Calla lily production in enrofloxacin-contaminated soil and manure: An attractive alternative coupling income generation with antimicrobial removal from the environment

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Enrofloxacin (Enro) is often detected in soil and animal manure used for crop production and phytotoxic responses have been observed in plants grown under antimicrobial presence. In the present paper, we investigated the effects of the presence of Enro in soils (1.9 mg kg^{-1}) and manure (50.4 mg kg^{-1}) in growth and flower production of calla lily plants (*Zantedeschia aethiopica*). We also reported the accumulation and distribution of Enro between plant tissues aiming to evaluate the safety of commercializing plants produced under Enro-contaminated conditions. The presence of Enro in soils and manure did not affect plant growth and flower production and did not induce any physiological effects in plants (as evaluated by photosynthetic pigment, hydrogen peroxide concentration, superoxide dismutase and catalase activity in leaves). Plants accumulated Enro mainly in their roots, restraining its translocation to shoots, which contributes to the safety of the commercialization of their flowers. However, when commercialized as pots, the amount of Enro carried by plants is a matter of concern, and therefore, selling pot plants must be avoided. Due to their tolerance and capacity to remove Enro (up to 14.76% of Enro from contaminated soil and/or manure), plants are indicated for phytoremediation programs.

KEYWORDS

Zantedeschia aethiopica, ornamental plant, contaminants, phytoremediation, soil

Introduction

Ornamental plants are an important sector in agribusiness, moving billions of dollars a year around the world (1–3). In addition to generating income, ornamental plants play important environmental roles: they offer shelter for wild fauna, act as biological filters and help in environmental matrix decontamination, in addition to being used in the landscaping of parks and gardens (4–8).

Among ornamental plants, calla lily (*Zantedeschia aethiopica* (L.) Spreng.) is of growing commercial interest, showing high commercialization value (9–12). This monocotyledon belonging to the family Araceae is marketed as pot plants, and mainly as cut flowers for flower arrangements and bouquets. Although the production of calla lily has increased worldwide due to its good environmental adaptation (13), these plants require high levels of fertilization, which is often achieved by organic fertilization using manure (14).

Although manuring has several beneficial effects in agricultural practice, its harmful and neutral effects on the trinity formed by plants, soil and organisms are expected (15). One of the major problems caused by manuring is placing organic contaminants into soil and plants. Some contaminants, such as antimicrobials, largely used in animal production, are not fully metabolized as they are administered, and are excreted in animal feces and urine (16), resulting in the high observed concentrations of antimicrobials in manure (17, 18).

Once applied to soil, manure can release antimicrobials into soil solution, which become available for plant uptake (19). Some antimicrobials are phytotoxic causing delayed germination (20) reductions on photosynthesis (21, 22) and mitochondrial respiration (23), in addition to causing oxidative stress damage (24, 25).

Fluoroquinolones (FQ) are one of the main classes of antimicrobials occurring in livestock (26–30), and once present in agricultural systems, they can be leached to water bodies or adsorbed in the colloidal fraction of the soil, remaining for long periods until they are degraded by biodegradation and photooxidation processes (31). These processes depend on the antimicrobial physical-chemical properties and concentration (molecular structure, size, form, solubility, speciation, hydrophobicity, etc.), soil characteristics (pH, texture, organic matter, mineralogy, microbial activity, etc.), climatic conditions (luminosity and temperature), manure source (32) and the exposure time of the area (26).

In soils, Enrofloxacin (Enro), an FQ commonly used in veterinary medicine, has been detected at levels of 1.03 mg kg^{-1} (30) while in animal manure, its concentrations reach up to 46 mg kg^{-1} (29). This antimicrobial is of interest as its toxicity has been seen in crops, such as soybeans, decreasing plant yields (19). In this context, the use of Enro contaminated manure in calla lily fertilization could result in decreased yields if this antimicrobial threatens plant growth.

However, some ornamental plants have demonstrated great tolerance to organic contaminants such as antimicrobials (33, 34). In a previous study, the presence of Enro in water used for irrigation did not affect the growth and flower production of *Zantedeschia rehmannii* and *Spathiphyllum wallisii* (unpublished data). It is important to note that the antimicrobial tolerance of ornamental plants is often followed by the plant capacity to accumulate the antimicrobial in their tissues. In this scenario, although yields may not be reduced by the presence of antimicrobials, ornamental plants could constitute a vehicle of antimicrobial contamination, transporting these molecules in their biomasses upon their commercialization (35).

On the other hand, this capacity for antibiotic accumulation can be used for decontamination purposes such as the use of ornamental plants to mitigate water and/or soil contamination (phytoremediation). Indeed, ornamental plants have emerged as potential phytoremediator species due to their high stress tolerance and are not intended for animal or human food consumption – which avoids the introduction of contaminants into the food web (35). Moreover, in addition to improving the environments with aesthetic value, ornamental plants provide multiple ecosystem services and promote human well-being (35).

Therefore, for both, the production and safety of commercialization of ornamental plants grown under contaminated conditions as well as for phytoremediation purposes, it is important to investigate the accumulation and distribution of contaminants between the different plant organs (mainly in those that are marketable). We therefore investigated the effects of the presence of Enro in soils and manure used for the growth of calla lily on plant growth and flower production. Moreover, we evaluated the accumulation and distribution of Enro among the different plant organs aiming to predict the safety of commercializing plants and to evaluate the phytoremediation capacity of plants.

Material and methods

Soil preparation and seedling production

Propagative material of *Z. aethiopica* (L.) Spreng. was acquired from Horto Botânico, Departamento de Agricultura, Federal University of Lavras (Lavras, Brazil). Seedlings were produced by burying the tubers in pots (5 L capacity) filled with substrate composed of soil + sand (2:1). The pots were irrigated daily for 45 days with distilled water. Once emerged, seedlings of 10 ± 2 cm size, with 2 leaves were transplanted to pots containing their respective treatments as described below.

The soil used in the experiment was collected in Curitiba, Paraná, Brazil, in the 0 - 20 cm soil layer, in a non-agricultural area. Prior to its use, the soil was tested for the presence of Enro (as described below) and no Enro was observed. Then, soil was corrected with dolomitic limestone until pH 5.5 ± 0.5. The physical-chemical characteristics of soil was as follows: pH ($CaCL_2$): 4.23; H+Al, Ca, Mg, K ($cmol_c$ dm^{-3}): 12.1, 3.3, 1.6, 0.05, respectively; P (mg dm^{-3}): 5.13; C (g dm^{-3}): 23.88; sand, silt, clay (%): 31.3, 7.4, 61.3. The bovine manure used was collected in an area of organic production, free of antimicrobial use (Centro Paranaense de Referência em Agroecologia - CPRA) in Curitiba (Paraná, Brazil). As for the soil, the manure was checked for Enro presence, and no antimicrobial was found.

Soil and manure were contaminated with Enro by using analytical grade Enro (Vetranal, Sigama-Aldrich, Canada). A stock solution (1 mg mL^{-1}) was firstly prepared in ultra-pure water and the correspondent aliquots used for the contamination of the matrices. Soil and manure were contaminated separately with 0 or 1.9 mg Enro kg^{-1} and 0 or 50 mg Enro kg^{-1}, respectively. These concentrations were selected based on the environmental representative concentrations of Enro in soils (30) and manure (29).

The ultrapure water was added in the same volume as the Enro-solution to the treatments without contamination. After 24h, the manure was incorporated into the soil in a ratio of 3:1. After mixing, samples of the substrate were taken to evaluate the Enro concentrations and then, the substrates were packed in plastic pots with a capacity of 12 liters. One seedling was then transplanted in each pot and considered as an experimental unit. A total of 25 experimental units were prepared in a completely randomized design, with 5 replicates per treatment. The plants were irrigated twice a week with 300 mL of distilled water. In parallel, three pots without plants were prepared and exposed to the same conditions to determine the antimicrobial degradation (photolysis, hydrolysis, and biodegradation). Treatments were as follows: soil + manure (S + M), contaminated soil + manure (CS + M), soil + contaminated manure (S + CM), contaminated soil + contaminated manure (CS + CM).

Plant production and physiological evaluations

Evaluations were performed in plants after the emission of the first flower (about 60 days after the transplantation). A hand-help SPAD (CCM-200 plus; Opti-Sciences; USA) was used to estimate chlorophyll content. SPAD readings were taken on second fully expanded leaves (from apex to the base) in a total of three readings per leaf. Leavers used for SPAD readings were healthy, turgid, flat, and homogeneous in color and size. The leaves from each plant were counted and after that, plants were harvested, washed thoroughly with distilled water, gently dried with absorbent paper and segmented into flowers, leaves, roots,

and rhizomes. Then, the fresh biomass of organs was measured, which was used to assess ornamental production of plants. Samples were then flash-frozen in liquid nitrogen and stored at -20 °C until being further evaluated.

Antioxidant enzymes and hydrogen peroxide (H_2O_2) concentrations (36) in leaves were determined using 0.1 g of plant tissues. Evaluations were performed in the same leaves used for SPAD readings. The enzymes were extracted in 1 mL of phosphate buffer containing 100 mM EDTA, 1 mL of L-ascorbic acid, and a 2% polyvinylpyrrolidone solution (PVP m/v) (37). The activities of superoxide dismutase (SOD) (38) and catalase (CAT) (39) were assessed after determining the total protein concentrations (40).

Chemical analyses

Enrofloxacin concentrations were investigated in dried samples (45 °C) using high performance liquid chromatography (HPLC). The antimicrobial was evaluated in substrates (soil and/or manure) at the beginning and at harvesting. For that purpose, at harvesting, substrate samples were collected at 10 cm depth, corresponding to the rhizosphere in pots with plants. Extraction was performed in 1 g substrate by adding 1 mL of 100 mM sodium phosphate buffer (pH 3) and 1 mL chloroform. After homogenization for 10 min in a rotating shaker (Firstlab, Brazil) at 25 rpm, samples were filtered in a syringe with cotton mesh and the organic phase (corresponding to chloroform) was then collected and dried in a SpeedVac machine (RC1010, Thermo).

For plant samples, dried organs were firstly homogenized, and the extraction of Enro was performed in three sub-replicates (1 g each) 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, following Migliori et al. (41). After extraction, the samples were dried in a SpeedVac machine. For both, substrate and plant samples, the obtained residues after extraction were suspended in acidified water (pH 4.5). Samples were then filtered through C18 solid phase extraction cartridges previously conditioned with 15 mL of methanol and 5 mL of water (pH 3.0). 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 (RC1010, Thermo), and the residues resuspended in 0.4% aqueous triethylamine pH 3.0, acetonitrile and methanol (75:10:15 v/v/v) (42).

The concentrations of Enro were determined following Shi et al. (42) (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 (88.5%), substrate and plant samples (without Enro treatment) were spiked with known concentrations of Enro, and then extracted and evaluated.

The Enro distribution among the different plant organs was analyzed by calculating the Translocation factor (TF) and Enro content (Enro concentration x organ DW) of the aboveground organs (shoots + flower) divided by the Enro content of the subterranean organs (roots + rhizomes) as follows:

$$\textbf{Translocation factor} \ (\textbf{TF})$$
$$= \frac{Shoot \ \ content + Flower \ \ content}{Root \ \ content + Rizhome \ \ content} \quad (1)$$

$$\textbf{Enro content}$$
$$= \ Enro \ \ concentration \ organ \ \star \ organ \ \ DW \quad (2)$$

Where DW: dry weight (*weight of the sample after drying*).

The natural degradation of Enro in the substrate and the plant removal efficiency of Enro (Removal Efficiency) was calculated as follows (43):

$$\textbf{Natural degradation} \ (\%)$$
$$= 100 - \frac{Cf \ without \ plants}{Ci \ without \ plants} * 100 \quad (3)$$

$$\textbf{Removal Efficiency} \ (\%)$$
$$= 100 - \frac{Cf \ with \ plants}{Ci \ with \ plants} * 100 - Degradation \quad (4)$$

Where: C_i and C_f are the initial and final concentration of Enro in the substrates. To study the Enro bioconcentration behavior, the bioconcentration factor (BCF) was calculated by dividing the Enro-concentration in plant tissues (ppm) at harvesting by the initial Enro concentration in substrate.

Data analysis

Statistical analyses were performed using JMP 7.0 software (SAS Institute Inc.). The results were expressed as the average of five replicates. Data were tested for normality (Shapiro Wilk) and homoscedasticity (Bartlett), and then statistically evaluated. Data for biomass production, physiological parameters, Enro-degradation in substrates and plant removal efficiency were submitted to one-way analysis of variance (ANOVA) and data for Enro accumulation in plants and Enro concentration in substrates were submitted to two-way ANOVA. The interactions between treatments (S + M), CS + M, S + CM and CS + CM) and plant organs (leaves, flowers, rhizome, and roots), as well as treatments and substrate concentrations (C_0, C_f -plants, C_f +plants) were evaluated using two-way ANOVA and the means were compared using the *post hoc* Tukey test (significance at P < 0.05).

Results

Initial concentration of Enro in substrates

No Enro was detected in substrate comprising soil and manure without artificial Enro contamination (S + M). The average concentration of Enro in contaminated soil was 1.9, 50.4, and 51.5 mg kg^{-1} for contaminated soil + manure, (CS + M), soil + contaminated manure (S + CM) and contaminated soil + contaminated manure (CS + CM), respectively.

Plant production and physiological responses

The number of flowers and leaves, the times until flowering, as well as the fresh biomass were not affected by manure addition or the presence of Enro in the substrate (Table 1; P>0.05).

TABLE 1 Number of leaves, SPAD index, dry biomass of distinct organs, H$_2$O$_2$ concentration and activities of Superoxide Dismutase (SOD) and Catalase (CAT) enzymes in calla lily plants grown in substrates with or without enrofloxacin addition.

Treatments	Number of leaves	SPAD	Leaves	Flowers	Roots	Rhizomes	H$_2$O$_2$	SOD	CAT
		Index		Dry Biomass (g)			µg g^{-1}	U mg prot^{-1}	
S+M	4.9 ± 2.0	797.2 ± 126.2	2.18 ± 5.5	0.99 ± 0.18	3.48 ± 1.58	3.42 ± 15.8	40.7 ± 13.9	0.26 ± 0.02	4.7 x10^{-7}± 2.8x10^{-6}
CS + M	4.5 ± 1.0	852.3 ± 117.6	2.08 ± 6.4	1.01 ± 0.25	3.54 ± 1.87	3.56 ± 1.74	28.5 ± 6.5	0.29 ± 0.05	4.02x10^{-6} ± 3.0x10^{-6}
S +CM	4.7 ± 2.0	833.4 ± 118.3	3.05 ± 3.2	1.27 ± 0.33	3.40 ± 0.95	4.60 ± 1.27	37.5 ± 6.0	0.27 ± 0.05	3.4x10^{-7} ± 2.4x10^{-7}
CS + CM	3.5 ± 0.8	806.0 ± 97.0	2.14 ± 8.1	0.10 ± 0.27	3.54 ± 1.70	3.32 ± 1.35	28.1 ± 8.5	0.23 ± 0.01	5.5x10^{-7} ± 2.8 x10^{-6}
F ratio	0.77	0.24	0.46 ns	1.33	0.009	0.99	2.35	1.83	5.41
Prob > F	0.52	0.86	0.45	0.29	0.99	0.42	0.11	0.18	0.25

Soil (S) = 0 mg Enro kg^{-1}; manure (M) = 0 mg Enro kg^{-1}; Contaminated soil (CS) = 1.9 mg Enro kg^{-1}; Contaminated manure (CM) = 50.4 mg Enro kg^{-1}.
Values represent means ± SD of five replicates.

Similarly, SPAD readings, SOD and CAT activity, as well as H_2O_2 concentrations were not affected by treatments (Table 1). Moreover, no visual symptoms of toxicity and good flower and foliage conditions (i.e., flower strength and color, absence of chlorotic or necrotic points and physical defects) were observed in plants grown in the presence of the antimicrobial (Figure 1).

Enrofloxacin concentration and distribution in plants

No Enro was detected in plant organs grown in non-contaminated substrate (S + M) (Figure 2). Regardless of the plant organ, greater Enro concentration was observed in plants grown in the presence of contaminated manure (S + CM and CS + CM) (Figure 2). Significant interaction between treatments and plant organs was observed for Enro concentration in plants (F=184.17; P<0.0001). When grown in contaminated substrate (CS + M, S + CM and CS + CM), the greatest concentration of Enro was detected in roots, followed by leaves while it did not significantly differ between flowers and rhizomes (Figure 2A).
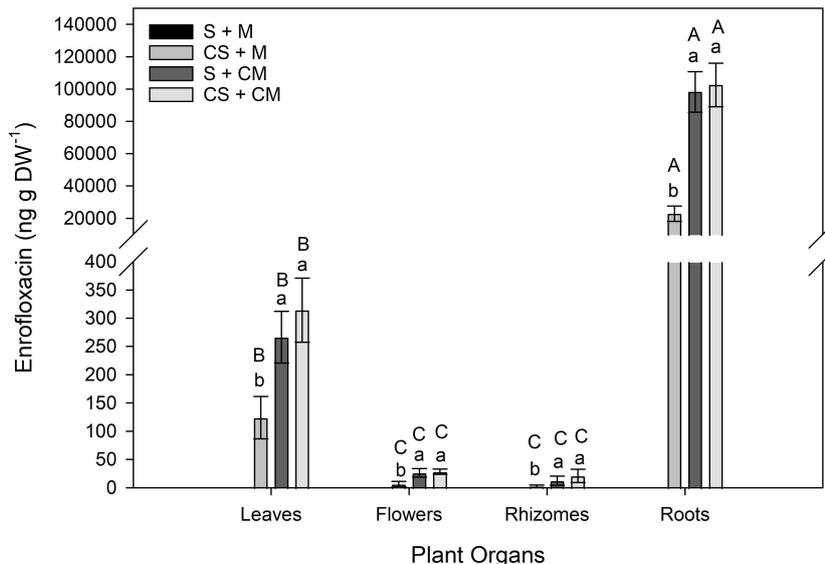
Phytoremediation capacity

Significantly lower (P<0.05) residual concentrations of Enro were observed in substrate in the presence of plants as compared

FIGURE 1
Aspect of calla lily grown in substrates with or without enrofloxacin addition. **(A)** Soil + manure; **(B)** Contaminated soil + manure; **(C)** Soil + contaminated manure; **(D)** Contaminated soil + contaminated manure.

FIGURE 2

Enrofloxacin concentration in different organs in calla lily plants grown in substrates with or without enrofloxacin addition. Bars represent the means $\pm$ SD of five replicates. Lower case letters indicate significant differences between treatments for the same plant organ; uppercase letters indicate significant differences in the pharmaceutical concentration of plant organs of the same treatment. Soil (S) = 0 mg Enro kg^{-1}; manure (M) = 0 mg Enro kg^{-1}; Contaminated soil (CS) = 1.9 mg Enro kg^{-1}; Contaminated manure (CM) = 50.4 mg Enro kg^{-1}.

to the pots without plants (Figure 3A). Natural Enro degradation (F = 255.20; P<0.0001) was the highest and the removal efficiency the lowest (F= 7.89; P<0.001) in substrate where only soil was contaminated with Enro (CS + M). Translocation (TF) and bioconcentration factor (BCF) values were lower than 1, regardless of the treatment (Table 2). For all the treatments, the BCF was higher in roots than in shoots (Table 2). The Enro-TF was greater in plants grown in CS + M, in relation to those grown in substrate receiving contaminated manure (S + CM and CS + CM) (Table 2).
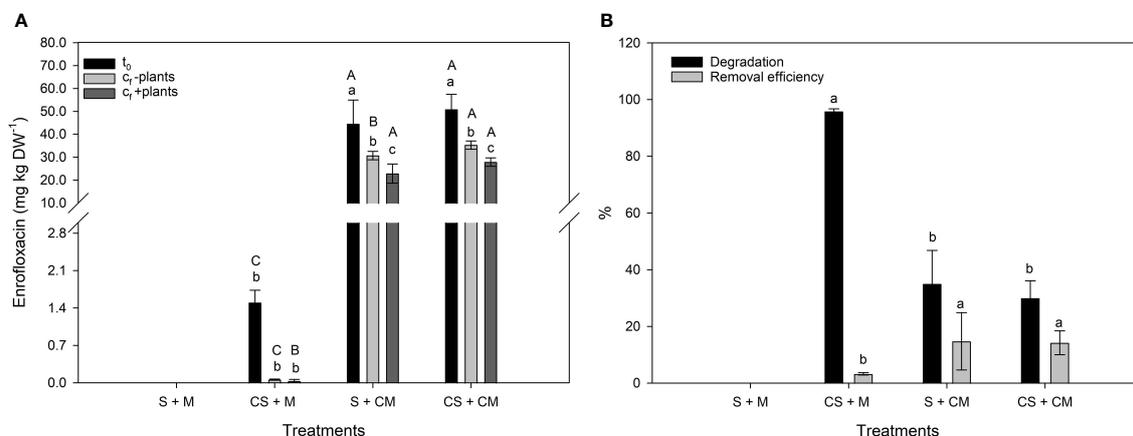
FIGURE 3

(A) Enrofloxacin concentrations in substrate at the initial (C_0) and final time (C_f) in the presence (+plants) or absence (-plants) of calla lily plants. Lower case letters indicate significant differences in Enro concentrations between t_0, C_f - plants and C_f + plants within the same treatment; uppercase letters indicate significant differences in the pharmaceutical concentration between treatments within t_0, C_f - plants and C_f + plants. **(B)** Natural degradation in substrates and Enro-removal efficiency of calla lily plants. Different letters indicate significant differences between treatments. Bars represent the means $\pm$ SD of five replicates. Soil (S) = 0 mg Enro kg^{-1}; manure (M) = 0 mg Enro kg^{-1}; Contaminated soil (CS) = 1.9 mg Enro kg^{-1}; Contaminated manure (CM) = 50.4 mg Enro kg^{-1}.

TABLE 2 Translocation factor (TF) and bioconcentration factor (BCF) in calla lily plants grown in substrates with or without enrofloxacin addition.

Treatments	TF	BCF		
		Shoots	Roots	Total
S + M	–	–	–	–
CS + M	4.77×10^{-3}	7.10×10^{-5} a	12.94×10^{-3} a	0.013a
S + CM	2.96×10^{-3}	6.90×10^{-5} b	2.34×10^{-3} b	0.0024b
S + CM	2.10×10^{-3}	6.90×10^{-5} b	2.06×10^{-3}b	0.0021b
F ratio	8.32	61.91	22.21	22.44
Prob > F	0.0015	< 0.0001	< 0.0001	< 0.0001

Soil (S) = 0 mg Enro kg^{-1}; manure (M) = 0 mg Enro kg^{-1}; Contaminated soil (CS) = 1.9 mg Enro kg^{-1}; Contaminated manure (CM) = 50.4 mg Enro kg^{-1}.
Values represent the means of five replicates. Different letters indicate significant differences between treatments.

Discussion

The non-observed effect of the investigated concentrations of Enro in substrates indicate the plant-tolerance to the pharmaceutical. Enro does not appear to interfere with the photosynthetic metabolism of plants, as it was observed in the aquatic macrophytes *Lemna minor* [44] and *Elodea canadensis* [45]. Similarly, here, the presence of the drug on plant substrates did not affect photosynthetic pigment levels (as evaluated by SPAD) and biomass production (Table 1).

Shoot biomass production is critical for predicting the commercial value of ornamental plants and is intrinsically related to photosynthetic rates [46] The calla lily tolerance to Enro was also seen by no-effects of Enro on H_2O_2 concentrations and antioxidant enzymes (SOD and CAT) in the plant leaves. Oxidative damage has been observed in plants exposed to Enro and were associated with Enro-phytotoxicity. For instance, increased ROS formation was followed by increased activity of antioxidant enzymes and decreased concentration of photosynthetic pigments in *Scenedesmus obliquus* [47] and *Chlorella vulgaris* [48].

Therefore, the absence of phytotoxic symptoms associated with the maintenance of plant biomass and flower production indicates that calla lily plants are tolerant to Enro and their commercial production may not be disrupted by the use of substrates (such as manure or soil) contaminated with the pharmaceutical.

Although the tolerance of calla lily plants to Enro brings good perspectives for the plant production aspect, the capacity of these plants to accumulate Enro could result in environmental problems as the plants may act as vehicles of the pharmaceutical, transporting it to uncontaminated areas. However, using ornamental plants in the bioaccumulation of Enro presents, to a certain extent, a lower risk to human health hazards because they avoid direct intoxication that could happen through crop plants [35, 49].

Accumulation of Enro in plant tissues have been observed in several agriculture important species, such as corn, soybeans, beans [19], lettuce, common barley (*Hordeum vulgare* L.), cucumber (*Cucumis sativus* L.) [50] and wheat (*Triticum aestivum*) [20]. According to Minden et al. [51], *Brassica napus* and *Triticum aestivum* are highly likely to be exposed to antibiotics due to soil fertilization with slurry or manure and antibiotics affected the characteristics of these plants, even in small concentrations. The absorption and accumulation of Enro in plants is related to the transpiration rate, involving both energy-dependent active and energy-independent passive processes [52, 53]. Once absorbed by roots, Enro can be moved through plant tissues and be transferred to the shoots using the transpiration current pathway [52]. The distribution in plant tissues will depend on the physiological and morphoanatomical differences of each species, in addition to the ability to absorb and metabolize antibiotics [19, 54]. In this context, the accumulation and distribution of Enro between plant organs must be investigated to attest the safety of the plant's marketable products - in our case, flowers, and potted plants, mainly.

The greatest part of the observed Enro in plant organs was found in roots, resulting in their greatest BCF in relation to shoots. As indicated by its lower TF (Figure 2B), indeed, Enro was preferentially allocated in subterranean organs, mainly in roots (Figure 2A). Yan et al. [55] also observed greater concentrations of the cipro, another FQ, in the roots of *Eichhornia crassipes* in relation to their shoots. As a result of the lower translocation of the antimicrobial to shoots, only residual concentrations of Enro were found in flowers (up to 28.37 ng g DW^{-1}). When considering the toxicological aspects, translocating less contaminants to the aerial parts is a desirable characteristic in a bioaccumulating species as the transfer of toxic elements to other links in the trophic chain is also minimized.

Furthermore, the residual concentration found in calla lily flowers is minimal compared to those that would be required to significantly contribute to environmental contamination and, therefore, the sale of calla lily flowers produced under Enro-contaminated conditions is safe. For instance, considering a

bouquet comprising 15 flowers (~1.6 g DW flower^{-1}), a maximum of only 680 ng of Enro will be transported by plants. However, special attention must be given when the plants are marketed in pots, because when considering the sum of all organs, leaves, flowers, roots and rhizome, the levels of Enro found were a matter of concern. Roots constitute up to 30% of the total biomass of calla lily plants during the initial period of production, although this percentage reduces overtime with plant maturity (56). Based on their maximum Enro contents, the leaves of calla lily carry up to 988 ng of Enro each; in addition, 3.5 g of roots and 3.7 g of rhizome (average for a plant), carry together, up to 35.9 μg of Enro. Therefore, considering a pot with four leaves and one flower, approximately 350 μg of Enro will be loaded (including the concentration of subterranean organs). This amount of Enro must be observed depending on the quantity and destination of plants sold in pots. Thus, pots that are sold alone do not pose an environmental risk, but if several plants are used, for example, to compose gardens, calla lilies can constitute a vehicle for Enro transmission to uncontaminated environments. The inappropriate use can raise environmental concerns (57). In this case, when they evaluated the use of ornamental plants in phytoremediation, Khan et al. (49) proposed establishing environmental quality standards to avoid secondary pollution using environmentally acceptable and sustainable methods. As a way of ensuring environmental safety, we suggest the commercialization of only calla lily flowers. In addition to attesting the safety of commercialization of calla lily flowers grown under substrate contaminated by Enro, our study also suggests the species to be considered for phytoremediation purposes. It can be observed that ornamental species are an excellent option to diversify crop production in small farms. In addition, these plants have high biomass production, tolerance to abiotic stresses and, particularly, do not enter the food chain (13, 58), which are desirable characteristics for phytoremediation programs.

Compared to other common species used in the phytoremediation system, the destination of plants is more flexible. In addition to the commercialization of cut flowers and foliage, small farms can use ornamental plants to attract tourism (59, 60). Besides that, rural tourism associated with constructed wetlands to reduce livestock contaminants provide new opportunities to familiar farms promoting greener activities for sustainable agriculture (61, 62). The use of ornamental plants in phytoremediation processes reduces the risk of economic losses for the horticultural market (49).

Several studies have reported the phytoremediator capacity of calla lily plants for metal reclaim (9, 11) and for wastewater treatment (12, 63). When considering total plant biomass, plants were able to accumulate up to 100 μg Enro in their tissues, from which up to 99% of Enro was found in plant roots. The BCF lower than 1 (typical in excluding species) associated with the much lower TF (Table 2) indicates the phytostabilization ability

of calla lily. In phytostabilization, contaminants are absorbed by plants and are prevented from migrating to shoots (64, 65). Once accumulated in roots, the contaminants are no longer available for moving through environmental matrices, avoiding them entering water, and mainly into the food chain (66–68). Moreover, by allocating Enro in their roots, plants prevent deleterious effects to photosynthetic organs (23, 45, 69), preventing negative effects of Enro on plant yields. Considering an average weight of 10 g of dry mass per plant, and a population density of 10,000 plants ha^{-1} in producing sites (70), the growth of calla lily in contaminated substrates can remove up to 10,000 mg of Enro per hectare until the first flower production, which can be safety sold. Interestingly, TF was greater in plants grown in CS + M in relation to those grown in substrate receiving contaminated manure (S + CM and CS + CM). This can be related to the total Enro-concentration found inside plants which was greater in S + CM (98511.11 ng g^{-1} DW) and CS + CM (102914.5 ng g^{-1} DW) than CS + M plants (22920.53 ng g^{-1} DW). Often, when in lower concentration inside plants, xenobiotics preset great translocation factor due to the availability of more sites in which they can be placed in plant shoots.

Although the plants have shown the ability to uptake and accumulate Enro, their calculated removal efficiency was < 15% (Figure 3B). It is important to note that the substrate plays an important role in the absorption and translocation of antimicrobials by plants (41), and the physical-chemical properties of the molecule determine the half-life in the environment. Overall, FQs present a high tendency to sorption to solids with slowly degraded rates (half-lives > 60 d), which agrees with our findings, as there was a significant amount in the soil after 180 days of exposure. This occurs as a result of the mechanisms, such as surface complexation, H-binding, cation bridge, ion exchange and hydrophobic partition affect antibiotic sorption in the surface and subsurface (71, 72). Generally, Brazilian soils have pH-dependent charges and low cation exchange capacities (CECs), and thus lower sorption potential for cationic species, such as FQs (72). However, studies have shown that the sorption of FQs is high and rapid (less than 24 h) in tropical soil (72).

The solubility of the compound plays an important role in the capture and translocation of chemicals by plants, because the absorption of the drug is higher when the compound is present in the solution of the soil pores, because water acts as a carrier of chemicals to the roots through symplastic and apoplastic pathways (66). Therefore, adsorption models for antibiotics in the soil still need further study, as often traditional Freundlich or Langmuir models may not exhibit antibiotic adsorption behaviors under different edaphoclimatic conditions, and thus they may not be able to reflect the precise adsorption mechanisms and the effect of plants on adsorption and transport behaviors of these pharmaceutics in the soil (73).

Conclusion

The use of Enro-contaminated soil or manure for growing calla lily plants did not result in impacts on plant productivity. Although plants uptake and accumulate the pharmaceutical in their tissues, up to 99% of the total Enro absorbed was retained in the roots, with only residual concentrations observed in flowers. The commercialization of cut flowers is safe, it offers a reduced risk of biomagnification compared to cultivated plants, but we emphasize that the production of plants under Enro contaminated condition to be sold as pots merits more attention as a relatively high amount of Enro can be transported in the whole plant body. Enro tolerance in plants associated with its ability to accumulate Enro places calla as a potential phytoremediation species, which should be considered when associating economic and environmental gains. Finally, the production of calla lilly plants has dual benefits, remedial and recreational at the same time, therefore, its use should be promoted when designing the phytoremediation strategy of soils and manures contaminated with Enro. However, the use of post-remediation biomass must be demonstrably safe.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

CSR, DNMC, MVR, PJ and MPG conceptualization. CSR, LYK., JCMB, LTM, AG, and MPG performed the experiments and analyzed the data; CSR, DNMC, LTM and MPG wrote the original draft; MPG and PJ were responsible for funding acquisition and project administration; DC, MR, and MG supervision; and CR, LM. and MG writing – review & editing. All authors contributed to the article and approved the submitted version.

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.

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