UNIVERSIDADE FEDERAL DO PARANÁ

CAMILA DA SILVA ROCHA

PLANTAS ORNAMENTAIS NO COMBATE À CONTAMINAÇÃO POR ENROFLOXACINO: DO BELO AO ÚTIL

CURITIBA
2022

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Tese apresentada ao curso de Pós-Graduação em Ciência do Solo, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutora em Ciência do Solo.

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CURITIBA

2022

TERMO DE APROVAÇÃO

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIA DO SOLO - 40001016014P4

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIA DO SOLO da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **CAMILA DA SILVA ROCHA** intitulada: **PLANTAS ORNAMENTAIS NO COMBATE À CONTAMINAÇÃO POR ENROFLOXACINO: DO BELO AO ÚTIL**, sob orientação do Prof. Dr. MARCELO PEDROSA GOMES, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua aprovação no rito de defesa.

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Avaliador Externo (UNIVERSIDADE FEDERAL DO PARANá- DPTO FITOTECNIA E FITOSSANITARISMO)

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DE CIÊNCIAS AGRÁRIAS

Rocha, Camila da Silva
Plantas ornamentais no combate à contaminação por enrofloxacino: do belo ao útil / Camila da Silva Rocha. – Curitiba, 2022.
1 recurso online: PDF.

Tese (Doutorado) – Universidade Federal do Paraná, Setor de Ciências Agrárias, Programa de Pós-Graduação em Ciência do Solo.
Orientador: Prof. Dr. Marcelo Pedrosa Gomes
Coorientadora: Profª. Drª. Daniella Carneiro Moraes Nogueira
Coorientador(a): Profª. Drª. Michele Valquiria dos Reis

1. Fitorremediação. 2. Antimicrobianos. 3. Plantas ornamentais. I. Gomes, Marcelo Pedrosa. II. Nogueira, Daniella Carneiro Moraes. III. Reis, Michele Valquiria dos. IV. Universidade Federal do Paraná. Programa de Pós-Graduação em Ciência do Solo. V. Título.

Bibliotecária: Telma Terezinha Stresser de Assis CRB-9/944

AGRADECIMENTOS

Agradeço a Deus por estar sempre ao meu lado durante esse tempo e cuidando de cada detalhe.

Especialmente aos meus pais Irene e João, esse trabalho não seria possível sem vocês.

À minha filha Maria Luiza, por ser meu combustível toda vez que pensei em desistir, e por ter aguentado toda sua infância com uma mãe pós-graduanda e ausente em muitos momentos, saiba que cheguei até aqui para que você tenha orgulho da sua origem e honre a educação e a ciência.

Ao meu orientador Marcelo, exemplo de profissional e amigo, a quem eu quero levar para a vida, por todos os momentos que passamos juntos, você mudou minha forma de ver o mundo e me ensinou a ter autoconfiança.

Aos amigos e professores do Programa de Pós-Graduação em Ciência do solo.

Aos amigos do Laboratório de Fisiologia de Plantas sob Estresse, que me proporcionaram tantos momentos de descontração e aprendizado científico.

Às coorientadoras Daniella e Michele.

Aos colegas de trabalho da T-Minas.

À Denise, por tantas vezes que foi um ombro amigo.

A todos meu muito obrigada!

RESUMO

As plantas ornamentais são espécies de expressiva importância econômica e com crescente demanda para o setor hortícola, pois geram renda para produtores em áreas relativamente pequenas. No entanto, muitos fatores estão envolvidos na produtividade e qualidade dos produtos comercializados. Entre as principais formas de manejo que garantem a alta produção está a adubação com esterco animal, que apesar de ser fonte de nutrientes pode atuar como veículo de contaminantes emergentes, como o Enrofloxacino (Enro). Esse antimicrobiano é usado em larga escala na pecuária e por isso, constantemente, são encontrados resíduos em áreas agrícolas. Nesse trabalho, foi investigado se a contaminação de diferentes matrizes (água ou solo) com enrofloxacino pode afetar a produtividade e qualidade de três importantes espécies ornamentais (*Zantedeschia rehmannii*, *Sapthiphyllum walisii* e *Z. aethiopica*). Observou-se que a contaminação da água de irrigação por Enro em concentrações crescentes (0, 5, 10, 100 e 1000 µg l^{-1}) não afetou a produção de flores e a biomassa de *Z. rehmannii* e *S. walisii*. Para essas duas espécies houve acúmulo de Enro majoritariamente nas raízes, enquanto uma quantidade insignificante do antimicrobiano foi observada na parte aérea das plantas (<120 ng g massa $seca^{-1}$) e, dado o baixo conteúdo total de Enro transportado por planta (<111 µg $planta^{-1}$), pode-se considerar que a comercialização dessas plantas é segura, tanto de flores de corte como plantas envasadas. Similarmente, quando concentrações de Enro,já encontradas no ambiente, foram adicionadas ao solo (1,9 mg kg^{-1}) e/ou esterco (50,4 mg kg^{-1}), não foram observados efeitos no crescimento, produção de flores e em parâmetros fisiológicos (concentração de pigmentos fotossintéticos e marcadores de estresse oxidativo) em plantas de *Z. aethiopica.* Assim como para *Z. rehmannii* e *S. walisii*, as plantas de *Z. aethiopica* acumularam mais Enro em suas raízes (até 10255 µg g massa $seca^{-1}$), com baixa translocação do antimicrobiano para a parte aérea (fator de translocação < 0,004), o que torna segura a venda das hastes florais de plantas produzidas em substrato contaminado por Enro. Entretanto, devido ao elevado acúmulo do antimicrobiano nas raízes (até 3.52 mg $planta^{-1}$) e dada a representatividade da biomassa radicular das plantas (~30%), a comercialização em vaso (como acontece na venda de flores destinadas a projetos paisagísticos e jardins) deve ser observada com cautela. Além de determinar se a produção é viável e a segurança da comercialização dos produtos advindos de plantas cultivadas sob condições de contaminação por Enro, a determinação dos parâmetros de produção de biomassa e de acúmulo de contaminantes (neste caso, Enro), permite avaliar o potencial fitorremediador de plantas ornamentais. Neste estudo, as três espécies avaliadas apresentaram tolerância e potencial bioacumulador de Enro, o que as tornam indicadas para programas de fitorremediação em diferentes matrizes ambientais na presença desse antimicrobiano. Dessa forma, o cultivo de *Z. rehmannii*, *S. walisii* e *Z. aethiopica* em áreas contaminadas por Enro pode atrelar ganhos econômicos (com a comercialização de seus produtos) à ganhos ambientais (descontaminação de Enro), fazendo do belo, um instrumento ambientalmente útil.

Palavras-chave: fitorremediação; copo-de-leite; antimicrobianos; lírio da paz; callas coloridas.

ABSTRACT

The ornamental plants are species of extreme importance and with increasing demand for the horticultural sector, it generates income for farmes in relatively small areas. However, many factors are involved in the productivity and quality of marketed products. Among the main forms of management that guarantee high production is fertilization with the animal manure, which despite an excellent source of nutrients may act as a vehicle for emerging contaminants, such as Enrofloxacin (Enro). This antimicrobial is used on a large scale in livestock and therefore constantly waste is found in agricultural areas. In this work, it was investigated whether contamination of different matrices (water or soil) with enrofloxacin can affect the production chain of three ornamental species (*Zantedeschia rehmannii*, *Sapthiphyllum walisii* and *Z. aethiopica*). It was observed that the contamination of the irrigation water by Enro in environmentally relevant contractions (0, 5, 10, 100 and 1000 µg l-1) did not significantly reduce biomass and the quality flowers of *Z.rehmannii* and *S. walisii*. The plants absorbed and accumulated Enro in their tissues, most of which were found in the roots. Since an insignificant amount of antimicrobial was observed in the shoots (<120 ng g dry mass^{-1}) and, given the low total content of Enro transported per plant (<111 µ g plant^{-1}), it is safe to commercialize in pots as well as of cutting flowers of *Z plants.* *rehmannii* and S. *walisii* produced under irrigation with water contaminated by Enro. Similarly, when environmentally relevant concentrations of Enro were added to the soil (1.9 mg kg^{-1}) and/or manure (50.4 mg kg^{-1}), no effects on growth, flower production and physiological parameters (concentration of photosynthetic pigments and oxidative stress markers) were observed in *Z aethiopica.* As well as *Z. rehmannii* and *S. walisii*, plants of *Z. aethiopica* accumulated more Enro in its roots (up to 102.55 µg g dry mass^{-1}), with low translocation of the antimicrobial to the aerial part (translocation factor < 0.004), which makes safe the sale of floral stems of plants produced in substrate contaminated by Enro. However, due to the high accumulation of antimicrobial in the roots (up to 3.52 mg plant^{-1}) and given the representativeness of root biomass in the body of plants (~30%), the commercialization of pot plants is not indicated. In addition to determining whether the production is feasible and the safety of the commercialization of products from plants grown under conditions of contamination by Enro, the determination of the parameters of biomass production and accumulation of contaminants (in this case, Enro), allows the evaluating potential phytomediator of ornamental plants. In this study, the three evaluated species presented tolerance and bioaccumulator potential of Enro, which make them indicated for phytoremediation programs of environmental matrices contaminated by this antimicrobial. Thus, the cultivation of *Z. rehmannii*, *S. walisii* and *Z. aethiopica* in areas contaminated by Enro may tow economic gains (with the commercialization of its products) to environmental gains (decontamination of Enro), making the beautiful, an environmentally useful instrument.

Keywords: phytoremediation; calla lily; antimicrobial; peace lily; color calla lily.

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Sumário

1 INTRODUÇÃO GERAL

A produção de flores e plantas ornamentais é uma atividade agrícola de importância social e econômica, principalmente em países emergentes como o Brasil. O comércio dessas espécies, movimenta bilhões de dólares ao redor do mundo (BRAINER, 2019; EUROPEAN COMMISION, 2020; USDA, 2020) com destaque para o setor de flores e folhagens de corte (PEREIRA et al., 2021), além da comercialização de plantas em vaso, mudas e materiais propagativos. Entre as espécies de maior interesse econômico estão as pertencentes à família Araceae, com boa adaptação ambiental, tolerância a estresse biótico (MOUNIKA; PANJA; SAHA, 2017) alta produção de biomassa e valor paisagístico, sendo comercializadas em vasos, como plantas de jardim, flores de corte, etc. Além disso, muitas delas já foram testadas como filtros naturais de compostos voláteis - como xyleno e tolueno- em ambientes fechados (PARSEH *et al.*, 2018; WANG, L. *et al.*, 2020; ZAMORA-CASTRO *et al.*, 2019) e como remediadoras de contaminantes orgânicos presentes no solo e na água e elementos traços (CHEN et al., 2009; MACCI et al., 2015). Nesse sentido, entre as espécies de Aracea mais estudadas e por isso escolhidas para esse estudo, estão o lírio da paz (*Spathiphyllum wallisii* Regel), copo-de-leite (*Zantedeschia aethiopica* (L.) *Spreng.*) e callas coloridas (*Zantedeschia rehmannii* Engl.) (CASIERRA-POSADA; BLANKE; GUERRERO-GUÍO, 2014; CHEN *et al.*, 2009; TEJEDA; ZURITA, 2020a).

Apesar da facilidade de adaptação ambiental, essas plantas necessitam de condições adequadas de solo para produção em escala comercial. Nesse sentido, a adubação, via fertilizantes orgânicos é uma das práticas fundamentais ao aumento da produção e à qualidade das plantas ornamentais (ALMEIDA *et al.*, 2008; FIGUEIREDO *et al.*, 2014). O dejeto animal além de fornecer nutrientes às plantas, também é uma ótima fonte de matéria orgânica, o que garante melhor condição estrutural e microbiológica ao solo (LIMA; DOMINGUES; SILVA, 2020). No entanto, a aplicação intensiva de adubação orgânica ao solo serve como veículo para diversos contaminantes, como os medicamentos de uso veterinário (LI, *et al.*, 2020). Em geral, os antimicrobianos, os quais são usados no tratamento de infecções bacterianas e como promotores de crescimento em animais, são as drogas mais frequentemente detectadas em solos agrícolas (SU *et al.*, 2018; VAN DOORSLAER *et al.*, 2014). Os antimicrobianos compreendem moléculas de origem natural, também, conhecidos como antibióticos ou sintética, e essas moléculas possuem ação bacteriostática ou bactericida. Dentro desse grupo de medicamentos, destaca-se a classe das fluoroquinolonas (FQ), a qual é efetiva no tratamento de infecções bacterianas (ZHANEL *et al.*, 2002), porém quando excretada nas fezes e urina dos animais, é degradada apenas na presença de luz ou por processos oxidativos onerosos (He et al., 2015), ou seja, os processos tradicionais de compostagem podem reduzir, mas não eliminar as moléculas de FQ em sua forma ativa (LIMA; DOMINGUES; SILVA, 2020).

Dessa forma, as FQ's podem permanecer no solo por longos períodos, adsorvidas à matéria orgânica e à fração argila de óxidos e hidróxidos de Fe e Al (Linke et al., 2010) e na água por ligações com compostos orgânicos e metálicos (AL-GHEETHI *et al.*, 2015). Uma vez presentes no solo, as FQ's podem ser transportadas aos corpos hídricos pelos processos erosivos (escoamento superficial) (REGITANO; LEAL, 2010). Isso ocorre, pois há baixa taxa de assimilação pelo organismo dos animais (NUNES *et al.*, 2019), o que favorece a permanência desses compostos no sistema e a consequente biodisponibilidade a organismos não-alvos, causando assim danos econômicos e ambientais (ARISTILDE et al., 2010b; GOMES et al., 2017; GOMES et al., 2019; GOMES et al., 2020; Panja et al., 2019; RIAZ, et al., 2017).

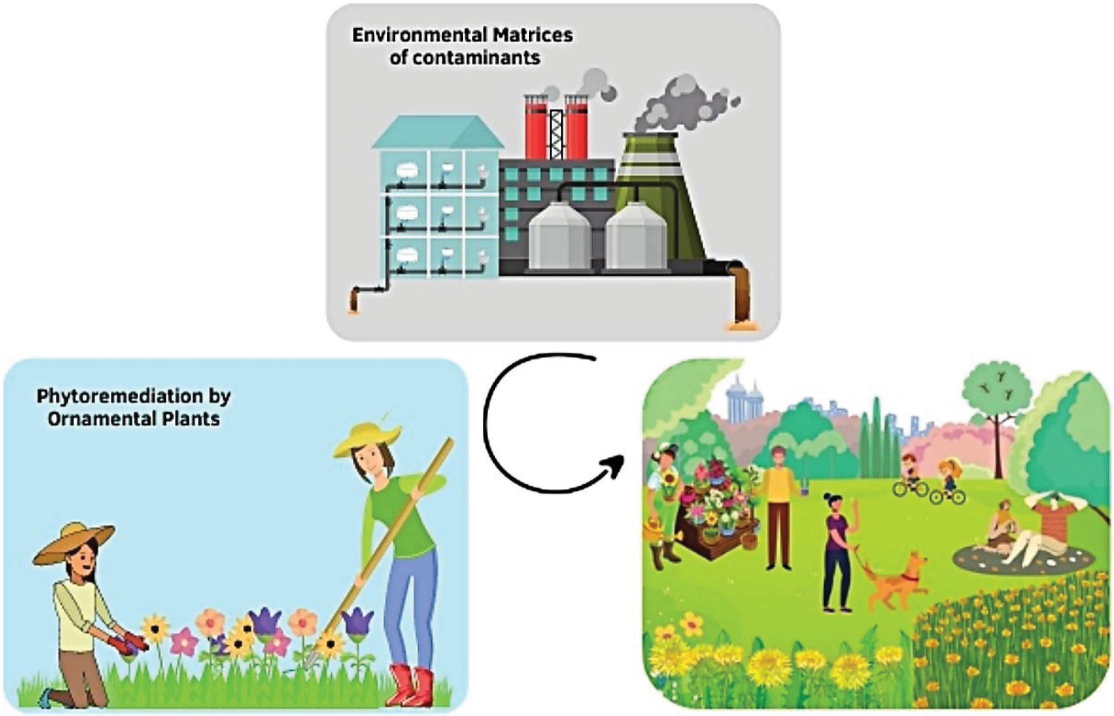
Uma FQ utilizada intensivamente na pecuária é o Enrofloxacino (Enro), e por isso, altas concentrações já foram encontradas em diferentes matrizes sólidas, com níveis de até 1,03 mg kg^{-1} em solos agrícolas (PARENTE, *et al.*, 2019) e até 46 mg kg^{-1} no esterco animal (Zhao et al., 2010), e também em água (LEAL *et al.*, 2012), com valores de até 800 ng L^{-1} em águas superficiais (GOMES *et al.*, 2022). Esses valores são preocupantes em relação ao desenvolvimento de resistência bacteriana a essa molécula, e embora a tolerância de algumas plantas ornamentais a contaminantes orgânicos tenha sido estudada (ROCHA *et al.*, 2022), não há estudos específicos sobre os efeitos dos fármacos, como o Enro, na produção e riscos associados à comercialização dessas plantas.

Algumas formas de reduzir os contaminantes inseridos no ambiente de formas mais sustentáveis, consiste no uso de plantas que conseguem bioacumular esses elementos em seus tecidos, técnica conhecida como fitorremediação. Nesse caso, as espécies a serem utilizadas devem possuir adaptação e tolerância ao estresse, bem como associações com microorganismos na região da rizosfera. O uso de plantas no combate a contaminação é uma estratégia baseada na natureza, ou mais conhecida como “Soluções baseadas na natureza (SBN)”, as quais compreendem ações de proteção, gerenciamento e restauração de ecossistemas naturais ou modificados, que abordam desafios sociais eficaz e adaptativamente, ao passo que fornece benefícios de bem-estar e biodiversidade (COHEN-SHACHAM, 2019). Dentre as principais formas de fitorremediação que ocorrem no organismo vegetal tem-se a rizodegradação, fitoextração, fitovolatilização etc. – as quais dependem tanto do tipo de contaminante como da espécie de planta e associações.

Considerando que as espécies ornamentais podem desempenhar um importante papel como uma SBN em projetos de fitorremediação acumulando contaminantes ambientais e nos sistemas agrícola como fonte de renda o presente trabalho teve como objetivo *i*) Avaliar os aspectos morfofisiológicos de espécies ornamentais com potencial de mitigação de contaminantes e discutir quais as principais características promovem ganhos econômicos atrelados aos processos de descontaminação*ii*) Mensurar os efeitos do antimicrobiano enrofloxacino, na produção de plantas ornamentais (*Zantedeschia rehmannii* e *Spathiphyllum wallisii*), bem como o potencial de acúmulo e translocação após irrigação com água contaminada; *iii*) Quantificar o efeito do solo e do esterco contaminado com enrofloxacino em plantas de copo-de-leite (*Zantedeschia aethiopica*), **e a investigação dos efeitos causados pelos contaminantes** tanto na produção e qualidade das plantas como seu potencial fitorremediador.

2 PHYTOREMEDIATION BY ORNAMENTAL PLANTS: A BEAUTIFUL AND ECOLOGICAL ALTERNATIVE[1]

2.1 GRAPHICAL ABSTRACT



2.2 RESUMO

A fitorremediação é uma tecnologia ecológica e econômica na qual as plantas são utilizadas para a remoção de contaminantes presentes no ambiente urbano e rural. Um dos desafios da técnica é o destino adequado da biomassa das plantas. Nesse contexto, o uso de plantas ornamentais em áreas sob tratamento de contaminação melhora a paisagem, servindo como opção turística e fonte de renda com alto valor agregado. Portanto, há um interesse particular de escolher plantas ornamentais para fins de fitorremediação. Além de sua alta tolerância ao estresse, rápido crescimento, alta produção de biomassa e bom desenvolvimento radicular, as espécies ornamentais não são destinadas ao consumo de alimentos animais e humanos, evitando a introdução de contaminantes na rede alimentar, além de melhorar os ambientes com valor estético. Além disso, as plantas ornamentais prestam múltiplos serviços ecossistêmicos e promovem o bem-estar humano, contribuindo para a conservação da biodiversidade. Nesta revisão, resumimos os principais usos das plantas ornamentais na fitorremediação do solo contaminado, ar e água. Discutimos o potencial uso de plantas ornamentais em faixas de tampão

[1] Artigo publicado: ROCHA, C. S.; ROCHA, D.C.; KOCHI, L.Y.; CARNEIRO, D.M.; REIS, M.V.; GOMES, M. P. Phytoremediation by ornamental plants: A beautiful and ecological alternative. Environmental Science and Pollution Research (2022), 29: 3336-3354.

construídas com o objetivo de mitigar a contaminação de terras agrícolas ocorridas nas proximidades de fontes de contaminantes. Além disso, fundamentamos os benefícios ecológicos e para a saúde do uso de plantas ornamentais em projetos paisagísticos urbanos e rurais. Espera-se que este estudo chame a atenção para uma tecnologia promissora de descontaminação combinada com o embelezamento das áreas urbanas e rurais, bem como uma possível fonte alternativa de renda e diversificação na horticultura.

Palavras-chave: Contaminação; Fitoextração; Elementos traços; Wetland.

2.3 ABSTRACT

Phytoremediation is an eco-friendly and economical technology in which plants are used for the removal of contaminants presents in the urban and rural environment. One of the challenges of the technique is the proper destination of the biomass of plants. In this context, the use of ornamental plants in areas under contamination treatment improve landscape, serving as a tourist option and source of income with high added value. Therefore, there is particular interest of choosing ornamental plants for phytoremediation purposes. In addition to their high stress tolerance, rapid growth, high biomass production and good root development, ornamental species are not intended for animal and human food consumption, avoiding the introduction of contaminants into the food web in addition to improving the environments with aesthetic value. Furthermore, ornamental plants provide multiple ecosystem services, and promote human well-being, while contributing to the conservation of biodiversity. In this review, we summarized the main uses of ornamental plants in phytoremediation of contaminated soil, air, and water. We discuss the potential use of ornamental plants in constructed buffer strips aiming to mitigate the contamination of agricultural lands occurring in the vicinity of sources of contaminants. Moreover, we underlie the ecological and health benefits of the use of ornamental plants in urban and rural landscape projects. This study is expected to draw attention to a promising decontamination technology combined with the beautification of urban and rural areas as well as a possible alternative source of income and diversification in horticultural production.

Keywords: Contamination; Phytoextraction; Trace elements; Wetlands.

2.4 INTRODUCTION

As biotechnology, phytoremediation uses the potential of plants to remove pollutants and contaminants from the environment, which occurs through different processes such as phytoextraction, phytostabilization, phytodegradation and phytovolatilization (HWANG *et al.*, 2020). In phytoextraction, the most well-known technique (ASGARI LAJAYER *et al.*, 2019) plants uptake and concentrate contaminants in their harvestable biomass, while phytodegradation occurs with mineralization of the contaminant inside plants or at the soil-root interface (with the help of microorganisms), normally decreasing its toxicity (LIU; CHEN; HE, 2018). Through the phytostabilization process, plants immobilize contaminants in the rhizosphere by its lignification or humidification (LEE; HADIBARATA; YUNIARTO, 2020). Phytovolatilization occurs directly or indirectly, by dilution and photochemical decay of the contaminant into the phyllosphere, with its later diffusion to the atmosphere via stomata (transpiration) (LEE; HADIBARATA; YUNIARTO, 2020).

Most phytoremediation studies for water purification focus on the use of aquatic macrophytes (GOMES *et al.*, 2020; MENDES *et al.*, 2021; SINGH; PANDEY; SUTHAR, 2019; YAN *et al.*, 2020). These plants have different levels of contaminant tolerance, and when used in combination in a decontamination system, they provide an environmentally sustainable and economically viable technology (MUN *et al.*, 2020). However, one of the few options for the destination of these contaminated plants is incineration. In soil phytoremediation programs, in turns, woody species are often used to reclaim contaminants (CRUZ, *et al.*, 2020; GOMES *et al.*, 2020; SANT'ANNA-SANTOS *et al.*, 2019). Nevertheless, due to their long-life cycle, the use of woody species can result in long-lived projects and slowly recuperation rates of contaminated areas. In this sense, the use of ornamental plants can fill these gaps, since these species present relatively rapid life cycles, in addition to high biomass production, and great efficiency in removing contaminants from waters, air and soils (CHEN *et al.*, 2009). In the case of species with phytodegradation capacity, ornamental plants can represent an incoming source via the commercialization as flowers or pots since contaminants may be mineralized or have lower toxicity once inside plant tissues. Ornamental plants can also be used for phytostabilization of abandoned mining areas (ZENG *et al.*, 2018) or intended for tourism in farms with contaminated soil from agricultural practices (LIU; XIN; ZHOU, 2018). In addition, ornamental trees with landscape values are also promising for the recovery of contaminated areas, acting as natural filters (HÉNAULT-ETHIER *et al.*, 2017) at the same time that allow the commercialization of their biomass for energetic purposes (HÉNAULT-ETHIER *et al.*, 2017).

The wide variety of shapes and colors of ornamental plants, bring to the environment the possibility of landscape intervention (LIU; XIN; ZHOU, 2018) offering advantages that include economic benefits, incentivating the maintainance of the treatment of areas (ASGARI LAJAYER *et al.*, 2019; HERNÁNDEZ; GALINDO-ZETINA; HERNÁNDEZ-HERNÁNDEZ, 2018; SANDOVAL-HERAZO *et al.*, 2018). The beatification of recovering areas by using ornamental plants improve the turism maily due to the promotion of experiences assossiated with well-being and healthy lifestyle (CIFTCIOGLU; EBEDI; ABAK, 2019; FISHER *et al.*, 2021; PAIVA; DE BRITO SOUSA; CARCAUD, 2020; REIS; DOS REIS; DO NASCIMENTO, 2020). Moreover, these species are not subject to biomagnification, since they do not enter the food chain (CHEN *et al.*, 2009). They also prevent erosion, reduce wind speed in addition to allowing greater infiltration of rainwater into the soil (TURKYILMAZ *et al.*, 2018).

The purpose of this review was to couple the potential of ornamental plants to remove different contaminants from soil, water, and air with their promoting economical, ecological and health benefits to ecosystems. We discuss the main morpho-physiological characteristics of species to be used in phytoremediation programs. Moreover, we report the potential of ornamental plants as barriers for mitigating the contamination of agricultural areas in the vicinity of sources of contaminants as well as green technologies to improve the phytoremediation capacity of ornamental plants. Finally, we highlighted the benefits of using ornamental remediation plants in landscape projects, contributing to the development of more economical and attractive phytoremediation approaches. In addition to reduce the gaps between the scientific results and practical application of ornamental plants, such information will be useful for public managers to generate more sustainable management models for urban green and rural areas. We also aimed to underlie the benefits of the formation of a vegetated polyculture, with flower production on a real scale, as a possibility of landscape use, while promoting a cleaner and more pleasant environment.

2.5 SPECIES SCREENING

2.5.1 Potential features in ornamental species

The choice of species to be used in phytoremediation programs starts with the observation of their ability to grow and survive in degraded or contaminated environments (ASGARI LAJAYER *et al.*, 2019) indicating the existence of some level of tolerance. Another important aspect is the rate of growth and productivity of biomass, as the use of some species may become impracticable due to reduced growth (LIU; XIN; ZHOU, 2018) Thus, the set of phenotypic characteristics such as edaphoclimatic acclimation, biomass production, root depth, growth rate, planting and maintenance feasibility and the ability to establish microsymbionts, directly influence the choice of species for phytoremediation (ASGARI LAJAYER *et al.*, 2019; LIU; CHEN; HE, 2018; SHIKHA; GAUBA, 2016).

Among the morphological characteristics, plant species must be evaluated for root length, density, and surface area, as the root system influences on the absorption and degradation of xenobiotics and on the growth and reproduction of microorganisms in the rhizosphere via root exudates (LIU; XIN; ZHOU, 2018). Microbial interactions are important as they contribute on the degradation (WEI *et al.*, 2018) as well as in the uptake and tolerance of plants to contaminants (GOMES *et al.*, 2020). Plant height, stem diameter and leaf area index are also positively related to the tolerance and accumulation of contaminants, since the impact on photosynthesis and biomass production influences the volatilization and excretion of contaminants during detoxification (LIU; XIN; ZHOU, 2018). For example, in constructed wetlands, the ideal species are those with broad leaves and a dense root system; these characteristics increase the plant transpiration surface and efficient distribution of the roots in the water column, assuring great uptake and removal of contaminants from water (HWANG *et al.*, 2020). Similarly, these characteristics are also observed during screening of plants for soil decontamination.

Plants must also be screened for their tolerance to biotic (such as diseases and pests) and abiotic stress (temperature, drought, salinity, and contaminants). This characteristic is related to the activation of cellular defense mechanisms. Often, when under stressful conditions, there is an increased production of reactive oxygen species (ROS) and consequent oxidative damage to cell structures (DEL-TORO-

SÁNCHEZ *et al.*, 2013b; LIU; XIN; ZHOU, 2018). In this context, the capacity of plants to cope with stress as well as the choice of plant species for phytoremediation have been related to their ability to activate antioxidant systems (GOMES *et al.*, 2013, 2016; GOMES; JUNEAU, 2016) Non-enzymatic antioxidants such as ascorbate, glutathione, phytoquelatins, tocopherols, phenolic compounds, etc., are substances produced by plant metabolism that can provide reducing equivalents or directly eliminate ROS. Among the main enzymes that act in the detoxification of ROS are peroxidases (for example, ascorbate peroxidase (APX) and peroxiredoxin), catalases (CAT), superoxide dismutase (SOD), thioredoxin and members of the glutathione system such as glutathione reductase (GR) and glutathione-*S*-transferases (GST) (DEL-TORO-SÁNCHEZ *et al.*, 2013b).. The amount of non-enzymatic antioxidant as well as the activity of antioxidant enzymes have been greatly used as biomarkers for plant contaminant tolerance and phytoremediation capacity (GOMES *et al*., 2013; 2017).

In this sense, several ornamental plants show relevant characteristics that pose them as potential phytoremediation species with an advantage of short growth cycle (LIU et al., 2018) and consequently, rapid removal of contaminants. It also allows observations and recording of physiological responses to stress after flowering - usually less than a year, so mainly herbaceous species with aesthetic value have been effectively used in decontamination processes for environments (Liu, 2018). In addition to the morphological characteristics, the remediation capacity of ornamental plants is causally related to the responses of the antioxidant system and the pattern of distribution and deposition of contaminants in cell walls, vacuoles and metabolically inactive tissues (ASGARI LAJAYER *et al*., 2019) which depends on the type and environmentally found concentrations of contaminants, as described below.

2.5.2 Ornamental species in constructed wetlands

The constructed wetlands (CWs) are structures designed to treat wastewater in a controlled manner, by combining physical, chemical, and biological processes, where the vegetation remains under saturation of wastewater at the roots, simulating the processes in natural humid areas (HERNÁNDEZ; GALINDO-ZETINA; HERNÁNDEZ-HERNÁNDEZ, 2018). It is an alternative to conventional water treatment that presents low cost, easy maintenance, good performance and a pleasant appearance (HERNÁNDEZ; GALINDO-ZETINA; HERNÁNDEZ-HERNÁNDEZ, 2018). Usually, in CWs aquatic macrophytes species are used. These species are considered important ecological indicators, but different from ornamental species, there is a difficulty in allocating biomass at the end of the cycle (KOCHI *et al.*, 2020). Moreover, after the uptake of significant numbers of contaminants, aquatic plants begin to senesce and rot, releasing the elements back to the water column, increasing their concentration again (WANG *et al.*, 2021). Therefore, the knowledge about the biology of the species and suitable management are important for the proper functioning of CWs (KOCHI *et al.*, 2020).

CWs can be classified as superficial and sub-surfaced flows. In the superficial flow, a layer of soil and the effluent are in contact with the atmosphere, which allows the presence of emergent plants to be planted in the soil and projecting from the water column, while the floating and submerged plants are planted in the soil or floating in the water column (SEHAR, 2020). The subsurface flow CWs, on the other hand, use only emergent plants and a porous substrate which favors the development of microbial films (ZAMORA *et al.*, 2019). Thus, it is observed that biodegradation and biotransformation processes in wetlands occur mainly in the rhizosphere, where exudates intensify microbial activity and reduce the bioavailability of xenobiotics (Influence of a new ornamental species (*Spathiphyllum blandum*) on the removal of COD, nitrogen, phosphorus and fecal coliforms: a mesocosm wetland study with PET and tezontle substrates (SANDOVAL *et al.*, 2020).

Ornamental plants have been shown to be effective when used in wetlands, especially when in polyculture systems (CALHEIROS *et al.*, 2015) (FIGURE 2-1 I-L). In an experiment with *Lavandula sp.* L., *Spathiphyllum wallisii* Regel and *Zantedeschia aethiopica* (L.) Spreng., a significant increase in water quality as indicated by decreased biochemical oxygen demand in five-day (BOD_5), nitrates, phosphates and fecal coliforms compared to tanks without plants (SANDOVAL-HERAZO *et al.*, 2018). However, only *S. wallisii* and *Z. aethiopica* tolerated the conditions and produced flowers, thus showing the importance of screening species (SANDOVAL-HERAZO *et al.*, 2018). Similarly, *Cyperus papyrus* L. and *Tulbaghia violacea* Harv. (BURGOS *et al.*, 2017) *Hedychium coronarium* J. Koening, *S. wallisi*

Regel (ZAMORA *et al.*, 2019) and *Spathiphyllum blandum* Schott (Influence of a new ornamental species (*Spathiphyllum blandum*) on the removal of COD, nitrogen, phosphorus and fecal coliforms: a mesocosm wetland study with PET and tezontle substrates (SANDOVAL *et al.*, 2020) also proved to be alternatives to improve indicators of residual water quality in mesocosms with efficiency of up to 70% in quality indicators.

When selecting plants for CWs, it is important to note the location of the structure, rainfall, the type of sewage, species growth rate, density and depth of the root system, seasonal growth pattern, drought or waterlogging tolerance and vegetative dominance, aiming to decrease interspecific competition (CALHEIROS *et al.*, 2015). Further, seasons with higher temperatures change the rates of removal nutrients and contaminants (MACCI *et al.,* 2015). As a result of appropriate planning, ornamental plants can promote sustainable landscaping and a fresher, purified environment, improving the visual and sensory aspect and making the space more attractive. Therefore, the application of this system is an excellent alternative in places where effluents are released, revitalizing the landscape, and reducing public health problems related to environmental contamination (ZAMORA *et al.*, 2019) (FIGURE 2-2).

FIGURE 2-2 - EXAMPLES OF ORNAMENTAL HERBACEOUS SPECIES MOST COMMON IN RESEARCH IN THE PHYTOREMEDIATION OF TRACE ELEMENTS (A-H): (A) CANNA INDICA L. (B) TAGETES PATULA L., (C) ALCEA ROSEA L., (D) ZINNIA ELEGANS JACQ. EXAMPLES OF ORNAMENTAL TREES WITH HIGH POTENTIAL FOR ACCUMULATION OF CONTAMINANTS: (E) PRUNUS CERASIFERA EHRH., (F) AILANTHUS ALTISSIMUS (MILL.) SWINGLE, (G) ELAEAGNUS ANGUSTIFOLIA L., (H) TILIA TOMENTOSA MOENCH. ORNAMENTAL PLANTS COMMONLY USED IN CONSTRUCTED WETLAND (I – L): (I) HELICONIA ROSTRATA RUIZ & PAV., (JPONTEDERIA CRASSIPES MART., (K) CANNA FLACCIDA SALISB., (L) ZANTEDESCHIA AETHIOPICA (L.) SPRENG.

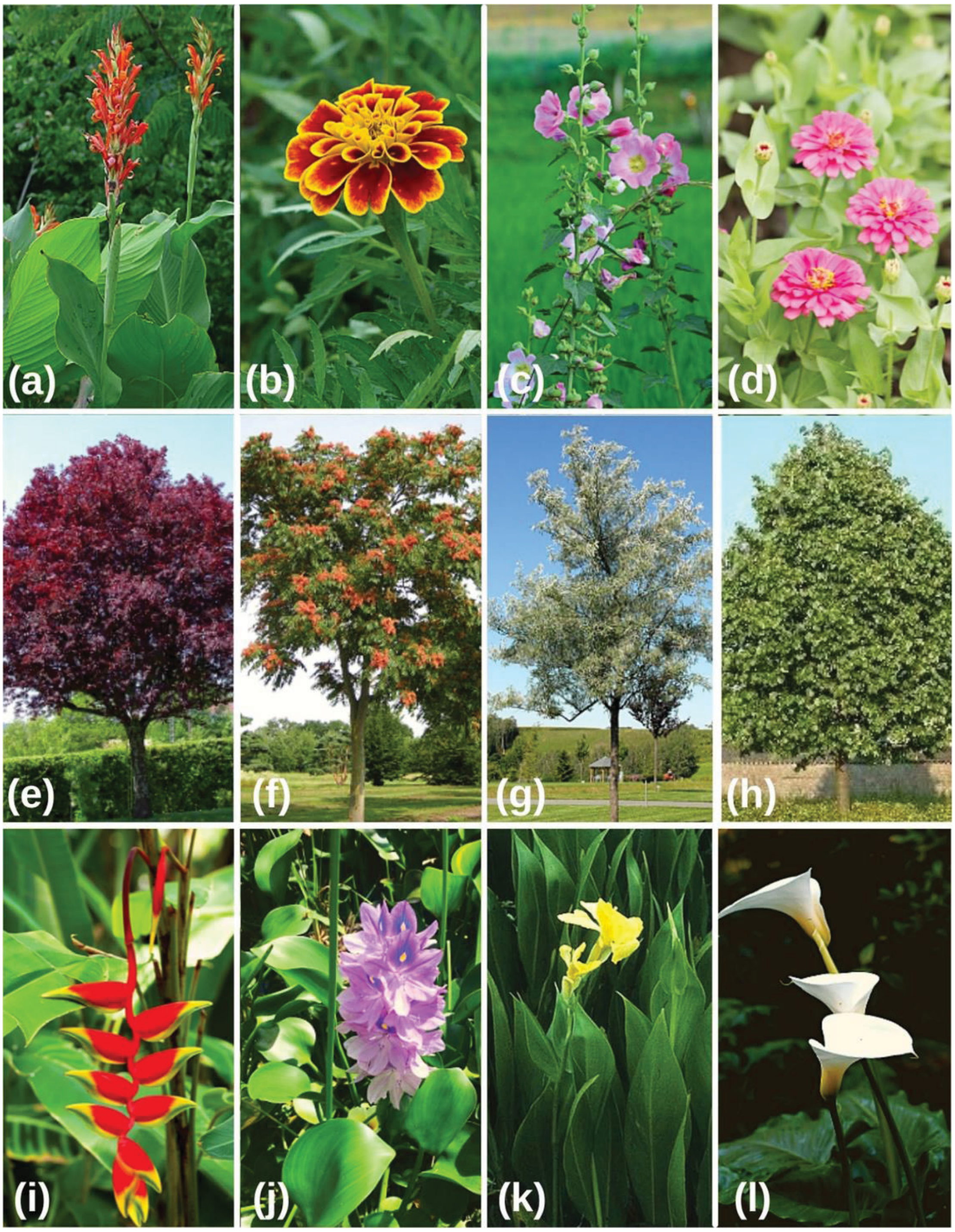
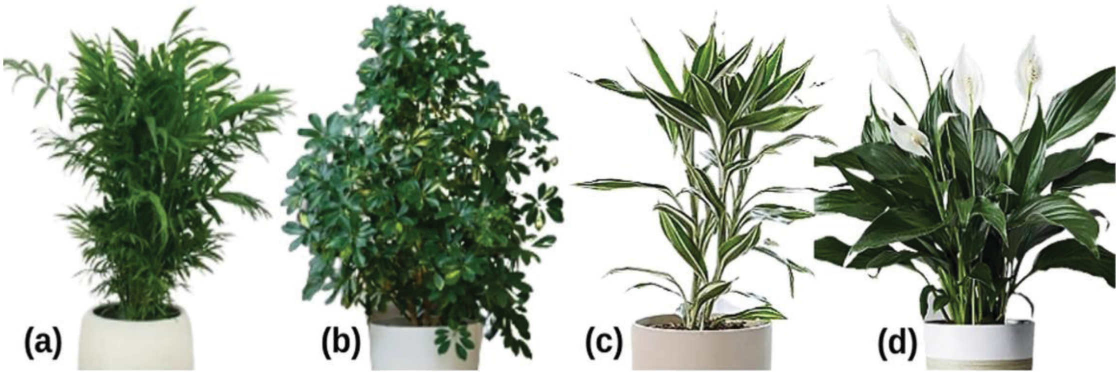


FIGURE 2-3 - POTTED PLANTS USED TO REMOVE VOCS: (A) CHAMAEDOREA ELEGANS MART., (B) SCHEFFLERA ARBORICOLA (HAYATA) MERR., (C) DRACAENA SANDERIANA SANDER EX MAST., (D) SPATHIPHYLLUM WALLISII REGEL

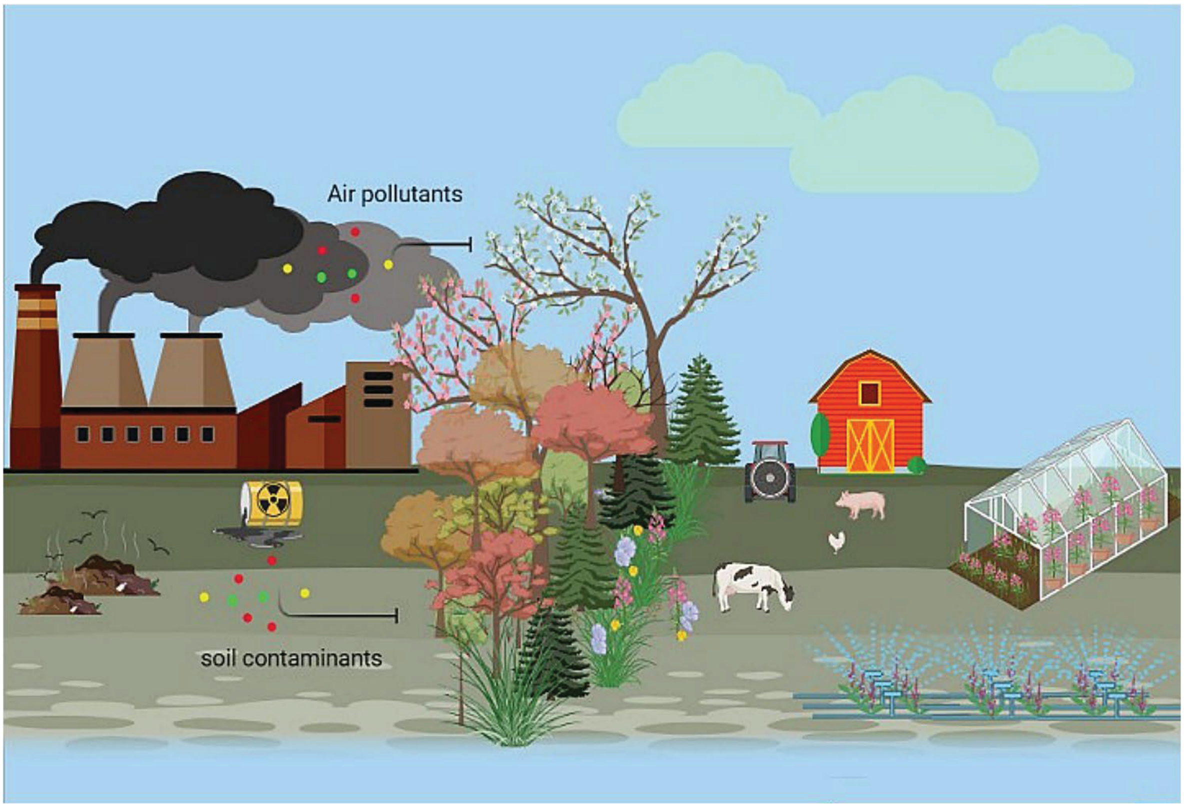


2.4.3 Ornamental species for mitigation of agricultural land contamination

Although agricultural practices are one of the major sources of contaminants to environment itself (by the use of fertilizers, pesticides and use of wastewater irrigation), farmers are particularly faced with problems when agricultural lands are in the closer vicinity of industries with high probability of exposure to industrial effluents. In the recent years, constructed buffer strips have been used as a green technology to minimize the export of chemicals from their source to nearby ecosystems (Herbaceous or Salix miyabeana 'SX64' narrow buffer strips as a means to minimize glyphosate and aminomethylphosphonic acid leaching from row crop fields (HÉNAULT-ETHIER *et al.*, 2017). This technology is limited, however, by the buffer's ability to intercept and attenuate chemicals traveling along the surface or subsurface pathways (MAYER *et al.*, 2007). Ornamental species is of particular interest in composing buffer strips at the interface of agricultural lands and industrial sites. Depending on their tolerance and extraction ability (as discussed below), these species may constitute a physical barrier, minimizing the contamination of agricultural lands by hazardous industrial compounds (FIGURE 2-4). Buffer strips act as a temporary or overtime buffer, diluting contaminants in water, soil and air, or as a definitive sink, stimulating contaminant sorption and degradation (mainly through microbial activity) or plant uptake, which results in contaminant sequestration, volatilization or decontamination (KRUTZ *et al.*, 2005). Recently, Hénault-Ethier et al., (2017), showed the potential of willow (*Salix miyabeana* Seemen) buffer strips as a means to minimize glyphosate, aminomethylphosphonic acid (HÉNAULT-ETHIER *et al.*, 2017) and nutrient runoff (HÉNAULT-ETHIER *et al.*, 2019). Willows has documented phytoremediation qualities been able to uptake soil pollutants, to filter water and to volatilize certain substances (BÖRJESSON, 1999; KUZOVKINA; VOLK, 2009; MIRCK *et al.*, 2005). In addition to its ecological benefit on mitigating contamination, the high biomass produced by the *S. miyabeana* buffer strips allows its harvesting, transformation on market opportunities, helping farmers assess the viability of this practice on their farm (HÉNAULT-ETHIER *et al.*, 2019). Willow biomass is of particular interest for bioenergy production, being an economically beneficial energy source for farmers and landowners (HÉNAULT-ETHIERA; GOMES 2017).

Herbaceous buffer strips may also help farmers to avoid agricultural land contamination by nearby potential hazardous industrial activities. This kind of buffer strip also shows phytoremediation abilities (HÉNAULT-ETHIER *et al.*, 2017) and the improvement of herbaceous buffer strips with flowering species not only result in aesthetic improvement but also improve habitat for animals, increasing, for instance, the abundance and species richness of butterflies (BLAKE *et al.,* 2011). Ornamental plants in buffer strips also contribute with insect landscape dynamics or genetics (ROSSI *et al*., 2016). The use of native ornamental plants in buffer strips also attract beneficial insects, increasing biodiversity and enhancing the delivery of insect-derived ecosystem services (GILL; COX; O'NEAL, 2014). In this context, the use of ornamental species in constructed buffer strips can constitute a green technology to improve the value of buffer strips, resulting in ecological and possible economic benefits to farmers at the same time to mitigating agricultural land contamination by surrounding sources of pollutants.

FIGURE 2-5 - ORNAMENTAL REMEDIATION PLANTS FOR MITIGATION OF AGRICULTURAL LAND CONTAMINATION. ORNAMENTAL PLANTS CAN BE USED TO COMPOSE BUFFER STRIPS AT THE INTERFACE OF AGRICULTURAL LANDS AND SOURCES OF CONTAMINANTS (SUCH AS INDUSTRIES) BY ATTENUATING CONTAMINANT



2.6 PHYTOREMEDIATION

2.6.1 Trace Elements

The introduction of trace elements into the environment by human activities such as mining, wastewater discharge, and use of chemical fertilizers and pesticides in agriculture (SUN *et al.*, 2011) leads to serious environmental problems. However, the use of plants by the phytoremediation method stands out for reducing the concentration of the pollutant in addition to contributing to the protection against erosion and to increase soil fertility (NAKBANPOTE; MEESUNGNOEN; PRASAD, 2016) - characteristics of an environmentally friendly technology.

The ability to remove and stabilize trace elements by plants can be evaluated by the bioaccumulation factor (BCF) which is defined as the concentration of the element in the plant (mg kg $^{-1}$) in relation to the concentration in the soil (mg kg $^{-1}$) or water (BUSCAROLI, 2017) as described in Equation 1. This is an important factor that indicates the phytoremediation potential since as it effectively measures what is being taken out of the soil by the plant. In general, BCF > 1 indicate significant quantitative transfer of metals from soil to plants, while values equal or less than 1 indicates that the plants may not be an accumulator or a metal excluder (SUBPIRAMANIYAM, 2021). Although BCF is species-dependent, it is most dependent on the metal concentration in the matrix than to plant physiological demands. Other available indexes to evaluate the potential of phytoextraction by plants is the translocation factor (TF) as described in Equation. 2 (CUI *et al.*, 2013), which indicates the mobility of metal from roots to shoots, where a value less than 1 indicates low contaminant translocation and greater than 1 indicates better phytoextraction capacity (LIU *et al.*, 2009). However, the use of TF varies with the stage of physiological maturation and with the evaporative demands of the plant and therefore its use is often criticized.

Similarly, some authors define the transfer of contaminants from the medium to the plants as Enrichment Coefficient (Equation 3) (EC) (KUMAR; SINGH; CHOPRA, 2018). The EC measures the mean concentration of the sample metal in relation to the metal concentration in the reference plants, where values from 2 to 5 are considered as moderate, 5 to 40 are significant values and above 40 they are extremely high EC (KUMAR; SINGH; CHOPRA, 2018). However, there is still no consensus in the scientific community regarding the exact definition of the terms and as reviewed by (BUSCAROLI, 2017) there are in the databases over nineteen terms with the same meaning, causing a great confusion to compare results.Another used indicator is the Tolerance Index (TI), calculated as the relation between the length of roots of plants exposed to metal stress and control condition (Equation 4) (CHAUHAN *et al.*, 2020). However, there are some differences in relation to the exact definition of this term, and some authors define TI in relation to the total biomass of plants and not just the length of the roots (LU *et al.*, 2020).

$$\mathrm{BCF} = \frac{\text{Metal concetration in plant tissue}}{\textit{Metal concentration} \text{ in medium}} \tag{1}$$

$$TF = \frac{Metal\ concentration\ in\ shoots}{Metal\ concentration\ in\ roots}$$

(2)

$$EC = \frac{Metal\ concentration\ in\ experimental\ plants}{Metal\ concentration\ in\ control\ plants}$$

(3)

$$TI\ (\%) = \frac{Length\ of\ roots\ in\ experimental\ plants\ \times\ 100}{Length\ of\ roots\ in\ control\ plants}$$

(4)

Some ornamental species have been tested for their ability to remove and concentrate trace elements from the environment (TABLE 2-2). Depending on their ability to remove metals, plants are also classified as hyperaccumulators. This classification criteria depends on the concentration of the element in the plant shoots, for instance, plants accumulating more than 10 mg Hg kg^{-1}, 100 mg Cd kg^{-1}, 1000 mg Co/Cr/Pb kg^{-1}, or 10,000 mg Zn/Ni kg^{-1} are considered as metal hyperaccumulators (LIANG *et al.*, 2009). Among them, the annual species *Tagetes erecta* L., *Tagetes patula* L. and *Zinnia elegans* Jacq. can accumulate cadmium (Cd) above 100 mg kg^{-1} (DW) in their shoots after exposure to 100 mg kg^{-1} of Cd in soil, being considered Cd-hyperaccumulator species (LIU; CHEN; HE, 2018). Similarly, the species *Alcea rosea* L., which have high value in landscape design, also showed Cd removal potential (with concentrations of 178.5 and 135.6 mg kg^{-1} in shoots and roots, respectively, when Cd in soil was 100 mg kg^{-1} (LIU *et al.,* 2009). *Chlorophytum comosum* (Thunb.) Jacques accumulated 1,522 and 865.5 mg Cd kg^{-1} in their shoots and roots respectively when exposed to 200 mg Cd kg^{-1} in soil (WANG *et al.*, 2012). In addition to their Cd-hyperaccumulation capacity, these species show highly ornamental value, posing them as great candidates for Cd reclamation from contaminated soils.

An alternative plant well known for its potential for hyperaccumulating metals is the sunflower (*Helianthus annuus* L.). In addition to the its ornamental destination, sunflowers are widely used to produce biodiesel and accumulate around 693.69 mg kg^{-1} of lead (Pb) (CHAUHAN *et al.*, 2020). This metal showed easy movement around sunflower, with a TF and an Enrichment Coefficient (EC) greater than 1 (ADESODUN *et al.*, 2010). This uptake capacity and ability to tolerate Pb, however, appears to be restricted to some plant species. For example, among the ornamental plants *Quamoclit pennata (Desr.) Bojer, Antirrhinum majus* L. and *Celosia cristata* L., only *C. cristata* was able to tolerate and accumulate Pb, with shoot concentrations as high as 1000 mg kg $^{-1}$ (CUI *et al.*, 2013).

Another trace element with growing worldwide concern is the arsenic (As). Humans can be exposed to chronic concentrations of the metalloid through contaminated air, food and drinking water (DEL-TORO-SÁNCHEZ *et al.*, 2013b). Two ornamental species able to accumulate As, *Z. aethiopica* (L.) Spreng. and *Anemopsis californica* (Nutt.) Hook. & Arn., proved to be excellent alternatives in the removal of this contaminant from drinking water, without any visual symptoms in leaves and stems (there was no evaluation on flower production) of toxicity after exposure to 34,711 µg As l^{-1} for 6 months (DEL-TORO-SÁNCHEZ *et al.*, 2013b). Such tolerance was related to their increased antioxidant enzyme activities, of such as APX, GR and CAT (DEL-TORO-SÁNCHEZ *et al.*, 2013b) which were responsible for the elimination of the excess of H_2O_2 and to maintain cellular thiol-redox balance - recognized mechanisms of As-tolerance.

In addition to the activation of enzymes such as SOD, POD and CAT (CUI *et al.*, 2013) ornamental plants exposed to metals, are favored by the activation of other protective systems, such as oxido-reducers, lignin peroxidase, laccase, veratryl alcohol oxidase, tyrosinase and azo-reductase (CHANDANSHIVE *et al.*, 2018). Furthermore, some genus of the Araceae family, such as *Zantedeschia* (CHEN *et al.*, 2009; DEL-TORO-SÁNCHEZ *et al.*, 2013b; TEJEDA; ZURITA, 2020b) and *Spathiphilum* (PARSEH *et al.*, 2018; SANDOVAL *et al.*, 2020) accumulate a large amount of calcium oxalate which acts in the protection against trace elements stress. In these plants, the synthesis of oxalic acid, a cellular constituent involved in the regulation of calcium (Ca) (DINEVA, 2019) is stimulated to maintain cellular ionic balance (GOUVEIA *et al.*, 2018). As a result, trace elements are incorporated to Ca oxalate crystals within the vacuoles of specialized cells (NAKATA, 2012), favoring their retention in plant tissues while reduce their toxicity.

Some ornamental plants are also favored by trace elements exposure. For instance, *Z. elegans* Jacq. produced a large number of flowers when grown in soils contaminated with 25 g kg^{-1} of chromium (Cr^{6+}) (PANDA *et al.*, 2020). However, few studies using ornamental plants for phytoremediation evaluated the effects of trace metals on flower production or their accumulation in flowers and therefore, more research is needed to relate the absorption capacity of contaminants with the quality of flowers (the main commercial product for many species; FIGURE 2-1 A- D) as well as the safety of their comercialization. In *Chysanthemum maximum* var. Shasta, for instance, Ni and Pb did not translocate to the flowers (GONZÁLEZ-CHÁVEZ; CARRILLO-GONZÁLEZ, 2013). Therefore, flowers of *C. maximum* plants grown under Ni or Pb contaminated sites, must be safe for commercialization, adding economic interest to its use for phytoremediation purpose.

Phytoremediation is not restraining to the use of garden and flowering plants; ornamental trees have also been used. In addition to returning the landscape beauty, ornamental trees improve air (by carbon sequestration) and soil quality (dendroremediation) (TURKYILMAZ *et al.*, 2018), promote protection of local fauna and thermal equilibrium by the release of water vapor (FIGURE 2-1 E-H), with the advantage of using biomass for wood, or pulp and paper production (KHAN *et al.*, 2021). In summary, the use of ornamental plants has gained attention for the reclaim of trace elements from contaminated areas, especially in urban environments, where they constitute a more viable option, promoting the improvement of aesthetics and cleanliness and landscape infrastructure.

TABLE 2-1 - ORNAMENTAL PLANTS USED IN PHYTOREMEDIATION OF TRACE-ELEMENTS

Element	Species	Exposition	Concentration in roots	Concentration in shoots	Region	References
			mg kg⁻¹			
Cd	*Alcea rosea L.*	100	136.00	100.00	China	(LIU *et al.*, 2009)
	Impatiens balsamina L.		325.00	148.00		
	Alcea rosea L.		135.60	178.50		(LIU *et al.*, 2009)
	Chlorophytum comosum (Thunb.) Jaques	200	1,522.00	865.50		(WANG *et al.*, 2012)
	Chysanthemum maximum var. Shasta	10 mg L^{-1}	320.00	64.00	Mexico	(GONZÁLEZ-CHÁVEZ; CARRILLO-GONZÁLEZ, 2013)
	Centaurea cyanus L.	100	85.22	>100.00	China	(LIU; CHEN; HE, 2018)
	Gerbera jamesonii Bolus ex Hook.f.		87.79	>100.00		
	Tagetes erecta L.		177.11	166.07		
	Tagetes patula L.		202.34	231.72		
	Taraxacum mongolicum Hand.-Mazz		109.13	>100.00		
	Zinnia elegans Jacq.		129.18	109.89		
	Alcea rosea L.	30	9.43	4.57	China	(HUANG *et al.*, 2020)
	Mirabilis jalapa L.		14.94	25.22		(LI, X. *et al.*, 2020)
	Tagetes patula L		30.27	60.23		
Cr	*Echinodorus cordifolius* (L.) Griseb.	8 mg L^{-1}	6,141.60	--	Thailand	(WORAHARN *et al.*, 2021)
	Helicónia psittacorum L.f. x *H . Spathocircinata*		≈2,500.00	≈250.00		
	Pontederia cordata L.		3,673.30	≈600.00		
	Tagetes erecta L.	12.5 mg L^{-1}	≈15,000.00	≈900.00	Brazil	(COELHO *et al.*, 2017)
	Euphorbia milli	75	634.00	231.00	India	(RAMANA *et al.*, 2015)
	Zinnia elegans L.		23.25	6.39		(PANDA *et al.*, 2020)
Cu	*Chysanthemum maximum* var.	10 mg L^{-1}	324.00	74.00	Mexico	(GONZÁLEZ-CHÁVEZ;

Ni	Shasta		172.00	38.00		CARRILLO-GONZÁLEZ, 2013)
	Antirrhinum majus L.		638.10	71.71		
	Celosia cristata L.	1,000	1,021.65	360.11	China	(CUI *et al.*, 2013)
	Ipomoea quamoclit L.		382.70	26.90		
	Chysanthemum maximum var. Shasta	100 mg L^{-1}	93.00	22.00	Mexico	(GONZÁLEZ-CHÁVEZ; CARRILLO-GONZÁLEZ, 2013)
Pb	*Helianthus annuus* L.	1,000	394.31	224.29	India	(CHAUHAN *et al.*, 2020)
	Calendula officinalis L.	1,000	14,251.70	961.50	China	(LU *et al.*, 2020)
	Chlorophytum comosum (Thunb.) Jacques		10,485.70	847.00		
	Iris lactea Pall.		11,156.70	1,077.50		
	Polygonum lapathifolium L.		12,220.50	713.50		
	Saponaria officinalis L.		15,466.30	672.50		
	Miscanthus sinensis Andersson	500	131.13	49.49		
	Hemerocallis fulva L.	1,000	788.32	301.42	China	(SONG *et al.*, 2020)
	Iris germanica L.		561.28	251.67		
	Canna x *generalis* L.H. Bailey		1,904.33	1,032.76		
	Pennisetum clandestinum Hochst. ex Chiov.		249.95	85.15		
	Viola × *wittrockiana* Gams	1,000	455.37	276.90	Poland	(SYCHTA; SŁOMKA; KUTA, 2020)
Sn	*Impatiens balsamina* L	2,000	244.8	114.4	China	(LIU *et al.*, 2021)
	Mirabilis jalapa L.		74.30	90.00		
	Tagetes erecta.L		95.80	11.30		
Zn	*Echinodorus cordifolius* (L.) Griseb.	40 mg L^{-1}	4,766.60	≈4,000.00	Thailand	(WORAHARN *et al.*, 2021)
	Helicónia psittacorum L.f. x *H . Spathocircinata*		4,313.50	<500.00		
	Pontederia cordata L.		≈2,500.00	≈2,500.00		
	Viola × *wittrockiana* Gams.	1,000	1,923.74	1,822.87	Poland	(SYCHTA; SŁOMKA; KUTA, 2020)

TABLE 2-2 - PLANTS USED FOR REMOVAL ORGANIC CONTAMINANTS AND THEIR EFFICIENCY

Species	Type System	Contaminants	Exposition (mg L^{-1})	Removal (%)	Region	References
		Wastewater				
Canna indica L. *Iris pseudacorus* L.	Monoculture	N (mg g^{-1} DW) P (mg g^{-1} DW)	14.56 1.93	>57.4 >37.06	China	(LI *et al.*, 2013)
Agapanthus africanus L. Hoffmanns. *Canna flaccida* Salisb. *Canna indica* L. *Zantedeschia aethiopica* (L.) Spreng. *Watsonia borbonica* (Pourr.) Goldblatt	Polyculture	COD BOD PO_4^{3-} NH_4^+ TSS	481 182 19.1 21.6 283	> 99 > 98 > 92 > 84 87	Portugal	(CALHEIROS *et al.*, 2015)
Spathiphyllum wallisii Regel *Zantedeschia aethiopica* (L.) Spreng.	Monoculture	BOD PO_4^- NO_3	115.96 11.75 11.95	>74.46 > 48 > 59	Mexico	(SANDOVAL-HERAZO *et al.*, 2018)
Chrysopogon zizanioides (L.) Roberty	Monoculture	Fecal coliforms NMP 100 mL^{-1} NO_3-N PO_4^{3-}	3319.31 29 10.5	> 65 89 97	USA	(PANJA; SARKAR; DATTA, 2020)
		Pharmaceuticals				
Helianthus annuus L.	Monoculture	Acetaminophen Carbamazepin	15	50 30	Czech Republic	(RYŠLAVÁ *et al.*, 2015)
Heliconia rostrata Ruiz & Pav.	Monoculture	Caffeine Ibuprofen	27	97 89	Brazil	(DE OLIVEIRA *et al.*, 2019)
Eichhornia crassipes (Mart.) Solms	Monoculture	Caffeine		94		

		Ibuprofen		89		
Canna flaccida Salisb.	Monoculture	Acetaminophen	5905.5	100	USA	(HWANG *et al.*, 2020)
		Carbamazepin	234.5	>36.7		
Iris sibirica L. *Zantedeschia aethiopica* (L.) Spreng.	Monoculture	Carbamazepin	15	31.1 20.9	Mexico	(TEJEDA; ZURITA, 2020b)
Cyperus haspan L. *Heliconea zingiberales*	Polyculture	Carbamazepin	0.2	10	Colombia	(DELGADO *et al.*, 2020)
		Sildenafil		>97		
Chrysopogon zizanioides (L.) Roberty	Monoculture	Ciprofloxacin	10	94	USA	(PANJA; SARKAR; DATTA, 2020)
		Tetracicline		100		
Mirabilis jalapa L. *Tagetes patula* L.	Monoculture	Tetracicline	5 mg kg^{-1}	99	China	(LI, X. *et al.*, 2020)
		Pesticides				
Eichhornia crassipes (Mart.) Solms	Monoculture	Organochlorine	1	>62	Pakistain	(RIAZ, *et al.*, 2017)
Pistia stratiotes L.		Pyrethroid	1	>72		
Canna flaccida Salisb.	Monoculture	Atrazine	226.5	>91.7	USA	(HWANG *et al.*, 2020)
		Others compounds				
Iris dichotoma Pall.	Monoculture	Petroleum Hydrocarbons	40,000 mg kg^{-1}	30.79	China	(CHENG *et al.*, 2017)
Iris lactea Pall.				25		

Tagetes patula L.	Monoculture	Benzo [a] pyrene	5 mg kg^{-1}	79.2 to 92.4	China	(Sun et al. 2011)
Dracaena sanderiana Sander ex Mast	Polyculture	Benzene	18 mg kg^{-1}	46	Thailand	(TREESUBSUNTORN; THIRAVETYAN, 2012)
Schefflera arboricola (Hayata)Merr. *Spathiphyllum wallisii* Regel	Monoculture		29.5 mg m^{-3}	94 93	Iran	(PARSEH *et al.*, 2018)
Hedera helix L.	Monoculture	Formaldehyde	1.0 mg kg^{-1}	70	Taiwan	(LIN; CHEN; CHUAH, 2017)
Chamaedorea elegans Mart.			16.4 mg m^{-3}	100	Iran	(TEIRI; POURZAMANI; HAJIZADEH, 2018)
Cyperus haspan L. *Heliconia ssp.*	Polyculture	Methylparaben	0.2 mg L^{-1}	>97	Colombia	(DELGADO *et al.*, 2020)

PAH: Polycyclic Aromatic Hydrocarbon; BOD: Biochemical Oxygen Demand; COD: Chemical Oxygen Demand; TSS (Total Suspended Solids); NMP: (Most probable number); ppm: parts per million; DW: Dry Weight. Fonte

2.6.2 Organic contaminants

The Emerging Contaminants are defined as synthetic or natural chemicals that are not currently monitored in the environment, or that have only recently been analyzed. Those contaminants have a high potential to enter the environment and cause adverse effects on human or ecological health (SPOSITO *et al.*, 2018). The category of emerging contaminants includes drugs and personal care products (PCPs), pesticides, solvents, fertilizer household cleaning products, polycyclic aromatic hydrocarbons, hormones among others (RATHI; KUMAR; SHOW, 2021). Frequently, the sewage containing these contaminants (at concentrations ranging from ng l^{-1} to mg l^{-1}) (DELGADO *et al.*, 2020) is discharged incorrectly in the soil or water bodies that supply cities, and the inefficiency of their depuration in traditional treatment processes, contribute to their environmental presence and contamination (CHRISTOU *et al.*, 2019).

Ornamental plants could act effectively in the removal of emerging contaminants. Here, we compile the main studies with ornamental plants, which search to mitigate the harmful effects caused by some environmental organic contaminants (TABLE 2-2). However, studies that assess the physiological behavior of plants during exposure to contaminants in soil and water are still scarce. As an example, the drug caffeine and ibuprofen, widely used by the population, were efficiently removed in CWs, by *Heliconia rostrata* Ruiz & Pav. and *Pontederia crassipes* Mart., with a removal efficiency greater than 90% of the initial concentration (27 mg l^{-1} after 204 days) (DE OLIVEIRA *et al.*, 2019). Similarly, *Canna flaccida* Salisb. removed all acetaminophen (5905.5 mg l^{-1}) residues from water in two weeks, and all atrazine residues (226.5 µg ml^{-1}) - a herbicide used on a large scale - in 12 weeks of treatment (HWANG *et al.*, 2020). In the same way, the sunflower (*H. annus*), completely removed ibuprofen (15 mg l^{-1}) in 48 hours and 50% of acetaminophen (15 mg l^{-1}) after 96 hours of water treatment, while the uptake of the anticonvulsant carbamazepine (15 mg l^{-1}) was 30% in 96 hours (RYŠLAVÁ *et al.*, 2015). Under hydroponic conditions, *Iris sibirica* L. and *Z. aethiopica* (L.) Spreng. also accumulated carbamazepine and showed high capacity (up to 70%) to degrade these drugs (Capacity of two ornamental species (*Iris sibirica* and *Zantedeschia* *aethiopica*) to take up, translocate, and accumulate carbamazepine under hydroponic conditions (TEJEDA; ZURITA, 2020b). This difference on absorption of distinct drugs occurs mainly because the absorption of organic xenobiotics depends on their physicochemical properties such as relative molecular mass, solubility in water, vapor pressure, Henry constant, partition coefficient octanol / water (K_{ow}) and acid dissociation constant (pKa) (RYŠLAVÁ *et al.*, 2015).

Recently, studies evaluated the removal of antibiotics by *Chrysopogon zizanioides* (L.) Roberty (PANJA; SARKAR; DATTA, 2020) and observed that when treated with 10 mg l^{-1} of ciprofloxacin and tetracycline, plant were able to reclaim 94% and 100% of these drugs, respectively. When present in contaminated soils, tetracycline (5.0 mg kg^{-1}) was efficiently removed by the ornamental species *Mirabilis jalapa* L. and *T. patula* L., with 99% efficiency (LI, X. *et al.*, 2020). Under laboratory conditions the antibiotic oxytetracycline (group of tetracyclines), was oxidized to compounds without antimicrobial action on the sunflower root (*H. annuus*) via the release of oxygen-reactive species (ROS) (GUJARATHI; LINDEN, 2005). In this sense, emerging contaminants are a group of drugs that can disrupt the oxidative metabolism of plants, which is closely related to its phytoremediation capacity. Furthermore, recent research has demonstrated enzymatic degradation as the main mechanism for removing PCPs by plants (KURADE *et al.*, 2021). For example, the increase in the activity of enzymes such as NADPH P450 reductase and glutathione-*S*-transferase are related to the process of detoxification of antibiotics by the metabolism of plants (TASHO; RYU; CHO, 2020), since the degradation of these drugs is associated with incresead ROS production as a response to stress (ROCHA *et al.*, 2021) In *T. patula* L. exposed to textile dye residues (20–100 mg L^{-1} Reactive Blue 160 for 4 days) there was a significant increase in intracellular and extracellular tyrosinase and NADH–DCIP reductase activity while laccase was found to be induced intracellularly (PATIL; JADHAV, 2013).

Moreover, the activity of cytochrome P450 is particularly involved in the metabolism of fluoroquinolones such as enrofloxacin in aquatic macrophytes (GOMES, Marcelo Pedrosa *et al.*, 2019). Cytochrome P450 is a phase I group enzymes of PCPs biotransformation, which drives oxidation reactions. These enzymes can also act as peroxidases in the presence of H_2O_2 (a type of ROS)

(KURADE *et al.*, 2021). However, the activity of these enzymes in ornamental species, specifically growing in contaminated environments has not yet been reported.

Another group of organic contaminants are Polycyclic Aromatic Hydrocarbons (PAHs) which are formed by incomplete combustion or pyrolysis of organic materials and found in the soil by atmospheric deposition (SUN *et al.*, 2011). For instance, Benzo [a] pyrene (B[a]P), is a PAH classified as a priority contaminant by the US Environmental Protection Agency (USEPA) for its carcinogenic potential and teratogenicity (SUN *et al.*, 2011). *T. patula* plants easily grew in low concentrations of B [a] P (≤10 mg kg^{-1}), which in addition to promote the plant growth (up to 49.7 % compared to the control) was greatly degraded into their tissues (79.2 to 92.4%) at 5 mg kg^{-1}. However, simultaneous contamination with Cd, Cu, and Pb, inhibited the growth of *T. patula* and the uptake and accumulation of B [a] P (SUN *et al.*, 2011). These results reinforce the importance of evaluating the combined effects of contaminants in plants screened for phytoremediation programs. In this context, the concomitant use of *T. patula* and *M. jalapa*, a plant with high biomass production, known for accumulating trace elements (WEI *et al.*, 2018), could be an alternative for B [a] P remediation. Indeed, the screening of plants for tolerance and removal of polluting substances often involves various species. For instance, the potential degradation of total petroleum hydrocarbons (TPHs) in soil was tested on 14 ornamental plants, including oil in the plant tissues (LIU *et al.*, 2012). Similarly, (CHENG *et al.*, 2017) indicate the species *Iris dichotoma* Pall. and *Iris lactea* Pall. for the remediation of petroleum hydrocarbons (PHCs)-contaminated saline-alkali soil; the remediation capacity of these plants was only limited when PHCs concentrations were high as 20,000 and 40,000 mg kg^{-1}, respectively.

We reinforce the importance of using several species simultaneously (polyculture) to effectively reduce the greatest number of contaminants present in the environment, (CALHEIROS *et al.*, 2015). The diversification of species in CWs is important because there is great variation over time between the sensitivity of plant species to changes in environmental conditions such as water levels, temperature, and herbivory. On the other hand, the polyculture can lead to the death of plants due to competition and greater production of biomass from some species, making it difficult to develop the other species in the consortium (DELL'OSBEL *et al.*, 2020).). Nevertheless, the polyculture system offers an advantage because the large production of biomass after phytoremediation may be used as plant pots, cut flowers, essential oils, perfumes, among others such as phytomining and biofuel production (KHAN *et al.*, 2021). Organic compounds must be metabolized or even fully mineralized in plant tissues, leading to their transformation in less toxic compounds, which may allow the commercialization of flowers or foliage of ornamental plants. For instance, we did not notice the accumulation of the antibiotic enrofloxacin in flowers of *Zantendeschia aethipica* (L.) Spreng when plants were submitted to irrigation with water contaminated with the antibiotic (unpublished data). Plants have mainly accumulated the antibiotic in their roots, restraining its translocation to flowers – assuring their commercialization. However, further studies regarding the accumulation of organic compounds in marketable parts of ornamental plants are needed to confirm the safety of their commercialization.

2.6.3 Air pollutants

Widely used indoors, ornamental plants can indicate and monitor atmospheric pollutants (CRUZ *et al.*, 2014; LIN; CHEN; CHUAH, 2017; PARSEH *et al.*, 2018). Volatile organic compounds (VOCs) are found in indoor air. However, many are carcinogenic and affect human health, such as formaldehyde and benzene (CRUZ *et al.*, 2014). Plants can alter VOC levels and therefore represent a potential green solution for improving air quality (CRUZ *et al.*, 2014). Removal of these substances can be induced by plants directly (absorption) and indirectly (biotransformation by rhizosphere-associated bacteria) (SRIPRAPAT; THIRAVETYAN, 2016).

Several factors influence the removal process such as the species used, the light intensity) and the concentration of VOCs in the environment (CRUZ *et al.*, 2014). Stomata are known to be important pathways for the absorption of VOCs and the duration of opening and closing stomata also directly influence the absorption rate. Thus, variations in the efficiency of VOC removal are expected between species with different photosynthetic mechanisms (C3, C4 and CAM), with greater efficiency expected among facultative CAM species, which absorb CO_2 in the dark, which may be associated with greater absorption of xylene in the dark period (SRIPRAPAT;

BORAPHECH, 2014). Xylene is also a VOC, and after the exposure of fifteen ornamental species to this contaminant, *Zamioculcas zamiifolia* (Lodd.) Engl. showed the highest removal efficiency, with LC50 around 3,464 ppm of xylene (SRIPRAPAT; BORAPHECH, 2014). When in low concentrations (20 ppm), xylene can cause small changes in chloroplasts, while in higher concentrations (20.000 ppm), plants manifest visual symptoms such as yellow leaf tips, holonecrosis, hydrosis and irreversible changes in the chloroplast's anatomy (SRIPRAPAT; BORAPHECH, 2014).

Formaldehyde is the most common VOC emitted from household materials and is associated with health risks. As biological purifiers, *Hedera helix* L. plants, decreased by 70% the time required to reach 1.0 ppm of formaldehyde gas compared to natural dissipation (LIN; CHEN; CHUAH, 2017), while *Chamaedorea elegans* Mart. removed 100% of compound (16.4 mg m^{-3}) in six days (TEIRI; POURZAMANI; HAJIZADEH, 2018). Similarly, *Schefflera arboricola* (Hayata) Merr. and *S. wallisii* reduced benzene in the air, with an average of efficiency removal (ER) greater than 90%, in addition to the absence of toxic effects on plants at concentrations of 3.5 to 30 μg m^{-3} (PARSEH *et al.*, 2018).

In order to select the greatest ornamental plants for the control of indoor air pollution, researchers should focus on the characteristics of morphological resistance, leaf damage rate, leaf damage time and survival rate when exposed to pollutants (WANG, L. *et al.*, 2020). Another important parameter is the characterization of microorganisms associated with the phylosphere, because the ephytic bacterial communities present in the leaves of host plants can harbor genes involved in hydrocarbon degradation, varying in quantity with the seasons (FRANZETTI *et al.*, 2020). However, in natural environments, the contamination by trace elements often coexists with organic contaminants, reducing phytoextraction capacity (LIU *et al.*, 2008; REDDY *et al.*, 2020). Further, the higher concentrations of contaminants negatively influenced plant growth, biomass, germination and survival (REDDY *et al.*, 2020).

In addition to mitigating air pollution in urban centers, ornamental plants can also be used on green roofs, acting as a strategy to reduce the temperature of cities, taking advantage of rainwater and could be incorporated as carbon trading credits to companies (ROWE, 2011). Removal percentages of the main species growing in soil, air and water contaminated is shown in (TABLE 2- 1).

2.7 MICROSYMBIONTS FOR ENHANCING PHYTOREMEDIATION CAPACITY OF ORNAMENTAL PLANTS

Many different technologies can be applied to boost phytoremediation capacity of ornamental plants. Several studies report the benefits of soil amendments, such as the use of chelators, to improve phytoremediation capacity of plants. This technique is based on the capacity of some molecules to facilitate the aqueous solubility of contaminants, decreasing their sorption to soil components and, in the case of organic contaminants, to promote their degradation (LIU; XIN; ZHOU, 2018). However, for several times, the chemicals used for soil amendments can be expensive, of controlled used and/or demand specialized technicians for their use. Moreover, for sometimes, the addition of new molecules to soil can result in a new source of contamination. In this context, microbial inoculation arises as a better option to assist phytoremediation. Inoculation with plant growth promoting microbes not only increase plant performance under stressful conditions as also can increase plant ability to reclaim contaminants (GOMES *et al.*, 2014). The inoculation of *H. annuus* with plant growth promoting rhizobacteria enhanced the plant Zn and Cd-phytoremediation ability (MARQUES *et al.*, 2013; PRAPAGDEE; CHANPRASERT; MONGKOLSUK, 2013). In *Anadenanthera peregrina* (L.) Speg., rhizobia inoculation increased arsenic (As) tolerance of plants, and when plants were double inoculated with the arbuscular mycorrhizal fungi (AMF) *Acaulospora scrobiculata* Trappe and rhizobia, the As-phytoextraction potential of plants were enhanced (GOMES *et al.*, 2020).

Microsymbionts effectively help plants to tolerate stress, promote growth and, by increasing the contact surface area (i.e. through fungus hypha), enhance the sequestering of contaminants. Moreover, the inoculation of ornamental plants with diazotrophic bacteria assures plant nitrogen nutrition via the biological fixation. Nitrogen is highly necessary for the biosynthesis of antioxidants (both enzymatic and non-enzymatic) necessary to scavenge ROS, often produced under situation of water, soil and air contamination (GOMES; SOARES; GARCIA, 2014; GOMES *et al.*, 2020). AMF can also promote phytoremediation capacity of plants by contributing with phosphorous (P) and nitrogen nutrition (THIRKELL; CAMERON; HODGE, 2016). Particularly in the case of arsenate contaminated matrices, increased P absorption contributes to the maintenance of high P/As ratios which is involved in plant As-tolerance (SMITH *et al.*, 2010). Moreover, AMF contributes to As uptake and accumulation in plants, enhancing their phytoextraction capacity (GOMES *et al.*, 2020). In the case of organic contaminants, microbes can assist phytoremediation by increasing the contaminant degradation. For instance, the AMF *Glomus caledonium* L. facilitates the degradation of B[a]P, increasing soil phytoremediation capacity of *Medicago sativa* L. (WU *et al.*, 2008).

2.8 ENVIRONMENTAL FACTORS INFLUENCING THE GROW AND PHYTOREMEDIATION CAPACITY

The increase of green spaces in urban regions will ensure greater quality of life due to positive effects on air quality and microclimates (ROLOFF; KORN; GILLNER, 2009). With the impacts of climate change, the selection of ideal species in recovery projects of degraded and/or contaminated areas is an important approach to increase efficiency and maintain plant development during the time established in the project. Thus, some parameters that alter the quality and health of plants include adequate soil conditions, such as moisture, fertility, salinity, among others (ROLOFF; KORN; GILLNER, 2009).

Agronomic fertilization techniques, especially with primary macronutrients, pH, and soil irrigation and drainage are essential to ensure the supply of essential plant growth elements and benefit greater absorption of polluting elements (bioaccumulation factor) (LI *et al.*, 2021). Due to the increase in biomass production, fertilization at adequate levels increases tolerance to trace elements and organic contaminants by increasing the activity of antioxidant enzymes, chlorophyll content and other pigments, soluble proteins, etc. (WANG, J. *et al.*, 2020). All these compounds are related to the quality of landscape species because pigments responsible for the attractiveness of flower colors are anthocyanins, flavonoids, carotenoids and betalains (NOMAN *et al.*, 2017). The extraordinary beauty of flowers, in addition to aesthetic value, is crucial to attract pollinators and ensure the survival of the species (NOMAN *et al.*, 2017).

Although the main effect of water stress on crops is the reduction of productivity, the most important effect in ornamental species is visual quality (NAZEMI RAFI; KAZEMI; TEHRANIFAR, 2019). In general, drought and salt stress decrease the number of inflorescences, delay the normal flowering period and the intensity of the color of flowers and foliage (ÁLVAREZ; SÁNCHEZ-BLANCO, 2015). This is mainly due to the two stressors occur most often simultaneously in the environment, and therefore it is essential to use tolerant plants and management to reduce these problems. Among the indexes found that plants develop to tolerate arid environments and reduce water loss are the modification of the shape or leaves orientation, reduction on leaf and shoot growth, closure of stomata, higher thickness, decreased leaf area and trichomes (NAZEMI RAFI; KAZEMI; TEHRANIFAR, 2019). Similar, the few porosity of the soil can also be a limiting factor to the development of plants, especially for ornamental trees planted in urban spaces, since soil compaction can lead to deformation and consequent loss of its landscape function as well as compromise the safety of residents (LEE; ZHANG; JIM, 2021).

Combined with edaphic factors, the climatic parameters such as temperature, CO_2 concentration and luminosity are determining the growth and development of species and therefore should be observed during the choice of ornamental plants. Even though the principal fuel is for photosynthesis, excess of CO_2 may be prejudicial to some species such as bromeliads, which may have reduced growth in addition to adverse effects on the ornamental value, such as leaf bleaching and a more compact form of the plant (CROONENBORGHS *et al.*, 2009). Also, temperature during the development is considered essential for the flowering phase and interferes in the post-production and marketing quality such as number flower buds, petals length and the time to start flowering (KARLSSON; WERNER, 2001).

In addition to the abiotic factors mentioned above, pathogens (fungi, viruses, and bacteria) and insect pests can severely affect the remediation capacity of plants, decreasing growth and biomass production. Thus, tolerance to biotic stress should be considered. An effective and environmentally strategy to protect plants from insects and reduce the use of insecticides is the use of resistant varieties, most often is conferred by the higher production of secondary chemical compounds such as terpenes, phenolic compounds and nitrogen compounds such as alkaloids (SUN *et al.*, 2015). In this sense, conventional plant breeding and genetic engineering are tools capable of improving the tolerance of ornamental plants to biotic stress (NOMAN *et al.*, 2017).

2.9 USE OF ORNAMENTAL PLANTS TO PROMOTE LIFE QUALITY

Ornamental plants are used in different environments and compositions, such as in landscaping, as pot plants, in floral arrangements, in the afforestation of roads, parks and others, contributing significantly to the human life quality, whether in the countryside or cities. Indeed, the contact with ornamental plants, through their cultivation or contemplation, contributes to people's physical and mental health (REIS; DOS REIS; DO NASCIMENTO, 2020). In this context, in addition to add value to the landscape, phytoremediation programs using ornamental plants affect the interaction of people with areas under remediation (CAPUANA, 2020).

In the Botanical Garden of Purwodadi in Indonesia, a model of phytoremediation of domestic sewage was developed, in which plants, in addition to treating surface water, create a pleasant environment for people, suitable for education and recreational activities (IRAWANTO, 2021).This was a good strategy to face with the reduction of green spaces in the urban area at the same time of creating an effective treatment station. The creation and maintenance of public green spaces are increasingly necessary in front of the disorderly urban growth. However, this need is limited by the availability of spaces in the cities or, by the contamination and/or degradation of the available spaces. In this context, the use of herbaceous and/or tree ornamental species in contaminated urban areas constitutes an important tool to solve the environmental problem related to the contamination while creates a green space for recreational use by the population. These spaces can also contribute to the promotion of environmental education.

This strategy was already applied in the Parque Moraiva, in Medellín (Colombia), in which an old dump located next to a river was transformed into a beautiful park using different ornamental remediation species. The garbage was replaced by flowers, contributing to environmental quality, creating a pleasant scenic landscape and leisure area, and thus, promoting the population's life quality.

Moreover, the space has also become a tourist place in the city, contributing to income generation (HOGLAND *et al.*, 2019) Another example is the Orrefors Park in Sweden, in which ornamental plants were used to remediate the contaminated soil. Currently, the park is considered a great success from an environmental and tourist point of view (HOGLAND *et al.*, 2019)

Flowers and garden tourism (such as visiting historic gardens, parks and botanical gardens) is a growing segment of the tourism industry (PAIVA; DE BRITO SOUSA; CARCAUD, 2020). The promotion of tourist activities is of strategic importance in phytoremediation projects using ornamental plants, as they have positive economic and social contributions in addition to incentive the maintenance and expansion of these systems. Phytoremediation with ornamental plants allows the creation of multifunctional landscapes that fulfill environmental, social and ecological functions, contributing the resilience of urban and rural areas. Therefore, more studies within this theme are needed to support phytoremediation projects at different scales and types of environments. Another important point to be highlighted is the need of studies involving the polyculture of ornamental remediation plants. The use of combined species in phytoremediation improves the removal of contaminants, for instance, as they play different ecological roles. These studies will allow the establishment of protocols and identify species to support landscape composition strategies. The identification of ornamental remediation species may also contribute to the commercial production chain for the supply of seeds and propagules for plant nurseries, for instance. It may favor environmental recovery projects in both urban and rural areas by indicating species to compose different habitats, with different edaphoclimatic conditions and management objectives (TURRIÓN *et al.*, 2021).

2.10 CONCLUSION AND FUTURE PERSPECTIVES

In summary, it is possible to observe that ornamental plants are excellent candidates to be used in the remediation of contaminated environments. In addition to not entering the food chain for human and animal food, these species are responsible for the beautification and serve as a source of income in rural and urban communities, including serving as an instrument of environmental education for the population around Both flowers and foliage can be inserted in landscaping projects, such as green roofs or trees in large urban centers, increasing the real estate value of the region, and even generating income by tourism. Moreover, ornamental plants can be used to compose buffer strips mitigating contamination of rural areas in the vicinity of contaminant sources and the resulted biomass used for bioenergy production (adding economic value to the phytoremediation purpose). In the future, plants will be increasingly required in a program of recovery of contaminated areas to return to a clean environment for the next generations and ornamental plants must be investigated as candidates for that purpose. However, more studies also need to assess the ornamental quality of plants produced in contaminated environments as well as the accumulation of contaminants in the marketable organs of ornamental plants, such as flowers and foliage, aiming to evaluate the feasibility and safety of their commercialization. In this context, when the trace element accumulation in marketable parts of plans is observed, we advise that the commercialization of plants produced under trace elements contamination must be avoided but can be used for incineration or energy production. Under that condition, ornamental plants can be used for phytostabilisation, promoting the beautification of contaminated sites, promoting tourism and environmental education. In contrast, when plants are able to transform or mineralize contaminants, such as the organic ones, leading to their absence on marketable organs, the sale of cut flowers and pot plant can represent a source of income to producers.

In addition, it is necessary to test the largest number of plants tolerant with landscape appeal to map native species with high potential for ornamentation and tolerance to contaminated environments. Genetic breeding programs, liming and soil fertility practices are essential tools to enhance the biomass production of ornamental plants and their visual quality. In a statement, this group composes local landscaping and can enable the diversification of products on the small farms, add more value to agricultural production, such as rural tourism, and permit the use of contaminated material (such as manure and wastewater in irrigation). Whether for use in landscaping, floriculture, wetlands, etc., we must consider the herbaceous and tree species mentioned here and deepen research on phytoremediation of organic contaminants and trace elements to obtain plants that are increasingly more tolerant and with greater potential for accumulation, increasing thus the well-being of the local population.

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3 IS IT SAFE TO COMMERCIALIZE ORNAMENTAL PLANTS IRRIGATED WITH ANTIMICROBIAL-CONTAMINATED WATER?[2]

3.1 RESUMO

Irrigamos duas espécies de plantas ornamentais (*Zantedeschia rehmannii* e *Spathiphyllum wallisii*) com água artificialmente contaminada com a o antimicrobiano enrofloxacino (Enro; 0, 5, 10, 100 e 1000 µg $^{L-1}$) para avaliar seus efeitos na produção de plantas ornamentais, bem como seu acúmulo e distribuição entre diferentes órgãos vegetais (raízes, folhas, bulbos e hastes florais), bem como examinar a segurança econômica e ambiental das plantas produzidas sob condições de contaminação com o fármaco. A presença de Enro na água de irrigação não alterou o crescimento das plantas (biomassa) ou a produção de flores. Ambas as espécies acumularam Enro, com suas concentrações internas distribuídas como seguintes: raízes > folhas >bulbos > hastes de flores. Além da tolerância vegetal, o conteúdo da Enro em órgãos vegetais indicou que tanto *Z. rehmannii* quanto *S. wallisii* poderiam ser produzidos sob condições contaminadas pela Enro e não contribuiriam significativamente para a transferência de contaminantes. A alta capacidade dessas plantas de acumular Enro em seus tecidos, associada à sua tolerância a ele, indica-as para uso em programas de fitoremediação de Enrofloxacino.

Palavras-chave: enrofloxacino; fluoroquinolone; contaminação da água; *Spathiphyllum wallisi; Zantedeschia rehmannii.*

3.2 ABSTRACT

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.

[2] Artigo aceito: ROCHA, C. S.; KOCHI, L. Y.; BRITO, J.C.M.; CARNEIRO, D.M.; REIS, M.V.; GOMES, M.P. Is it safe to commercialize ornamental plants irrigated with antimicrobial-contaminated water? Environmental Science and Pollution Research (2022), *In press*.

Key words: enrofloxacin; fluoroquinolone; water contamination; *Spathiphyllum wallisii; Zantedeschia rehmannii.*

3.3 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, C. E. *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; LEE; CHO, 2012). FQ degradation occurs 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). Considering their environmental persistence and widespread occurrence (GOMES *et al.*, 2022; GOMES, Marcelo Pedrosa *et al.*, 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 4^{th} 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.*, 2022), no studies focusing on the effects of pharmaceuticals on the commercial production of plant parts (such as bulbs, foliage, and flowers) have apparently been undertaken. In addition to production aspects, it will be 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.*, 2022).

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; 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; TESTCENTRUM; THE NETHERLANS, 2010). Similarly, *Spathiphyllum*, commonly known as peace lilies, are also economically important throughout the world as ornamental plants (MOUNIKA; PANJA; SAHA, 2017). We therefore investigated the effects of Enro-contaminated irrigation water on the production of *Zantedeschia rehmannii* and *Spathiphyllum 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.

3.4 MATERIAL AND METHODS

3.4.1 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 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; 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.

3.4.2 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.

3.4.3 Chemical analyses

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, following Spanking et al. (2000). 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 (RC1010, Thermo), 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, ciprofloxacin (Cipro, a metabolite of Enro) 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 (94.5%), plant samples (without Enro treatment) were spiked with known concentrations of Enro, 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:

$$Content = Enro\ concentration\ organ * organ\ DW$$

$$Translocation\ factor = \frac{(Shoot\ content + Flower\ content)}{(Root\ content + propagative\ organ\ content)}$$

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

3.4.4 Statistical analysis

The results are expressed here as the averages of five replicates. 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.

3.5 RESULTS

3.5.1 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 (FIGURE 3-2). 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 (FIGURE 3-1). 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, except for 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 (TABLE 3-1; 3-2). Dry weight production by *S. wallisii* was reduced, however, in plants exposed to 1000 μg L^{-1} Enro in relation to the control (TABLE 3-2).

FIGURE 3-1 - 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 MG ENRO L^{-1} (CONTROL); B AND E =1000 MG ENRO L^{-1}; CAND F = ALL TREATMENTS TOGETHER

TABLE 3-1- 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**
$\mu g\ L^{-1}$					$g.plant^{-1}$			
0	12.40±5.41	18.40±1.67	61.8±13.00	92.60±19.00	1.24± 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 repl

TABLE 3-2 - BIOMASS PRODUCTION BY DIFFERENT ORGANS OF *S. WALLISII* PLANTS EXPOSED TO INCREASING CONCENTRATIONS OF ENROFLOXACIN THROUGH IRRIGATION WITH ENROFLOXACIN-CONTAMINATED WATER.

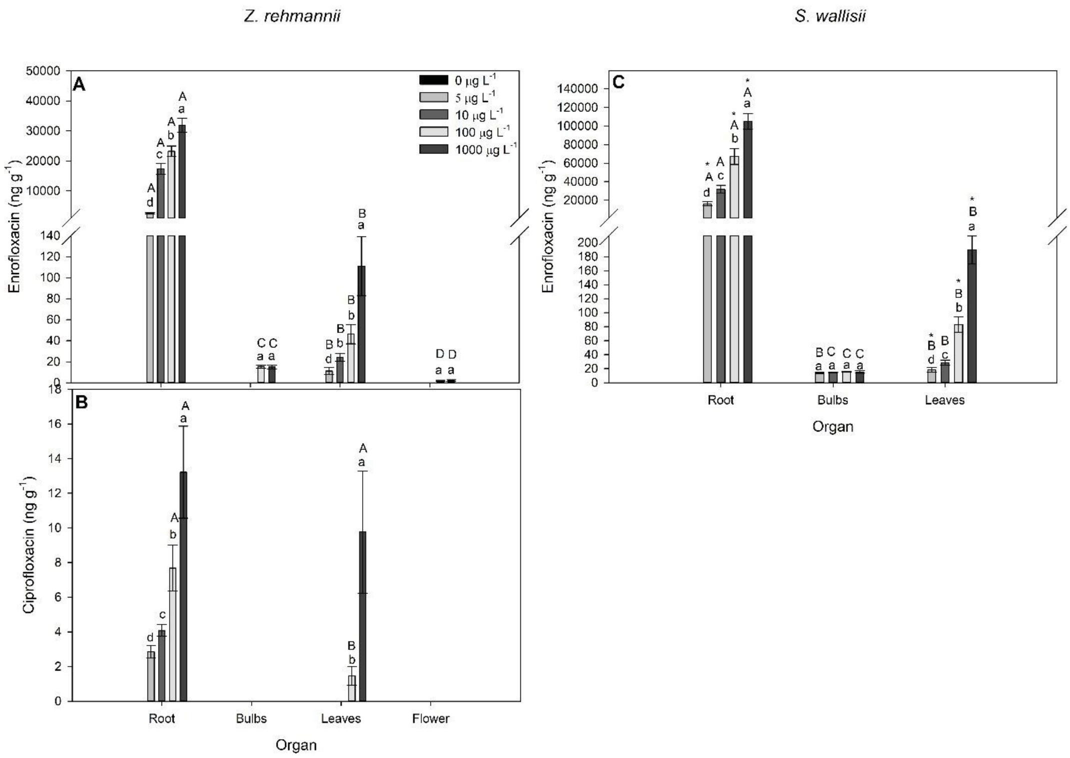
	Fresh weight				Dry weight			
Enro	**Leaves**	**Roots**	**Rhizomes**	**Total**	**Leaves**	**Roots**	**Rhizomes**	**Total**
μg L^{-1}					g $plant^{-1}$			
0	11.50±3.53	5.49±2.29	23.00±7.07	39.99±12.90	1.46 ± 0.01 a	1.48±0.20	1.79±0.72	4.72±0.95
5	8.56± 2.56	4.26±0.48	12.84±3.52	25.66±6.35	1.36 ± 0.14 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
C.V (%)	45.98	30.89	34.73	3.,39	34.19	39.81	25.43	24.76
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.

3.5.2 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 (GRÁFICO 3-1). The greatest concentrations of Enro in both plant species were encountered in the roots (GRÁFICO 3-1 A 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 (GRÁFICO 3-1 B); in leaves, however, Cipro was detected only in plants submitted to 100 and 1000 µg Enro L^{-1} (GRÁFICO 3-1 B). 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}; (GRÁFICO 3-1 A). 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* (GRÁFICO 3-1 A and C).

FIGURE 3-1 - 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.

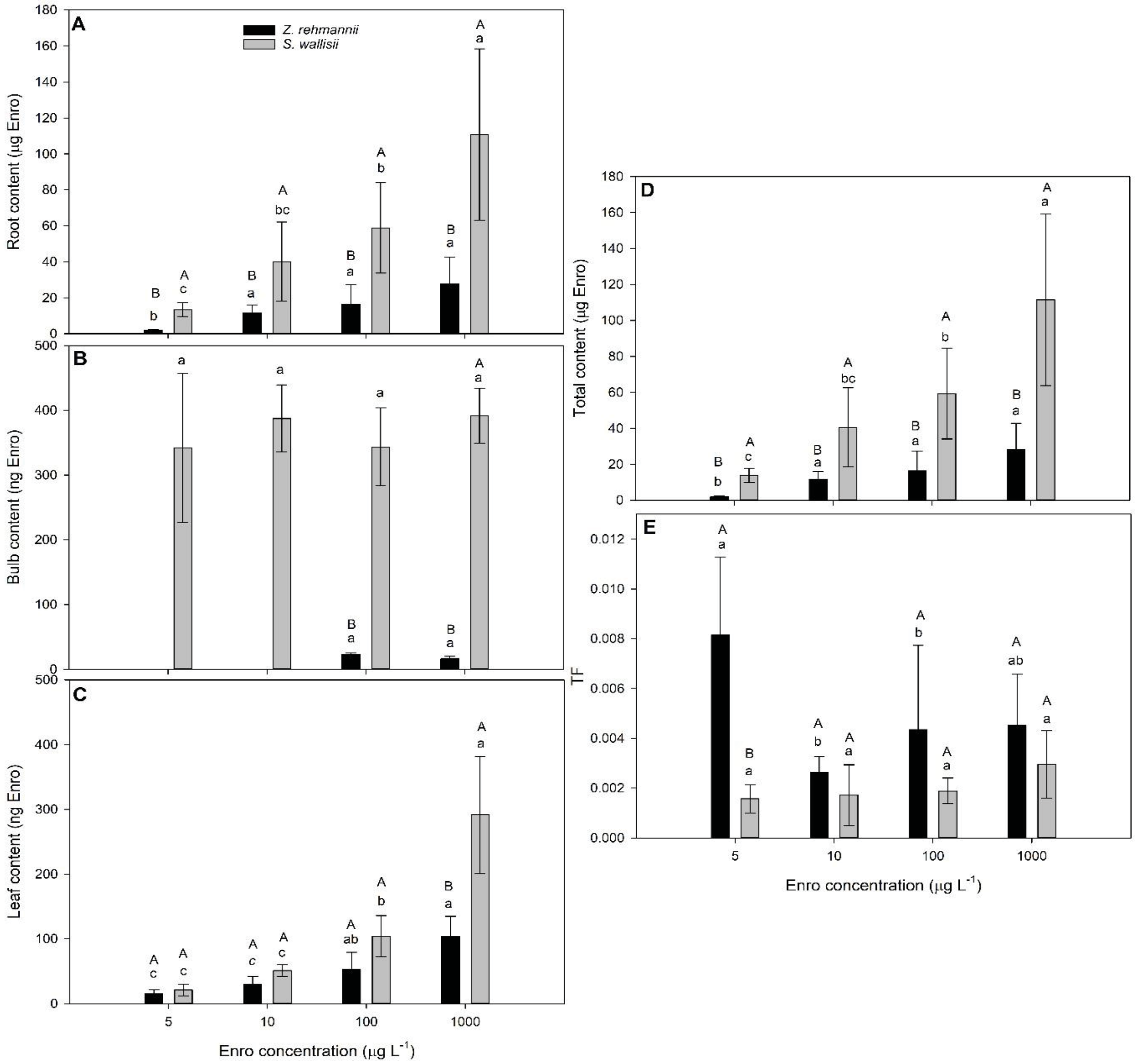


Values represent the means ± standard deviations of five replicates. Lower case letters indicate significant differences in the pharmaceutical concentration of plant organs of the same plant species;

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 ($p < 0.05$).

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) (FIGURE 3-2 E). The Enro contents of roots (FIGURE 3-2 A) and leaves (FIGURE 3-2 C) 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 (FIGURE 3-2 A) and leaf (FIGURE 3-2 C) 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* (FIGURE 3-2 A). 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* (FIGURE 3-2 C). 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} (FIGURE 3-2 B). Regardless of the Enro treatment, the total content of Enro was greater in *S. wallisii* plants than in *Z. rehmannii* (FIGURE 3-2 D). In contrast, the TF only differ in plants treated with 5 µg Enro L^{-1} (being greater in *Z. rehmannii*) (FIGURE 3-2 E).

FIGURE 3-2 ENROFLOXACIN CONTENTS OF DIFFERENT PLANT ORGANS (**A**, **B** AND **C**), TOTAL CONTENT (**D**), AND THE TRANSLOCATION FACTOR (**E**), 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 ($p < 0.05$).

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; XIN; ZHOU, 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 (TABLE 3-1; 3-2), 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; 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.

Enro has been widely encountered in natural water sources (HE *et al.*, 2015; MARQUES *et al.*, 2021; PARENTE, C. E. *et al.*, 2019; YIRUHAN *et al.*, 2010), and that water is often used for irrigation in crop production systems (MARQUES *et al.*, 2021). Due to the inefficiency of water treatment plants for removing antibiotics (AL-GHEETHI *et al.*, 2015), Enro residues will be expected even in irrigation systems using treated water. However, 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; MELIS; SPOSITO, 2010; GOMES, Marcelo Pedrosa *et al.*, 2017, 2019; HOANG *et al.*, 2012; 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, 2019). Here, Cipro was only found in roots and leaves of *Z. rehmannii* (GRÁFICO 3-1 A 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). 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* (GRÁFICO 3-1). 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 those flower stems considered together. That minimal concentration could not significantly contribute to environmental contamination with the eventual disposal of those flower stem biomasses. 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, although Enro concentrations in leaves must be considered when marketing potted flowers.

Based on their respective Enro contents (GRÁFICO 3-2), the leaves of *S. wallisii* carry up to 291.56 ng of Enro each, while a leaf of *Z. rehmannii* can carry 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. 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.

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. Ornamental plants have been used to reclaim contaminants from polluted waters and soils (ROCHA *et al.*, 2022). 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.*, 2022), 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; XIN; ZHOU, 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*. However, regardless of the species, the roots were the main organ of Enro accumulation (GRÁFICO 3-2). 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.*, 2012; 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; 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; 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 contamination. 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, 2020).

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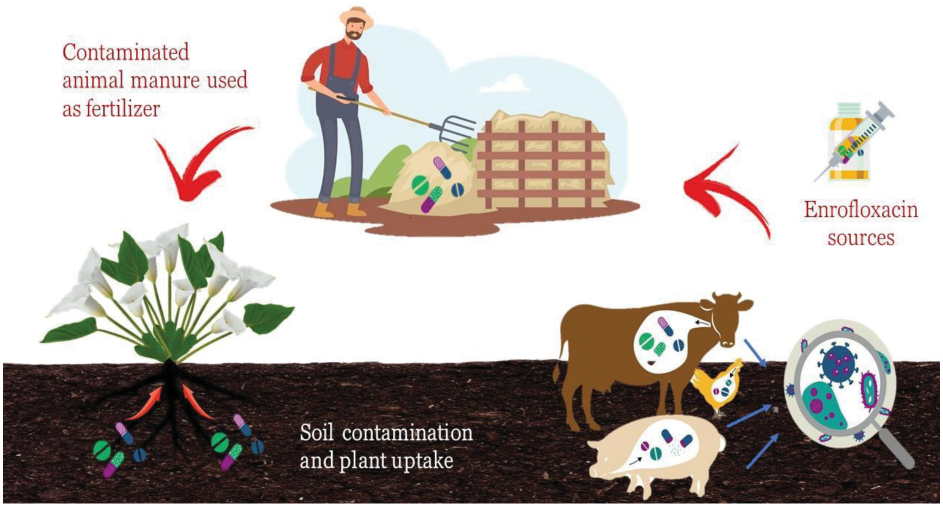
4 PRODUCTIVITY AND PHYTOREMEDIATION POTENTIAL OF CALLA LILY (ZANTEDESCHIA AETIOPHICA L. ON SOIL CONTAMINATED WITH ENROFLOXACIN

4.1 GRAPHICAL ABSTRACT



4.2 RESUMO

A enrofloxacina (Enro) é frequentemente detectada no solo e no esterco animal utilizado para a produção de culturas, e assim respostas fitotóxicas têm sido observadas em plantas cultivadas sob a presença antimicrobiana. Aqui, investigamos os efeitos da presença de Enro em solos (1,9 mg kg^{-1}) e esterco (50,4 mg kg^{-1}) utilizados para o crescimento de plantas de copo-de-leite (*Zantedeschia aethiopica*), uma importante espécie ornamental, no crescimento vegetal e na produção de flores. Também foi avaliado o acúmulo e distribuição da Enro entre tecidos vegetais com o objetivo de avaliar a segurança das plantas comercializadas produzidas sob condições contaminadas pela Enro. As concentrações representativas ambientais de Enro em solos e esterco não afetaram o crescimento vegetal e a produção de flores e não induziram qualquer perturbação fisiológica das plantas (pigmentos fotossintéticos, concentração de peróxido de hidrogênio, e atividade de superóxido dismutase e catalase nas folhas). As plantas acumularam a Enro principalmente em suas raízes, restringindo sua translocação para parte aérea, o que contribui para a segurança da comercialização de suas flores. No entanto, quando comercializado como vasos, a quantidade de Enro transportada por plantas é preocupante e, portanto, a venda de plantas envasada deve ser evitada. Devido à sua tolerância e capacidade de absorção da Enro, as plantas são indicadas para programas de fitorremediação podendo remover até 14,76% da Enro do solo contaminado e/ou esterco.

Palavras-chave: *Zantedeschia aethiopica*; plantas ornamentais.; contaminantes. fitotorremediação.

4.3 ABSTRACT

Enrofloxacin (Enro) is frequently detected in soil and animal manure used for crop production and phytotoxic responses have been observed in plants grown under the antimicrobial presence. Here, we investigated the effects of the presence of Enro in soils (1.9 mg kg^{-1}) and manure (50.4 mg kg^{-1}) used for the growth of calla lily plants (*Zantedeschia aethiopica*), an important ornamental species, on plant growth and flower production. 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. Environmental representative concentrations of Enro in soils and manure did not affect plant growth and flower production and did not induce any physiological perturbation 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 worrisome, and therefore, selling of pot plants must be avoided. Due to their tolerance and capacity to uptake Enro, plants are indicated for phytoremediation programs being able to remove up to 14.76% of Enro from contaminated soil and/or manure.

Keywords *Zantedeschia aethiopica*; Ornamental plants; Contaminants; Phytoremediation.

4.4 INTRODUCTION

Ornamental plants represent an important sector within agribusiness, moving billions of dollars a year around the world (BRAINER, 2019; EUROPEAN COMMISION, 2020; USDA, 2020). 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 matrices decontamination, in addition to being used in the landscaping of parks and gardens (ERICKSON *et al.*, 2020; JUNQUEIRA; PEETZ, 2018; LOWENSTEIN; MATTESON; MINOR, 2019; REIS; DOS REIS; DO NASCIMENTO, 2020; ROSSI *et al.*, 2016). Among ornamental plants, calla lily (*Zantedeschia aethiopica* (L.) Spreng.) is of growing commercial interest, showing high commercialization value (CASIERRA-POSADA; BLANKE; GUERRERO-GUÍO, 2014; CHEN *et al.*, 2009; DEL-TORO-SÁNCHEZ *et al.*, 2013b;

TEJEDA; ZURITA, 2020b). *Z. Aethiopica* is a monocotyledon belonging to the family Araceae is marketed in pots plantlets, and mainly like cut flowers to compose arrangements and bouquets. Although the production of calla lily has increased worldwide due to its good environmental adaptation (CASIERRA POSADA; NIETO; ULRICHS, 2012), this plant require high levels of fertilization, which is often achieved by organic fertilization using manure (ALMEIDA *et al.*, 2012).

Although manuring has several beneficial effects as agricultural practice, its harmful and neutral effects on the trinity formed by plants, soil and organisms are expected (MANDAL *et al.*, 2007). One of the major problems caused by manuring is the insertion of organic contaminants to soil and plants. Some contaminants, such as antimicrobials, largely used in animal production, are not fully metabolized once administered, being excreted in animal feces and urine (ZHOU *et al.*, 2020), resulting in the high observed concentrations of antimicrobials in manure (CHECCUCCI *et al.*, 2020; LIMA; DOMINGUES; SILVA, 2020). Once applied to soil, manure can release antimicrobials to the soil solution, which become available for plant uptake (MARQUES *et al.*, 2021). Some antimicrobials are phytotoxic causing delayed germination (RIAZ, *et al.*, 2017) reductions on photosynthesis (ARISTILDE; MELIS; SPOSITO, 2010; NUNES *et al.*, 2019) and mitochondrial respiration (GOMES *et al.*, 2018), in addition to promoting oxidative stress damages (GOMES, *et al.*, 2017; GOMES, 2017; PANJA *et al.*, 2019).

Fluoroquinolones (FQ) are one of the main classes of antimicrobials occurring in livestock (CAMOTTI BASTOS *et al.*, 2018; GAO *et al.*, 2012; LEAL *et al.*, 2012; PARENTE, *et al.*, 2019; ZHAO; DONG; WANG, 2010), and once present in agricultural systems, they can be transported by surface runoff to the water bodies or adsorbed in the colloidal fraction of the soil, remaining for long periods until they are degraded by the processes of biodegradation and photooxidation (TANDON; KUMAR; YADAV, 2013). Such processes depend on the antimicrobial and 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) source of the manure (REGITANO; LEAL, 2010) and the exposure time of the area (CAMOTTI BASTOS *et al.*, 2018).

In soils, Enrofloxacin (Enro), a FQ commonly used in veterinary medicine, has been detected at levels of 1.03 mg kg^{-1} (PARENTE *et al.*, 2019) while in animal manure, its concentrations reach up to 46 mg kg^{-1} (ZHAO; DONG; WANG, 2010). This antimicrobial is of such interest since its toxicity has been seen in crops, such as soybeans, decreasing plant yields (MARQUES *et al.*, 2021). In this context, the use of Enro contaminated manure in the fertilization of calla lily 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. 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* (ROCHA et al., 2022 – in press). 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 biomass upon their commercialization (ROCHA *et al.*, 2022).

In another 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) (ROCHA *et al.*, 2022). Indeed, ornamental plants have emerged as potential phytoremediator species due to their high stress tolerance in addition to not being intended for animal or human food consumption – which avoids the introduction of contaminants into the food web (ROCHA *et al.*, 2022). Moreover, in addition to improving the environments with aesthetic value, ornamental plants provide multiple ecosystem services and promote human well-being (ROCHA *et al.*, 2022). 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 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 produced under Enro-contaminated conditions and to evaluate the phytoremediation capacity of plants.

4.5 MATERIAL AND METHODS

4.5.1 Soil preparation and seedling production

The 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 substrates 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. Priory its use, the soil was tested for the presence of Enro (as described below) with no Enro being observed. Then, soil was dried, sieved (2 mm mesh) and corrected with dolomitic limestone until pH 5.5 ± 0.5. The physical-chemical characteristics of soil was as following: 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).

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 mg Enro kg^{-1} and 0 or 46 mg Enro kg^{-1}, respectively. These concentrations were selected based on the environmental representative concentrations of Enro in soils (PARENTE *et al.*, 2019) and manure (ZHAO; DONG; WANG, 2010). 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 for evaluation of 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 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).

4.5.2 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 the second fully expanded, healthy, turgid, flat, and homogeneous in color and size leaves per plant, in a total of three readings per leaf. The number of leaves from each plant was 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 ºC for until further evaluations.

Antioxidant enzymes as well as hydrogen peroxide (H_2O_2) concentrations (VELIKOVA; YORDANOV; EDREVA, 2000) 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) (GOMES *et al.*, 2016-). The activities of superoxide dismutase (SOD) (BEYER; FRIDOVICH, 1987) and catalase (CAT) (AEBI, 1984) were assessed after determining the total protein concentrations (BRADFORD, 1976).

4.5.3 Chemical analyses

Enrofloxacin concentrations were investigated in dried samples (45 ºC) 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. The extraction was performed in 1 g substrate by the addition of 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 syringe with cotton meshes 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. 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) (Shi et al. 2009). The concentrations of Enro 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 (88.5%), substrate and plant samples (without Enro treatment) were spiked with known concentrations of Enro, and 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) was divided by the Enro content of the subterranean organs (roots + rhizomes) as follows:

$$\textbf{Content} = Enro\ concentration\ organ * organ\ DW$$

$$\textbf{Translocation factor} = \frac{Shoot\ content + Flower\ content}{Root\ content + Rizhome\ content}$$

The natural degradation of Enro in the substrate (degradation) as well as the efficiency of plant removal of Enro was calculated as following (MENDES *et al.*, 2021):

$$\textbf{Degradation (\%)} = 100 - \frac{Cf\ without\ plants}{Ci\ without\ plants} * 100$$

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

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 the substrate.

4.5.4 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). While 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 $P < 0.05$).

4.6 RESULTS

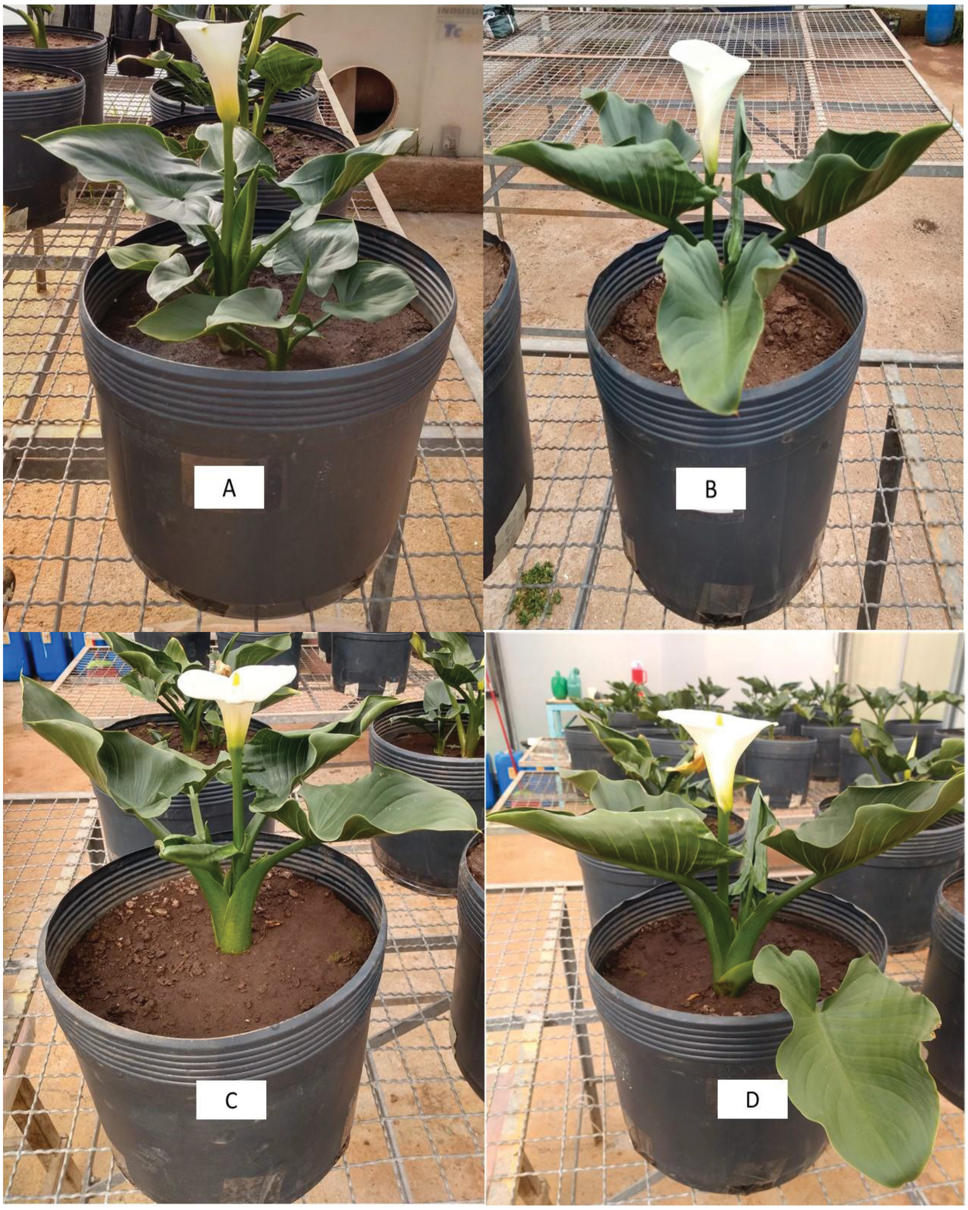
4.6.1 Initial concentration of Enro in substrates

No Enro was detected in the substrate consisted of 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.

3.2 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 4- 1; $P>0.05$). Similarly, SPAD readings, SOD and CAT activity as well as H_2O_2 concentrations were not affected by treatments (TABLE 4-1). 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 4-1).

FIGURE 4-2 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.

Table 4-1 Number of leaves, SPAD index, dry biomass of distinct organs, H_2O_2 concentration and activities of enzymes Superoxide Dismutase (SOD) and Catalase (CAT) in calla lily plants grown in substrates with or without enrofloxacin addition. Values represent means ± SD of five replicates.

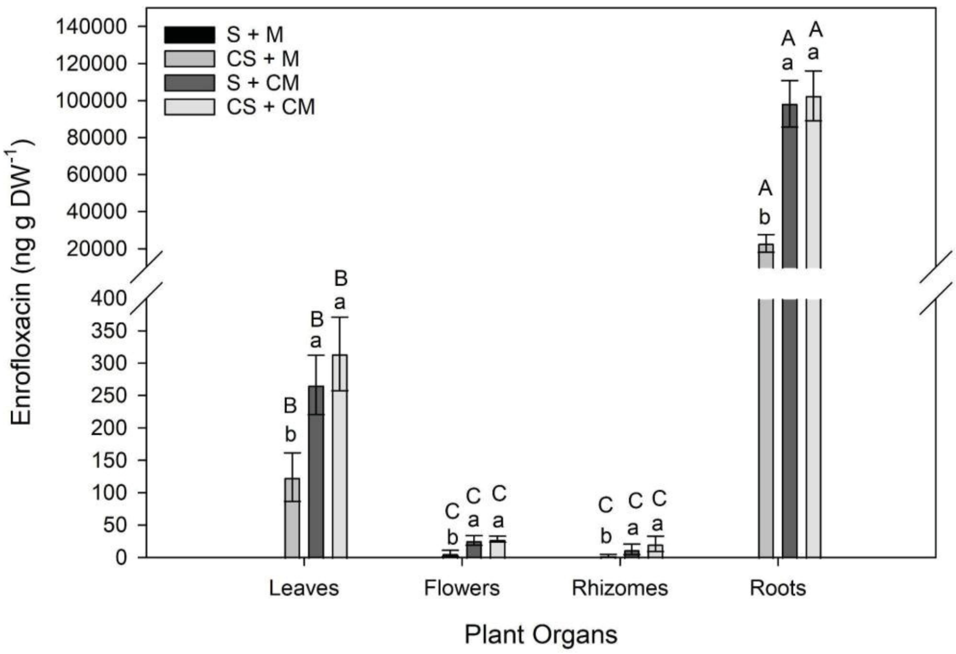
	Number of leaves	SPAD	Leaves	Flowers	Roots	Rhizomes	H_2O_2	SOD	CAT
Treatment		index			Dry Biomass (g)		$\mu g\ g^{-1}$		U mg $prot^{-1}$
S+M	4.9±2.0	797.2±126.2	2.18±5.5	0.99±0.18	3.48±1.58	3.42±15.8	40.7±13.9	0.26±0.02	$4.7\ x10^{-7}\pm 2.8x10^{-6}$
CS + M	4.5±1.0	852.3±117.6	2.08±6.4	1.01±0.25	3.54±1.87	3.56±1.74	28.5±6.5	0.29±0.05	$4.02x10^{-6}\pm 3.0x10^{-6}$
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	$3.4x10^{-7}\pm 2.4x10^{-7}$
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	$5.5x10^{-7}\pm 2.8\ x10^{-6}$
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

4.6.2 Enrofloxacin concentration and distribution in plants

No Enro was detected in organs of plants grown in non-contaminated substrate (S + M) (FIGURE 4-1). 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 4-1). 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 4-1).

FIGURE 4-1 ENROFLOXACIN CONCENTRATION (**A**) IN DIFFERENT ORGANS IN CALLA LILY PLANTS GROWN IN SUBSTRATES WITH OR WITHOUT ENROFLOXACIN

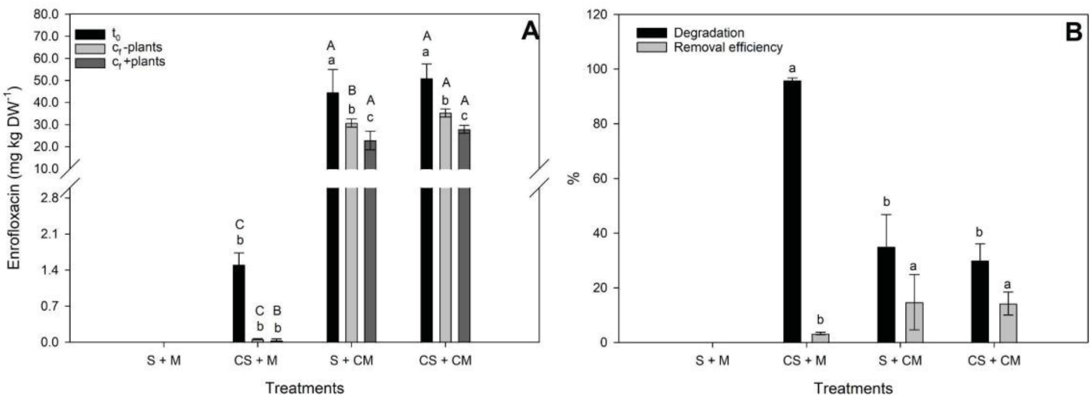


Bars represent the means ± SD of five replicates. Lower case letters indicate significant differences between treatments for the same plant organ; uppercase letters indicate significant differences in the pharmaceutical concentration of plant organs of the same treatment. Soil (s) = 0 mg Enro kg^{-1}; 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}.

4.6.3 Phytoremediation capacity

Significantly lower ($P<0.05$) residual concentrations of Enro were observed in the substrate in presence of plants as compared to the pots without plants (FIGURE 4-2A). Natural Enro degradation ($F = 255.20$; $P<0.0001$) was the greatest and the removal efficiency the lowest ($F= 7.89$; $P<0.001$) in substrate where only soil was contaminated with Enro (CS + M) (GRÁFICO 4-2B). Translocation (TF) and bioconcentration factor (BCF) values were lower than 1, regardless of the treatment (TABLE 4-2). For all the treatments, the BCF was greater in roots than in shoots (TABLE 4-2). 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 4-2).

FIGURE 4-2 **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. **B)** NATURAL DEGRADATION IN SUBSTRATES AND ENRO-REMOVAL EFFICIENCY OF CALLA LILY PLANTS.



Lower case letters indicate significant differences in Enro concentrations between c_0, c_f - plants and c_f + plants; uppercase letters indicate significant differences in the pharmaceutical concentration between treatments within c_0, c_f - plants and c_f + plants. Different letters indicate significant differences between treatments. Bars represent the means ± 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 4-2 - TRANSLOCATION FACTOR (TF) AND BIOCONCENTRATION FACTOR (BCF) IN CALLA LILY PLANTS GROWN IN SUBSTRATES WITH OR WITHOUT ENROFLOXACIN.

Treatment	TF	BCF		
		Shoots	Roots	Total
S + M	-	-	-	-
CS + M	4.77×10^{-3} a	7.10×10^{-5} a	12.94×10^{-3} a	0.013a
S + CM	2.96×10^{-3} b	6.90×10^{-5} b	2.34×10^{-3} b	0.0024b
S + CM	2.10×10^{-3} b	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

Values represent the means of five replicates. Different letters indicate significant differences between treatments 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}.

4.7 DISCUSSION

The non-observed effect of the investigated concentrations of Enro in substrates indicates 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 macrophyte *Lemna minor* and *Elodea canadensis* (GOMES *et al.*, 2019). Similarly, here, the presence of the drug on substrates of plants did not affect photosynthetic pigment levels (as evaluated by SPAD) and biomass production (TABLE 4-1). Shoot biomass production is critical for predicting the commercial value of ornamental plants and is intrinsically related to photosynthetic rates (FERRANTE *et al.*, 2015) 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 damages have 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 algae like *Scenedesmus obliquus* (QIN *et al.*, 2012) and *Chlorella vulgaris* (WANG *et al.*, 2019). 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. Accumulation of Enro in plant tissues have been observed in several agriculture important species, such as corn, soybeans, beans (MARQUES *et al.*, 2021), lettuce, common barley (*Hordeum vulgare* L.), cucumber (*Cucumis sativus* L.) (LILLENBERG *et al.*, 2010) and wheat (*Triticum aestivum*) (RIAZ *et al.*, 2017). The absorption and accumulation of Enro in plants are related to the transpiration rate, involving both energy-dependent active and energy-independent passive processes (DODGEN *et al.*, 2015; ZHANG *et al.*, 2019). Once absorbed by roots, Enro can be moved through plant tissues, being transferred to the shoots using the transpiration current pathway (DODGEN *et al.*, 2015). 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 (GOMES *et al.*, 2020; MARQUES *et al.*, 2021). In this context, the accumulation and distribution of Enro between plant organs must be investigated to attest the safety of the plant 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 4-2B), indeed, Enro was preferentially allocated in subterranean organs, mainly in roots also observed greater concentrations of the ciprofloxacin, another FQ, in the roots of Eichhornia crassipes in relation to their shoots (YAN *et al.*, 2020). 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}). This concentration is minimal in comparison 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 consisted of 15 flowers (~1.6 g DW $flower^{-1}$), a maximum of only 680 ng of Enro will be transported by plants. However, this perspective changes when considering the sale of plants in pots, since the accumulation of Enro in all organs (leaves, flowers, roots, and rhizome) must be considered, particularly due to the alarming levels of Enro found in plant roots. 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 (CARNEIRO, 2009). 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 carried on (including the concentration of subterranean organs). This amount of Enro is worrisome. Depending on the number and destination of plants acquired as pots, calla lily may constitute a vehicle of Enro contamination. We therefore highlight that, when produced in conditions of Enro contamination, only flowers must be commercialized aiming to guarantee the environmental safety of their sold.

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 is noticed that ornamental species are an excellent option to diversify crop production in small farms; moreover, these plants present high biomass production and tolerance to abiotic stress and, especially, they do not enter in the food web (CASIERRA-POSADA; , PAOLA J. NIETO, 2012; FONSECA; SEGEREN, 2013) desirable characteristics for phytoremediation programs. Compared to other common species used in the phytoremediation system, the destination of plants is more flexible, moreover, in addition to the commercialization of cut flowers and foliage, small farms can use ornamental plants to attract tourism (CHASE *et al.*, 2018; GAO; BARBIERI; VALDIVIA, 2014). Besides that, rural tourism associated with constructed wetlands to reduce livestock contaminants provide new opportunities to familiar farms promoting greener activities for sustainable agriculture (RAYNOLDS, 2021; XU *et al.*, 2018). Several studies have reported the phytoremediator capacity of calla lily plants for metal reclaim (CASIERRA-POSADA; BLANKE; GUERRERO-GUÍO, 2014; Modulation of antioxidant defense system after long term arsenic exposure in *Zantedeschia aethiopica* and *Anemopsis californica*DEL-TORO-SÁNCHEZ *et al.*, 2013b) and for wastewater treatment (CHEN *et al.*, 2009; FIGUEIREDO *et al.*, 2014). 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 4- 2) indicates the phytostabilization ability of calla lily. In phytostabilization, contaminants are absorbed by plants and are prevented from migrating to shoots (typical in photoextractor species) (BUSCAROLI, 2017; MCCUTCHEON; SUSARLA; MEDINA, 2002). Once accumulated in roots, the contaminants are no longer available for moving through environmental matrices, avoiding their entrance on water, and, mainly, into the food chain (CHRISTOU *et al.*, 2019; PULLAGURALA *et al.*, 2018; YU *et al.*, 2022). Moreover, by allocating Enro in their roots, plants prevent its deleterious effects to photosynthetic organs (GOMES *et al.*, 2018; GOMES *et al.*, 2019; LINKE *et al.*, 2010), 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 (SILVA, 2017), the growth of calla lily in contaminated substrates can remove up to 10,000 mg of Enro per hectare until the first flower production, while flowers can be safety sold.

Although the plants have shown the ability to uptake and accumulate Enro, their calculated removal efficiency was < 15% (Figure. 4-2B). It is important to note that the substrate plays an important role in the absorption and translocation of antimicrobials by plants (MIGLIORE; COZZOLINO; FIORI, 2003), and the physical-chemical properties of the molecule determine the half-life in the environment. Overall, FQ's 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 surface and subsurface (LEAL *et al.*, 2013; PARENTE, C. E. *et al.*, 2019). Generally, Brazilian soils have pH-dependent charges and low cation exchange capacities (CECs) and thus lower sorption potential for cationic species, such as FQ's (LEAL *et al.*, 2013). However, studies have shown that the sorption of FQ’s is high and rapid (less than 24 h) in tropical soil (LEAL *et al.*, 2013). 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 (YU *et al.*, 2022).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 pharmaceutics in the soil (ZHI *et al.*, 2019).

4.8 Conclusion

The use of Enro-contaminated soil or manure for growing calla lily plants did not result in impacts on plant productivity. Although plant 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 being observed in flowers. Although the commercialization of cut flowers is safe, we highlight that the production of plants under Enro contaminated condition to be sold as pots merits more attention, since a relatively high amount of Enro can be transported in the whole plant body. However, the Enro-tolerance of plants associated with their ability to accumulate Enro pose the calla lily as a potential phytoremediator species, which must be considered for coupling economic and environmental gains.

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5 CONCLUSÃO GERAL

Em concentrações ambientalmente relevantes, a presença de Enro na água utilizada para irrigação de *Zantedeschia rehmannii* e *Sapthiphyllum walisii* bem como no substrato utilizado para o crescimento de plantas de *Z. aethiopica* não afeta a produtividade das plantas e a qualidade das flores produzidas. As espécies estudadas empregam a estratégia de baixa translocação de Enro para a parte aérea, evitando os possíveis danos do fármaco aos tecidos fotossintéticos. Justamente por isso, as flores das três espécies produzidas em condições de contaminação por Enro apresentam concentrações ínfimas do antimicrobiano, garantindo a segurança de sua comercialização. Enquanto a venda em vasos é segura para *Z. rehmannii* e *S. walisii*, para *Z. aethiopica* ela não é aconselhável, devido ao elevado acúmulo de Enro nas plantas- efeito do significativo volume de raízes em relação a massa total da planta-, o que pode resultar no carreamento de concentrações significativamente elevadas do fármaco. Tendo sido reportada a tolerância e o potencial bioacumulador das três espécies, conclui-se que elas podem ser indicadas para programas de fitorremediação de Enro e que, desta forma, além de representar uma fonte de ganhos econômicos, o cultivo de *Z. rehmannii*, *S. walisii* e *Z. aethiopica* em áreas contaminadas por Enro auxilia na descontaminação ambiental ao mesmo tempo que adiciona beleza às áreas em que se encontram. A escolha dessas espécies considerou a sua importância econômica aos produtores de flores e consequentemente maior facilidade e conhecimento a manutenção de jardins construídos em ambientes contaminados. No entanto, para estudos futuros, é necessário que se investigue outras plantas, em especial as nativas, para que seja possível construir ambientes regenerativos com maior biodiversidade de espécies vegetais e microbiota associada, como preconiza as estratégias conhecidas como SBN (soluções baseadas na natureza).

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