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Article

Antibiotics in the Environment: Prescribing Risks to Non-Target Organisms

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Abstract: Background: The cephalosporins class is among the most widely used group of antimicrobials worldwide. Antibiotics, together with other drugs and personal care products, make up a group of emerging contaminants. The effects of exposure to this group of chemical contaminants on non-target organisms are not well understood, as they are still poorly studied. Therefore, this study evaluated the phytotoxicity of five cephalosporins in *Lactuca sativa*. **Methods**: Lettuce seeds were exposed to different concentrations of antibiotics (25 to 500 mg/L) for 5 days in the dark. After this period, the germination percentage and the wet and dry weights were recorded. **Results**: The highest tested concentration (500 mg/L) inhibited the germination of lettuce seeds ($p < 0.05$); there was a decrease in dry weight when exposed to a first-generation cephalosporin ($p < 0.05$). Additionally, there was a significantly negative influence ($p < 0.05$) on the fresh weight, especially in the group that evaluated the exposure of seeds to 25 mg/L of Cefepime. **Conclusions**: We emphasize that there is no record of environmental concentrations of cephalosporins in soil, and therefore, we can indicate that it is possible to have environmental damage resulting from the inappropriate and constant disposal of cephalosporins in the environment.

Keywords: cephalosporins; antimicrobials; phytotoxicity; lettuce

Citation: da Silva Freitas, L.; Honscha, L.C.; Volcão, L.M.; de Lima Brum, R.; da Silva Júnior, F.M.R.; Ramos, D.F. Antibiotics in the Environment: Prescribing Risks to Non-Target Organisms. *Pollutants* **2022**, 2, 435–443. https://doi.org/10.3390/pollutants2040029

Academic Editor: Paolo Pastorino

Received: 15 July 2022
Accepted: 21 October 2022
Published: 27 October 2022

1. Introduction

Since their discovery, antibiotics have been widely used to treat infectious human and animal diseases and improve agro-industrial performance [1]. The indiscriminate and erroneous use of these compounds in these areas, combined with the low efficacy of effluent treatment, have been pointed out as the main factors related to antimicrobial resistance. This can be attributed to the spread through mobile genetic elements, which facilitate the transfer of these genes between microorganisms [2]. According to estimates by the World Health Organization (WHO), this scenario will culminate in antibiotic resistance being among the leading causes of mortality worldwide by 2050 [3]. The number of deaths could reach 10 million, and the economic damages could be even greater, approximately USD 10 trillion [4].

Once in the environment, these compounds can act on non-target organisms (plants, animals, or microorganisms) and cause damage to populations, communities, and ecosystems. Antibiotics, other pharmaceuticals, and personal care products, also called Pharmaceutical and Personal Care Products (PPCP's), are an important group of emerging contaminants that can produce physiological effects in humans, even at low concentrations. This group includes medicines such as analgesics, antibiotics, and antidepressants, among others, and personal hygiene products such as tooth-pastes, mouthwashes, face creams, perfumes, etc. [5,6]. These compounds have been detected in all environmental areas, from residual water, soil, organic fertilizer, and sewage sludge, among others, and they are co-responsible for accelerating the spread of antimicrobial resistance and increasing human, animal, and ecological risks [7,8].

In Brazil, this scenario is even worse. A recent review, whose objective was to create a global map of bovine antibiotic residues in water and soil, showed that, although Brazil is the largest meat producer and the second largest consumer of antibiotics in the world, there is only one study that mentions the residues of these compounds in the water and soil [9]. A qualitative study also carried out in Brazil explored the use of antibiotics on a pig farm. This study showed that 67% of farmers produced their feed, and when they found it convenient, they added powdered antibiotics to the feed for pig treatment or prophylactic use. Moreover, 45% of respondents reported that they did not know the differences between human and veterinary antibiotics, and 21% reported that there were no differences between the drugs. In the same study, according to the information provided, it was suggested that pigs were exposed to large amounts of antibiotics for a long period, and yet, almost half of the producers still considered the use of antibiotics indiscriminate [10]. To further aggravate the Brazilian scenario, according to data from the National Health Information System, in 2020, only 55% of the population had access to the sewage network, and only 50.8% of this collected sewage was treated [9,11].

Beta-lactams are a group of antibiotics widely used in community and hospital infections. This group includes carbapenems, monobactams, penicillins, and cephalosporins. Cephalosporins have a broad spectrum of action and have emerged significantly in human, animal, and environmental samples, including hospital wastewater and food products, as well as in animals for human consumption [10–12].

Cephalosporins are an important class of antibiotics, the second most consumed group in Europe [13], and have been growing in another niche, gaining even more visibility for being an option in the treatment of infections and prophylaxis in dental procedures [14]. In Japan, first-generation cephalosporins were the most prescribed by dentists, around 66% from 2015 to 2017 [15].

Although a recent review has shown the toxicity and degradation of cephalosporins in the aquatic environment [14], the dynamic of this class of contaminants for terrestrial organisms is not yet fully understood. Combined with the continuous input of these compounds and the lack of knowledge of the effects of this group of antibiotics on terrestrial organisms, studies point to a longer half-life of some cephalosporins in the terrestrial environment than in the aquatic environment (over 40 days) [16–18].

Among environmental spaces, the soil has been highlighted to be an excellent niche for the growth of numerous microorganisms and probably has the largest and most divergent resistome (set of all antibiotic resistance genes) comprised of bacteria with intrinsic and acquired resistance to antibiotics [19,20]. From an economic point of view, this compartment has direct and indirect effects on the growth and development of plants, livestock, and food products, in general [21]. Once in the soil, the behavior of these compounds can be diverse and may undergo leaching, be transported to water bodies, or even accumulate in plants or in the soil itself [22].

In this context, managing the potential risk of these substances on non-target organisms of economic interest is essential to broaden the view of the potentially harmful aspects of the inappropriate disposal of antibiotics in the environment and their environmental and economic consequences [23]. Many PPCPs can be absorbed by vegetables during chronic exposure, even at a low level, and consequently, can be transferred along the food production chain [24]. In this sense, mimosa lettuce, the most consumed vegetable in the world and, according to the US Environmental Protection Agency [25], a species indicated for phytotoxicity studies in standardized protocols, was chosen as an experimental model. Lettuce (*Lactuca sativa* Mill.) is among the most consumed leafy vegetables worldwide and has its culture widely distributed throughout Brazil. This fact is directly related to its wide adaptation to severe climatic conditions, which has favored its prominence among the species of greatest economic and social importance [26]. In addition, to maintain the exponential and qualitative increase of this species, farmers have invested in complementary resources to make seedlings healthy and vigorous, as well as phytosanitary strategies.

Thus, the present study evaluated the influence of three generations of cephalosporins on the germinative potential of lettuce seeds and the fresh and dry weight of the seedlings after five days of exposure, using the acute toxicity test with *L. sativa* seeds.

2. Materials and Methods

2.1. Plant Species and Antibiotics Tested

Hanson lettuce seeds (*Lactuca sativa*) commercially obtained, brand ISLA Ltda., Brazil (pesticide free), were used. The seeds were selected manually, verifying the uniformity of size, weight, and color. Wilted, moldy, stained, discolored, and damaged seeds were excluded. The cephalosporins of the first (Cephalothin and Cefazolin), third (Ceftriaxone and Ceftazidime), and fourth generation (Cefepime) with 100% purity and obtained from Sigma Aldrich were used.

2.2. Experimental Design

Phytotoxicity tests were conducted by assessing the acute toxicity of antimicrobials in lettuce seeds in the following concentrations: 25, 50, 100, 250, and 500 mg/L, diluted in mineral water, according to OECD 208 [27]. In addition, mineral water was used as a negative control. The experiments were carried out in three independent replicates, using 25 lettuce seeds in each 9 cm diameter Petri dish containing a paper filter moistened with 3 mL of each corresponding concentration and no addition of pesticides. The Petri dishes containing the seeds and antibiotics remained in a BOD-type germination chamber, at a constant temperature of 25 ºC, in the dark, and the standards for phytotoxicity of each test were measured after five days (germination rate, fresh weight, and dry weight of seedlings) [28,29]. According to the Seed Analysis Rules of the Brazilian Ministry of Agriculture, Livestock, and Supply [30], the presence of visible root protrusion was considered a germination criterion. The experiments were carried out in triplicate.

2.3. Data Analysis

The results were expressed as the mean ± standard deviation. To compare the means, analysis of variance (ANOVA) was performed, and when necessary, the a posteriori test (Tukey) was applied for comparison between the groups and the control (5% of statistical significance, $p < 0.05$). GraphPad Prism 4 software was used for data analysis and to build the graphs.

3. Results

Considering the percentage of seed germination, the highest tested concentration (500 mg/L) of antibiotics significantly reduced germination compared to the negative control, after five days of exposure (Table S1). Also, the 3rd and 4th generation cephalosporins reduced the germination rate to zero at a concentration of 500 mg/L (Figures 1 and 2).

Similar to germination, fresh weight (parameter related to initial seedling growth) was not altered by exposure to Cephalothin in any of the concentrations (Figure 3, Tables S2 and S3). The dry and fresh weights were obtained by weighing the seedling on an analytical balance, but the dry weight was weighed after drying in an oven at 105 °C for 24 h.

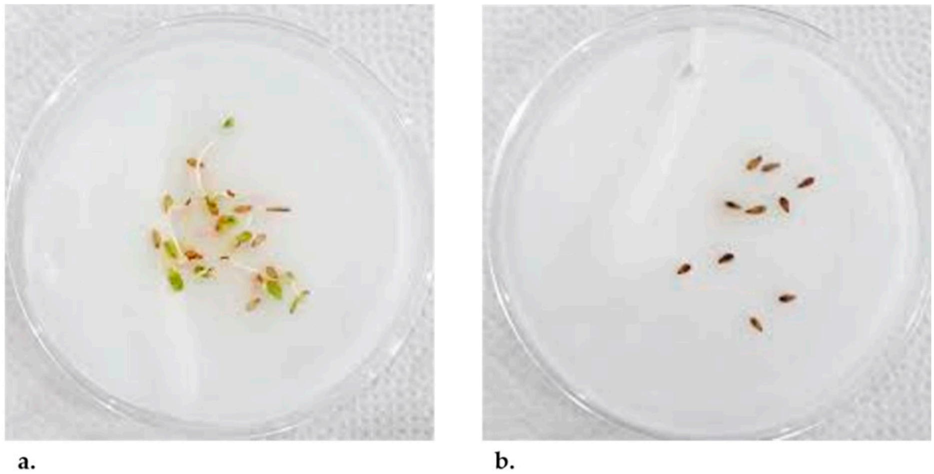

Figure 1. Petri dishes containing seeds exposed to different concentrations of cephalosporins demonstrating (**a**) *Lactuca sativa* seeds germinated after five days of exposure to 25 mg/L of Ceftazidime and (**b**) the absence of germination of *L. sativa* seeds exposed to 500 mg/L Ceftazidime.

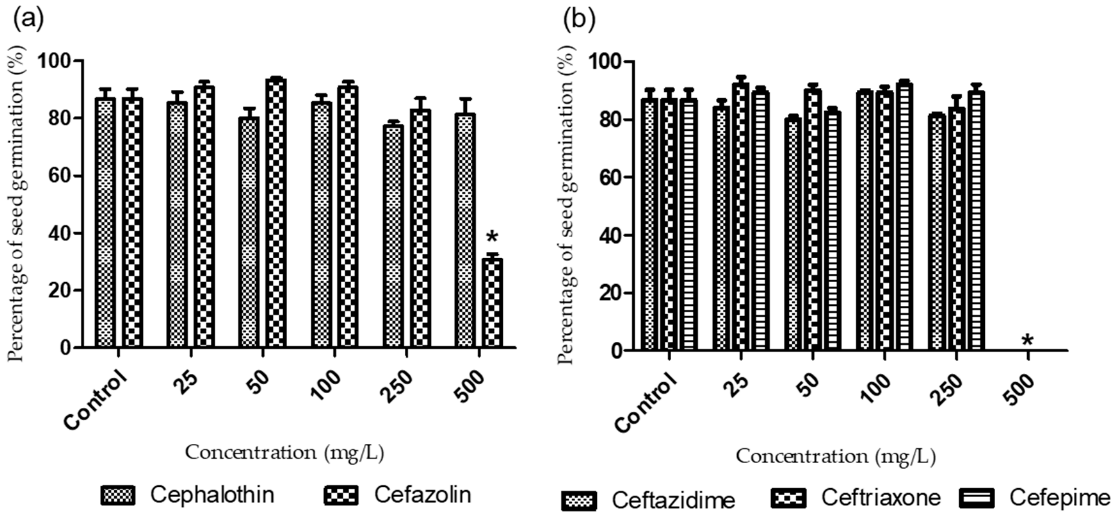

Figure 2. Average germination percentage of *L. sativa* seeds exposed to different cephalosporins. (**a**) average germination in cephalosporins of the first generation (Cephalotin and Cefazolin), (**b**) average germination in cephalosporins of the third (Ceftazidime and Ceftriaxone) and fourth generation (Cefepime). * indicates significance at the $p < 0.05$ level compared to the control.

On the other hand, for the other antibiotics, especially the 1st, 3rd, and 4th generations, there was a statistical difference between the concentrations evaluated with regard to fresh weight. The reduction in fresh weight for Cefepime occurred from the lowest concentration tested (25 mg/L) ($p < 0.05$), from the 50 mg/L of Cefazoline ($p < 0.01$) and from the 250 mg/L of Ceftazidime and Ceftriaxone ($p < 0.001$).

The dry weight results are shown in Figure 3. Considering the Cephalothin antibiotic, the dry weight of the seedlings was increased in all concentrations in relation to the negative control ($p < 0.05$). On the other hand, the other antibiotics reduced dry weight by at least one concentration tested compared to the control. The antibiotic Cefepime reduced the dry weight at a concentration of 250 mg/L, and for the other antibiotics, only the highest concentration had a significant reduction compared to the control.

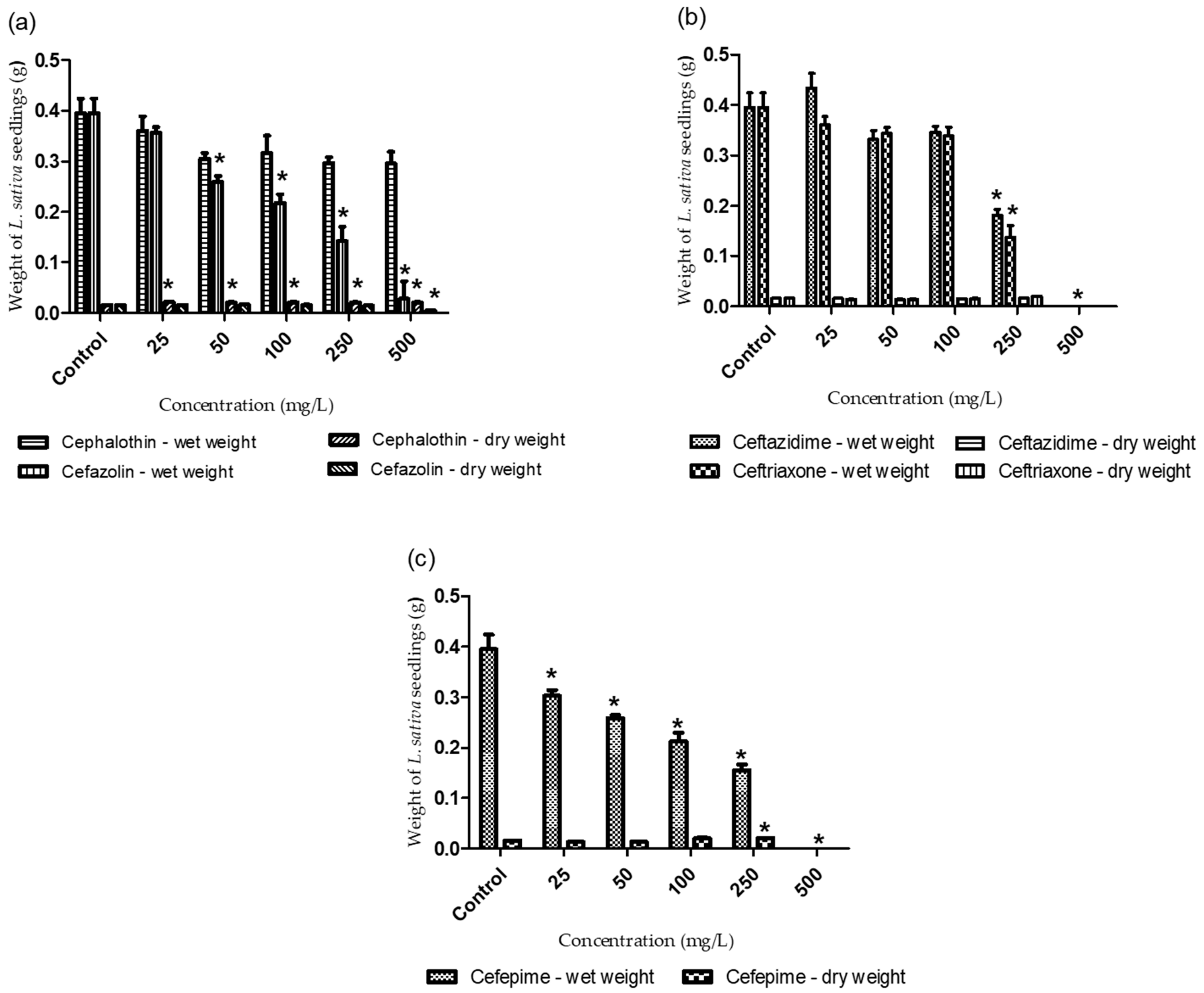

Figure 3. Wet and dry weight of *L. sativa* seedlings exposed to different cephalosporins. (**a**) wet and dry weight in cephalosporins of the first generation, (**b**) wet and dry weight in cephalosporins of the third, and (**c**) wet and dry weight fourth generation. * indicates significance at the $p < 0.05$ level compared to the control.

4. Discussion

Despite some studies that point to the low potential phytotoxic of antibiotics [31,32], the present study showed antibiotic toxicity for at least one of the evaluated parameters. High concentrations of cephalosporins significantly influenced seed germination; however, Cephalothin was the only antimicrobial agent evaluated that maintained the stability of the germination process even after exposure to 500 mg/L.

The fact that the highest concentration evaluated prevented the germination of *L. sativa* for most of the cephalosporins evaluated could be related to the degree of adsorption of these compounds since, as recently mentioned in the study by An et al. [33], the increase in the concentration of ceftiofur (the third-generation cephalosporin) reduces the degree of adsorption and the desorption capacity and facilitates the antibiotic reaching the surface and underground environments [34].

Other studies have reported that although there is a tendency for cephalosporins to be stable in an aquatic environment, for example, the rates of hydrolysis and photolysis can vary according to the antibiotic evaluated and act on the ecotoxicity of these compounds [35,36]. Therefore, similar to the findings of our study, cephalexin, a first-generation cephalosporin, such as Cephalothin, tends to exhibit lower acute toxicity against *Vibrio fischeri* than other generations of cephalosporins, which could be associated with a more intense hydrolysis process of these antimicrobials [36].

The germinative process involves different stages, starting with the seed imbibition, when the metabolic activity of the seed is restored, briefly paralyzed due to physiological maturation, followed by the absorption period and culminating with the protrusion of the primary root. Considering that each of these stages is crucial for seedling development and, therefore, the production of viable vegetables, in this study, in addition to root protrusion as a germination indicator, we also evaluated fresh weight (before starting treatment with the antibiotics) and dry weight (after total germination) [37].

The comparison of results based on fresh and dry weight indicated that fresh sprouting biomass is a more sensitive outcome than dry sprouting biomass, similar to other studies that showed the low responsiveness of the germination rate of plants exposed to antimicrobials [28,38,39].

Additionally, it should be noted that the concentrations tested in the present study are high, in the range of mg/L and that antibiotics, in general, are detected in environmental samples in the order of ng or µg per liter or per kile [40], including cephalosporins in an aquatic environment [36]. As far as we know, there are no reports of environmental cephalosporin concentrations in soil samples or plants, but several studies point to concentrations of some non-cephalosporin antibiotics in the soil in the order of mg/kg and mg/L [34,41–43]. According to Das et al. [13], the presence of third-generation cephalosporins, such as ceftriaxone, in pharmaceutical effluent has already been reported in the range of 125–175 mg/L. Moreover, Ye et al. [44] and Cycoń et al. [34] have reported the low biodegradability of these antimicrobials in wastewater, which favors their persistently high concentration.

Even though no studies identified the environmental concentration of these antimicrobials in soil, Pagaling et al. [45] showed that bacteria isolated from the soil after exposure to ceftriaxone show inhibitory concentrations in the order of mg/L. In addition, Qian et al. [46] showed that high concentrations of ceftriaxone (25–50 mg/mL, including those evaluated in this study) contributed to the death of *Zebrafish* embryos, reinforcing the need to investigate possible selective pressure that high concentrations of these antimicrobials may be exerting both directly on non-target organisms such as plants and animals, but also indirectly influencing the microbial composition of this microenvironment.

Recently, Wilkinson et al. [47] evaluated the role of pharmaceutical products in rivers around the world, emphasizing that antibiotics are among the compounds found as a pollutant in this most frequent environment, including concentration ranges of four to five orders of magnitude. Moreover, they point out that this is probably related to the failure of regulatory oversight and inadequate use and sales of these compounds in human and animal health, especially in low- and middle-income countries, where the occurrence and concentrations of antibiotics in the environment are higher [9,10].

Once antibiotics are released on agricultural land, the crops are exposed to them due to their persistence, and the level of exposure depends on the physicochemical properties of the compounds, sorption potential, and environmental conditions. Therefore, as identified in this study, the significant interference of different cephalosporins in the germination process of a vegetable of extreme economic relevance worldwide and nationally, combined with the ability of antibiotics to affect diverse environments by reducing their biochemical activities and diversity and modifying the microbial community, could impact directly or indirectly agroindustrial losses, in addition to reducing the therapeutic options in human and animal health by the selection of resistant pathogens.

5. Conclusions

This study evidenced that cephalosporin antibiotics can cause toxicity in *L. sativa*, although the perceived effects were in high concentrations. The fourth-generation cephalosporins were more toxic, considering the parameters evaluated. Toxicity to plants due to exposure to PPCP's must be monitored, which may indicate a possible risk that these residues move through the food chain. Added to this, the lack of information on environmental concentrations of cephalosporins in soil, despite its widespread use worldwide, alerts us to a potential hazard to non-target organisms. Further research is needed to evaluate the effects of PPCP's on plants in realistic field practice, such as irrigation with treated wastewater containing residues of these products.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pollutants2040029/s1, Table S1. Average germination percentage of *L. sativa* seeds exposed to different cephalosporins; Table S2. Wet weight of *L. sativa* seedlings exposed to different cephalosporins; Table S3. Dry weight of *L. sativa* seedlings exposed to different cephalosporins.

Author Contributions: Conceptualization, F.M.R.d.S.J. and D.F.R.; Formal analysis, L.M.V. and R.d.L.B.; Funding acquisition, F.M.R.d.S.J. and D.F.R.; Investigation, L.C.H.; Methodology, L.d.S.F.; Supervision, L.M.V., F.M.R.d.S.J. and D.F.R.; Writing—original draft, L.d.S.F. and L.C.H.; Writing—review and editing, F.M.R.d.S.J. and D.F.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001 and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq): Edital Universal 2018 Grant 408303/2018-2, Research Productivity Grant 310856/2020-5)—Flavio Manoel Rodrigues da Silva Júnior and CNPq (Edital Universal, 2014, Grant 442381/2014-0 and Research Productivity, Grant 305921/2019-3—Daniela Fernandes Ramos).

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, for the Doctoral scholarships.

Conflicts of Interest: The authors declare no conflict of interest.

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