Natural degradation of Enro in soil and water occurs mainly due to photodegradation (Sciscenko et al., 2020; Sturini et al., 2012), and, in an interval of 84h, Enro-degradation in the substrate varied from 30 to 43%, depending on the Enro-concentration in the contaminated water (Fig. 2A). However, in the presence of plants, the Enro concentrations in substrate at harvested reduced from 52 to 79% in pots with *Z. rehmannii* and from 78 to 87% in pots with *S. wallisii* in relation to those without plants (Fig. 3C). Ornamental plants have been used to reclaim contaminants from polluted waters and soils (Rocha et al., 2022b). Many species of ornamental plants, such as *Althaea rosea* and *Chrysanthemum maximum*, can tolerate contaminated environments and still show high biomass production (Asgari Lajayer et al., 2019; Liu et al., 2008; Sandoval-Herazo et al., 2018) – an important feature in terms of the selection of species for phytoremediation purposes. Additionally, ornamental plants only infrequently enter the food chain - especially phytotoxic plants such as *Zantedeschia* and *Spatiphyllum* - a fact that prevents the spread of antimicrobial resistance genes (Chen et al., 2009). Finally, in addition to lending aesthetic value to recuperating environments, the use of ornamental plants in phytoremediation programs could provide options for tourism and sources of income with high added value (Rocha et al., 2022b), and the viability of additional income through ecotourism related to phytoremediation activities has already been demonstrated (Asgari Lajayer et al., 2019; Khan et al., 2021; Liu et al., 2018; Sandoval et al., 2019).

According to our results, both *S. wallisii* and *Z. rehmannii* plants are indicated for reclaiming Enro. Due to its greater biomass production, *S. wallisii* plants accumulated more Enro than *Z. rehmannii* – which explain the lower Enro final concentration observed in the substrate of *S. wallisii* in relation to *Z. rehmannii* (Fig. 2C) However, regardless of the species, the roots were the main organ of Enro accumulation (Fig. 3). Indeed, the TF of plants was lower than 1, indicating that Enro was preferentially accumulated by subterranean plant organs. The allocation of pharmaceutical contaminants to the roots

is a plant strategy to avoid their possible deleterious effects on photosynthetic tissues (Hoang et al., 2013; Pomati et al., 2004; Yan et al., 2020; Zhang et al., 2019), assuring their continued viability. In addition, and regarding market safety, the reduced translocation of pharmaceuticals to the shoots among plants being cultivated for flower stem production will avoid their transport when those organs are commercialized – so that the Enro-phytoextraction capacity of *S. wallisii* and *Z. rehmannii* plants can be coupled with their economic production.

When used in phytoremediation programs, ornamental plants add aesthetic value, and serve to diversify the production of rural properties with plants that can often have greater retail values than traditional crops (Pandey & Souza-Alonso, 2018). Those plans provide the opportunity to improve landscapes for ecotourism while at the same time marketing flowers and foliage for decorative purposes in homes, gardens, offices, shopping malls, and for landscape design projects, including living walls, green roofs, and rain gardens (Pandey and Souza-Alonso, 2018). Floriculture represents an interesting and sustainable option for remedying contaminated areas, especially in small rural properties, while generating stable incomes – especially for women – who make up most of the workforce on flower farms (Junqueira and Peetz, 2018). Promoting the individual empowerment of those women in the field is a precondition for collective empowerment (Raynolds, 2021) and can ensure a lower migration rate to large urban centers, especially as this sector encourages rural women to actively participate in entrepreneurism related to tourism (Xu et al., 2018). Finally, in addition to economic aspects, the ability of some ornamental species, such as *S. wallisii* and *Z. rehmannii*, to reclaim antimicrobials may help reduce the development of microbes resistant to antibiotics. Antimicrobials present at sublethal levels in the environment contribute to the development and dissemination of antibiotic resistance by microorganisms, which can lead to public health problems (Camotti Bastos et al., 2018; Lillenberg et al., 2010). Antimicrobial resistance poses a major threat to human health the world over (Lima et al., 2020), and it is estimated that those resistant microorganisms were responsible for 1.27 million deaths in 2019 (Murray et al., 2022). The development of technologies (such as the use or ornamental plants) aiming to reclaim antimicrobials from the environment is therefore of extreme importance.

5. Conclusion

We demonstrated here the safety of irrigating *S. wallisii* and *Z. rehmannii* plants with Enro-contaminated water. The quality and yields of flower stems produced by those plants were not affected by the pharmaceutical. Although the plants accumulate Enro in marketable organs (bulbs, leaves, and flower stems), the quantities of that antimicrobial compound in those organs would not significantly contribute to environmental risks. The capacities of those plants to reclaim Enro make them suitable for phytoremediation programs that, in addition to reclaiming contaminated environments, can result in economic gains. Customer demands in the near future for ecologically favorable products will positively affect cultivation and sales supported by environmental concerns and lower production costs (Darras, 2021). Thus, the production and sales of products resulting from decontamination efforts can significantly increase if those new products are supported by dynamic marketing campaigns that focus on their beneficial natures (as compared to traditional cut flowers) (Darras, 2021; Havardi-Burger et al.,

2020). To obtain the greatest success using ornamental plants for phytoremediation purposes, it will be important to identify native species with ornamental potential that are tolerant to antimicrobials, stimulate the regionalization of large-scale production, and focus on increasing employment opportunities for local workers.

Gardening practices as a hobby, biophilia incorporated into architectural design, and the cultivation and contemplation of flowers and ornamental plants can be an option for caring for the mental health of the population, especially after the drastic changes caused by the pandemic of covid 19 pandemic (Reis et al., 2020).

Declarations

Competing interests The author declare that they have no competing interests

Funding This research was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) – Finance Code 406190/2018-6. M.P. Gomes received research productivity grants from CNPq.

Conflict of Interest The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author contributions C.S.R, D.M.C, M.V.R and M.P.G conceptualization. C.S.R, L.Y.K., J.C.M.B., R.S.A.K., and M.P.G. performed the experiments and analyzed the data; C.S.R, D.M.C, M.V.R and M.P.G wrote the original draft; M.P.G was responsible for funding acquisition and project administration; D.M.C, M.V.R, and M.P.G supervision; and C.S.R and M.P.G writing – review & editing.

Ethical approval: Not applicable.

Consent to participate: The authors declare have consented to the submission

Consent to publish: The authors give their consent for the publication

Availability of data and materials: Not applicable.

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Figures

Figure 1

S. wallisii (A, B and C) *Z. rehmannii* (D, E and F) plants exposed to increasing concentrations of enrofloxacin through irrigation with enrofloxacin-contaminated water. A and D = 0 µg Enro L^{-1} (control); B and E =1000 µg Enro L^{-1}; D and F = all treatments together.

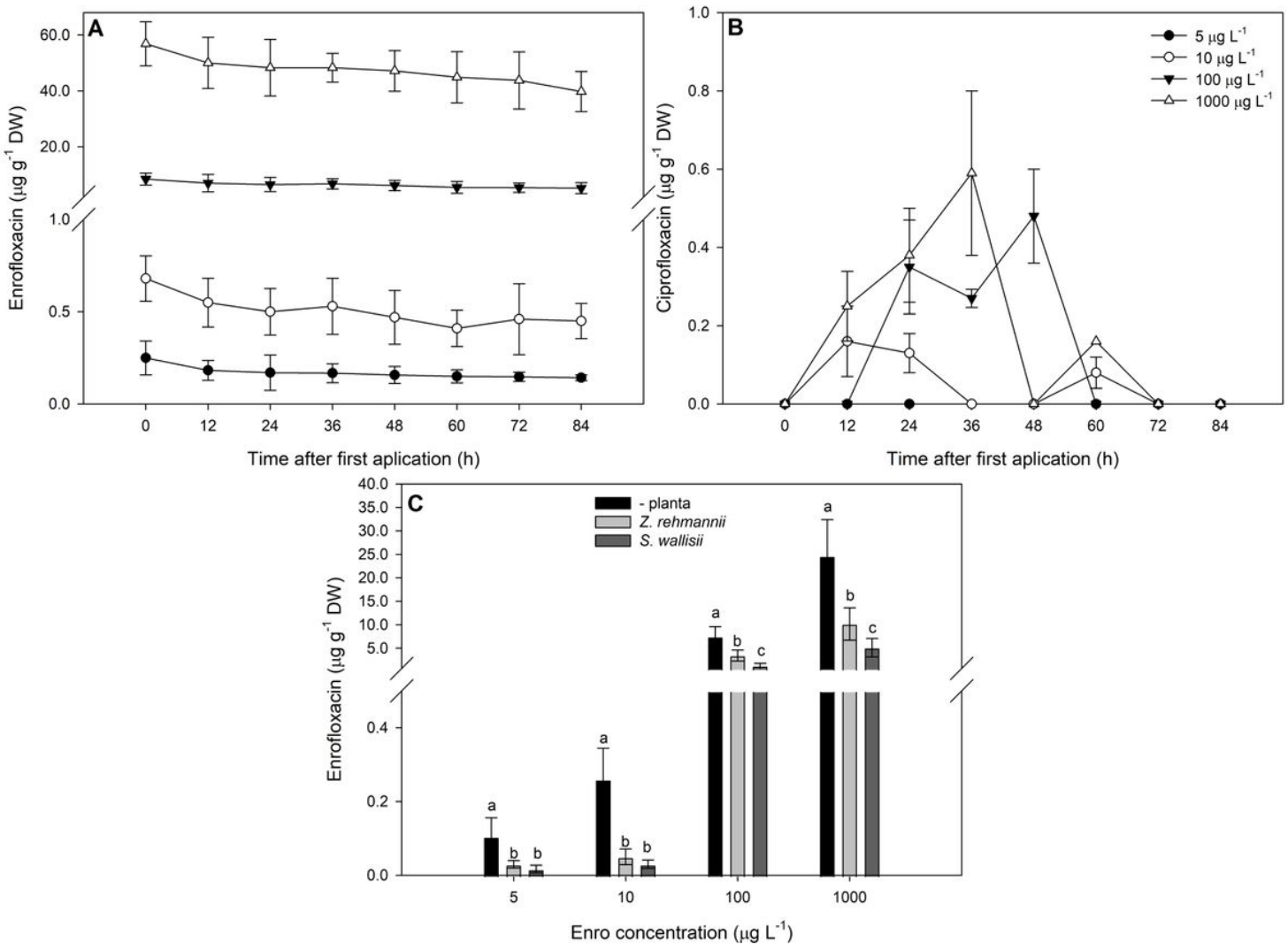

Figure 2

Time course of enrofloxacin (**A**) and ciprofloxacin (**B**) concentrations in substrate of pots without plants and antibiotic concentrations at harvesting in the substrate of pots without (- plants) and with plants (*Z. rahmannii* and *S. wallisii*) (**C**) exposed to increasing concentrations of enrofloxacin through irrigation with enrofloxacin-contaminated water. Values represent the means ± standard deviations of three replicates. Letters indicate significant differences in the pharmaceutical concentration in substrates of pots without and with plants within the same Enro concentration by the Contrast Test ($P < 0.05$).

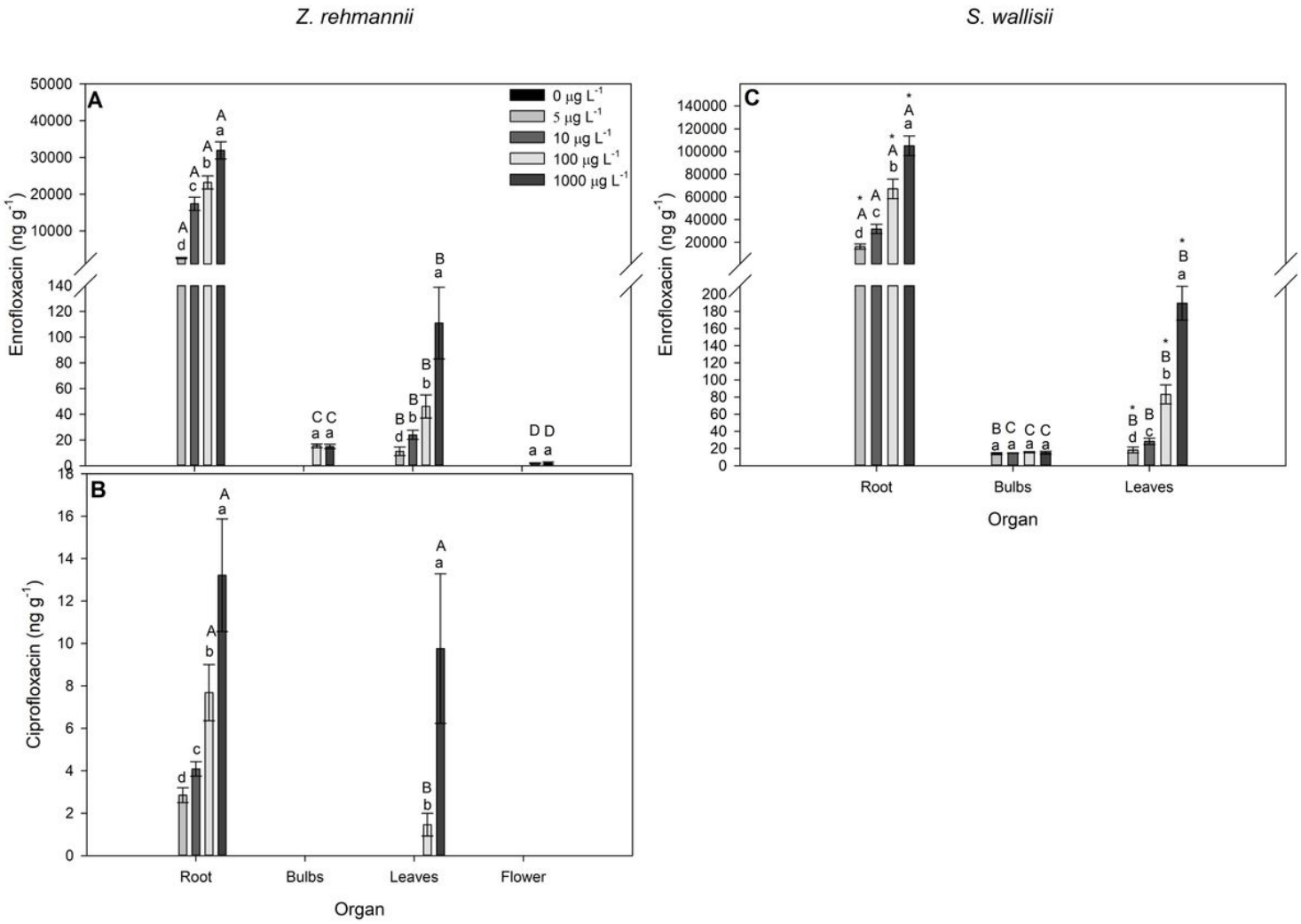

Figure 3

Enrofloxacin (**A** and **C**) and ciprofloxacin (**B**) concentrations in different organs of *Z. rehmannii* and *S. wallisii* plants exposed to increasing concentrations of enrofloxacin through irrigation with enrofloxacin-contaminated water. Values represent the means ± standard deviations of five replicates. Lower case letters indicate significant differences in the pharmaceutical concentration of plant organs of the same plant species; uppercase letters indicate significant differences between the organs of the same species, and * indicate significant differences between the organs of the different species by the Contrast Test ($P < 0.05$).

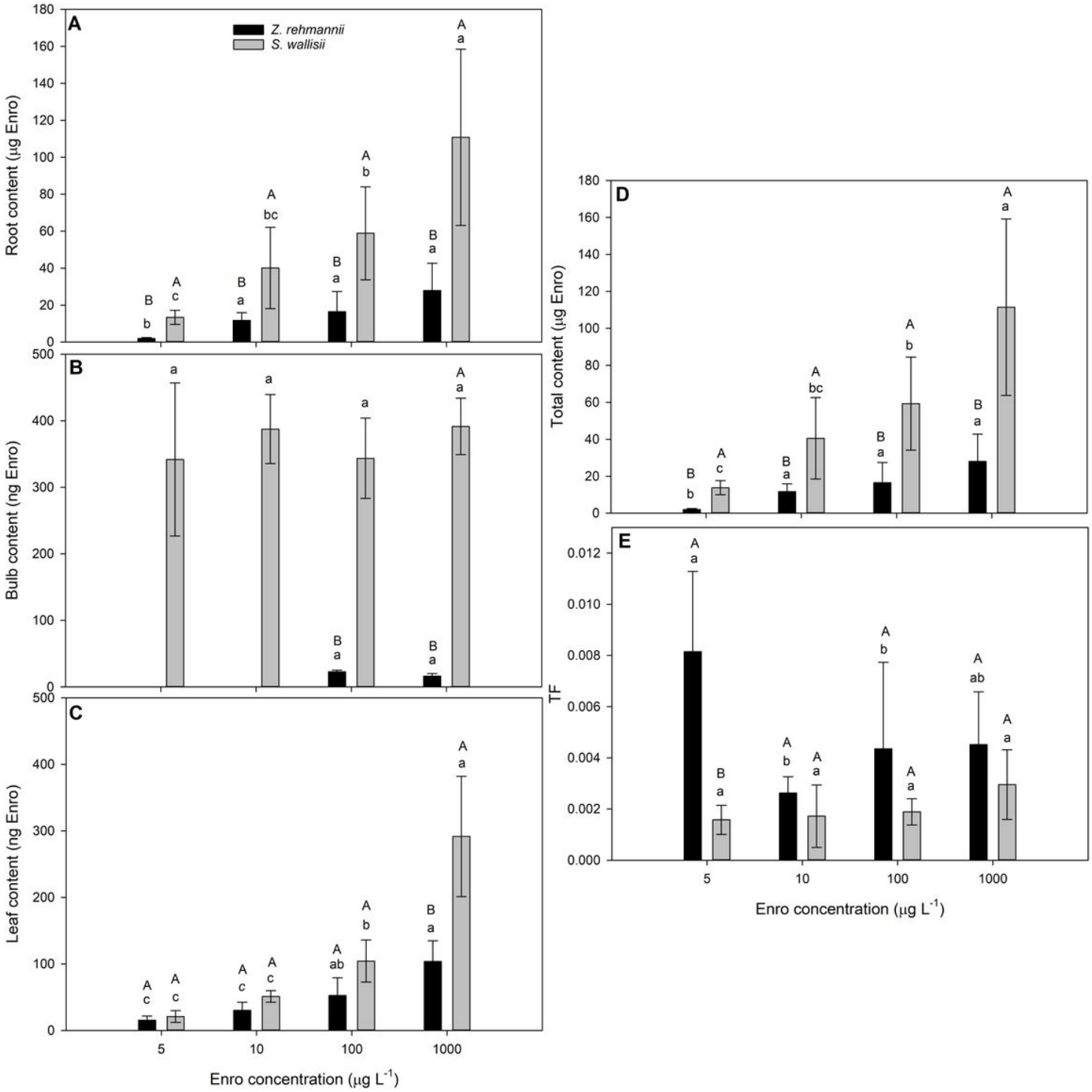

Figure 4

Enrofloxacin contents of different plant organs (**A**, **B** and **C**), total content (**D**), and the translocation factor I, of *Z. rehmannii* and *S. wallisii* plants exposed to increasing concentrations of enrofloxacin through irrigation with enrofloxacin-contaminated water. Values represent means ± standard deviations of five replicates. Lower case letters indicate significant differences in the pharmaceutical content of the same species; uppercase letters indicate significant differences between the species within the same Enrofloxacin treatment by the Contrast Test ($P < 0.05$).

Supplementary Files

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- GA.jpg
- TABLE1S.docx
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