

Article

Investigation of the Effect of Twelve Pharmaceuticals on Germination and Growth Parameters of Basil (*Ocimum basilicum* L.)

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Abstract: Pharmaceuticals (PhACs) are chemical substances that, after their use, can reach wastewater treatment plants, but the resulting treated wastewater (TWW) can still contain these contaminants. If TWWs are used for irrigation, PhACs can contaminate crops and also hinder their growth. The aim of this work was to evaluate the effects of 12 PhACs and their mixture at different doses on basil germination and early growth and on its photosynthetic pigment content. The germination percentage was not affected by PhACs even when applied at the highest doses. The results showed that the germination speed cannot be considered as an index of vigor of future seedlings as not all seeds that germinated first developed the best. PhACs between 25 and 100 ppb did not show negative effects on early growth and photosynthetic pigments of basil; in fact, in some cases the seeds even benefitted from their application as if it were a chemical treatment developed for the seeds. The highest assessed dose of PhACs always caused a reduction in growth parameters and the photosynthetic pigment content of basil, especially with climbazole, naproxen, triclosan, and the mixture of PhACs. In general, basil can be considered a species tolerant to PhACs after taking into account their average content in wastewater; however, more studies are needed to evaluate the long-term effects of PhACs and their translocation to edible parts.

Keywords: contaminant of emerging concern; shoots and roots length; chlorophyll; carotenoids; seeds



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1. Introduction

Pharmaceuticals (PhACs) are different chemical substances that are used in both human and veterinary medicine. Some of these have been recognized as contaminants of emerging concern (CECs) as they are increasingly widespread in the environment and have potential adverse effects on non-target organisms and humans [1]. Furthermore, the use of PhACs is increasing worldwide and will continue to increase in the future [2]. After their use, PhACs and/or their metabolites reach wastewater treatment plants through feces and urine where they undergo physical, chemical, and biological processes. The resulting treated wastewater (TWW) can still contain these contaminants [3,4]. Therefore, the use of TWW for irrigation, an agricultural practice already widespread in several regions affected by drought, can expose the soil environment to PhACs contamination [5].

Plants have the ability to absorb and accumulate PhACs in their tissues and is confirmed by several studies [6–9]. The effects vary in relation to plant species and the chemical and physical properties of the molecules [10,11]. Few studies have investigated the toxic effects of PhACs on seed germination, including reduced shoot growth and metabolic disorders [12–14]. In several experiments, hydrophobicity was considered the crucial factor that most affects the phytotoxicity of these compounds toward seeds [15]. Pino et al. [13] investigated the ecotoxicity of 15 PhACs in *Lactuca sativa* L. seeds and *Chlamydomonas reinhardtii*. They found that propranolol was the most toxic drug for root and hypocotyl elongation, followed by nonsteroidal anti-inflammatory drugs (NSAIDs), gemfibrozil, and tetracycline. However, the highest concentrations tested did not affect seed germination.

Similarly, Bellino et al. [12] reported that chloramphenicol, spiramycin, spectinomycin, and vancomycin, belonging to four different chemical groups, adversely affected tomato root development but not seed germination. In contrast, Rude et al. [14] showed that other PhACs, such as paracetamol, ibuprofen, and amoxicillin, did not induce toxicity in the early growth of lettuce seeds. Therefore, the potential effects of PhACs on plant growth and development need to be fully assessed. Such studies would be of paramount importance considering the possible effects of PhACs on food chains, ecosystems, and human health.

Seed germination and root elongation tests are simple, sensitive, and inexpensive environmental bioassays [16,17], which are commonly used to evaluate the phytotoxicity of organic and/or inorganic compounds on plants [18]. Evaluation of the biological responses of crops to PhACs is important because plants could be increasingly irrigated with reclaimed wastewater. This hypothesis could represent a sustainable alternative to face the water shortage in drought areas, such as the Mediterranean basin.

Sweet basil (*Ocimum basilicum* L.) is an annual herb belonging to the Lamiaceae family, and the *Ocimum* genus represents the most widely used medicinal and aromatic plants [19]. In fact, basil is distributed throughout the world and comprises more than 60 cultivars [20]. Aromatic plants, such as sweet basil, can absorb and accumulate PhACs [21], which could alter their growth and yield. Although the toxic effects of a single pharmaceutical have been described at different trophic levels, current knowledge of the effects of a mixture of pharmaceuticals is still scarce. Therefore, the objective of this study was to evaluate the effects of 12 widely consumed pharmaceuticals from different therapeutic groups, alone or in combination, on the germination, early growth, and photosynthetic pigment content of *Ocimum basilicum* L. The authors wanted to simulate, at the laboratory level, the contact between basil seeds and TWW containing PhACs as TWW can be used for basil irrigation and, therefore, could be a threat that needs further evaluation.

2. Materials and Methods

2.1. Pharmaceuticals

The PhACs used in the present study were carbamazepine (CBZ), clarithromycin (CLR), climbazole (CBZ), diclofenac (DCF), fluconazole (FCZ), gemfibrozil (GFZ), ketoprofen (KET), metoprolol (MTP), naproxen (NPX), sulfamethoxazole (SMX), triclosan (TCS), and trimethoprim (TMP). CLR, TMP, and SMX are antibiotics, CBZ and FCZ are antifungal drugs, DCF, KET, and NPX are anti-inflammatory drugs, and finally, TCS, MTP, GFZ, and CBZ are antibacterial, beta blocker, antilipemic, and antidepressant drugs, respectively. PhACs were selected considering their common presence in European wastewaters [22,23]. The chemical structures and properties of the selected PhACs are listed in Table 1.

Solutions of each pharmaceutical were prepared at five different concentrations: 25, 50, 100, 200, and 600 ppb. To study a more realistic scenario, *Ocimum basilicum* seeds were also exposed to PhACs mixture solutions (MIX) at same concentrations.

Analytical standards (purity > 99%), used to prepare all solutions, were provided by Lab Instruments (Italy). Fresh stock solutions of pharmaceuticals were prepared in high purity water.

Table 1. Physicochemical properties (Molecular Weight; Chemical Structure; Chemical Class Water Solubility; KOW, Octanol/Water Coefficient; and pKa, Acid Ionization Constant) of selected CECs.

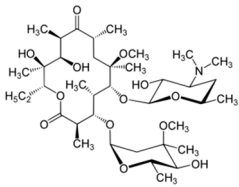
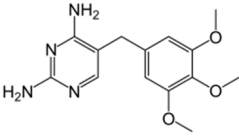
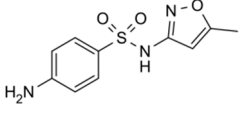
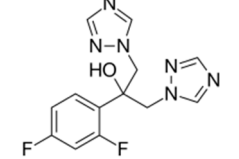
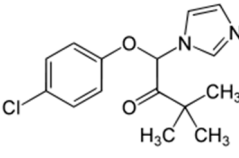
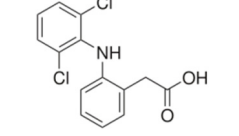
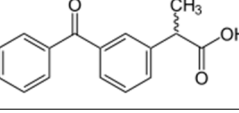
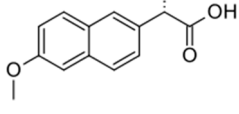
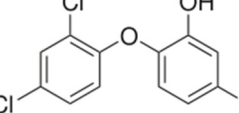
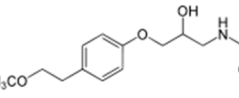
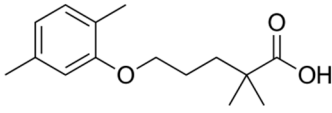
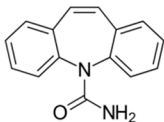
CECs	Molecular Weight g/mol	Chemical Structure	Chemical Class	Water Solubility mg/L	KOW	pKa
Clarithromycin	748		antibiotic	1.69 at 25 °C	3.16	8.99
Trimethoprim	290.32		antibiotic	400 at 25 °C	0.91	7.12
Sulfamethoxazole	253.28		antibiotic	610 at 37 °C	0.89	1.6
Fluconazole	306.27		antifungal	4.36 at 25 °C	0.25	2.27
Climbazole	292.76		antifungal	58 at 25 °C	3.76	6.49
Diclofenac	296.1		anti-inflammatory	2.37 at 25 °C	4.15	4.15
Ketoprofene	254.28		anti-inflammatory	51 at 22 °C	3.12	4.45
Naproxen	230.26		anti-inflammatory	15.9 at 25 °C	3.18	4.15
Triclosan	289.5		antibacterial	10 at 20 °C	4.76	7.9
Metoprolol	267.36		beta blocker	0.4 at 25 °C	1.88	9.7

Table 1. Cont.

CECs	Molecular Weight g/mol	Chemical Structure	Chemical Class	Water Solubility mg/L	KOW	pKa
Gemfibrozil	250.33		antilipemic	11 at 25 °C	4.77	4.5
Carbamazepine	236.27		antidepressant	18 at 25 °C	2.45	13.9

2.2. Experimental Procedure

Ten basil seeds were placed on Petri plates on Filter-Lab paper (85 mm) and moistened with 10 mL of each solution. Five replicates were tested for every solution, including controls without PhACs. The experiment was carried out in a growth chamber with Petri dishes randomly arranged. The first two days, the seeds remained in the dark to induce germination, then they were subjected to a photoperiod of 16:08 h light:dark with a light intensity of 10,000 lx at 24 °C and 60 % humidity. The germination parameters were recorded daily for the first 9 days, while the length of the roots and shoots, the fresh and dry weight, and the photosynthetic pigment content were determined on the 17th day of the experiment.

2.3. Germination Parameters

The parameters used to compare the germination data for representation and accuracy were as follows:

1. Germination Energy (GE)
2. Final Germination Percentage (FGP)
3. Mean Germination Time (MGT)
4. Seed Vigor Index I (SVI-I)
5. Seed Vigor Index-II (SVI-II)

Details, measurement units, and calculation methods of each parameter are shown in Table 2.

Table 2. Description of various parameters used to study seed germination.

Germination Parameter	Symbol	Unit	Calculation	Formula Description	Parameter Description
Germination Energy	GE	%	$GE = (n/N) \times 100$	N = number of seeds germinated after four days N = number of seeds tested	GE is the parameter that gives the percentage of fast-germinating seeds. On day 4, an evaluation of the germination energy is conducted [24]. When plants have high germinative energy, they have constant growth that limits competition with weeds and ensures maximum use of the cultivated land.
Final Germination Percentage	FGP	%	$FGP = (nT/N) \times 100$	nT = total number of germinated seeds N = number of seeds tested	Higher FGP values correspond to higher seed germination [25].

Table 2. Cont.

Germination Parameter	Symbol	Unit	Calculation	Formula Description	Parameter Description
Mean Germination Time	MGT	day	$MGT = \sum(n \times d)/N$	N = number of seeds germinated on each day d = number of days from the beginning of the test N = total number of seeds germinated at the end of the experiment	At low MGT values, seed germination is faster [26].
Seed Vigor Index-I	SVI-I	-	$SVI-I = SL \times FGP$	SL = seedling length (cm) FGP = Final Germination Percentage	The seed vigor index was determined by multiplying the FGP and the length of the seedling (mm) or seedling dry weight of the seedling (g). The seed lot showing higher seed vigor index was considered more vigorous [27].
Seed Vigor Index-II	SVI-II	-	$SVI-II = SDW \times FGP$	SDW = Seedling dry weight (mg) FGP = Final Germination Percentage	

2.4. Morphological Parameters and Photosynthetic Pigment Analysis

After 17 days of growth, the length of seedlings was measured using a ruler. Then, they were separated into roots and shoots to determine the fresh weight of the two seedling portions. Afterwards, roots and shoots were placed in paper bags, dried in an oven at 80 °C for 24 h, and weighed as described by Bibi et al. [28]. Seed Vigor Index I (SDI-I) and Seed Vigor Index II (SDI-II) were obtained by multiplying seedling length and seedling dry weight, respectively, using FPG [27].

For the determination of chlorophyll a (CHA) and b (CHB) and total carotenoids (TCA), 25 mg of dried plant material and 25 mg of MgO (to prevent phaeophytin formation) were placed in 15 mL tubes. Subsequently, 5 mL of methanol was added and the whole mixture was homogenized with stirring for 2 h. The samples were then centrifuged for 5 min at 4000 rpm. The supernatant was transferred in a 1 cm path length cuvette. The absorbance was measured using methanol solvent as a blank in a UV-Vis spectrophotometer at three different wavelengths: 666, 653, and 470 nm. According to the method described by Lichtenthaler and Wellburn [29], photosynthetic pigments were calculated as follows:

$$CHA = 15.65 OD_{666} - 7.340 OD_{653}$$

$$CHB = 27.05 OD_{653} - 11.21 OD_{666}$$

$$TCA = (1000 OD_{470} - 2.860 CHA - 129.2 CHB)/245$$

where OD represents the absorbance at a given wavelength.

2.5. Statistical Analysis

All experimental data were tested against the normal distribution of variables (Shapiro–Wilk test) and the homogeneity of variance (Bartlett test) using R studio software, version 4.1.3. The variables were then subjected to a suitable ANOVA and post-hoc test. The difference was significant when the *p* value ≤ 0.05 .

3. Results and Discussion

3.1. Germination Parameters

Figure 1 shows the percentage of basil germination as a function of each PhAC at different doses and of MIX solutions at same concentrations. The statistically lowest percentages of germination were observed for CLZ and MIX only at the highest dose, followed by NPX and TCS, which showed slightly lower percentages of germination relative to other treatments. The remaining these did not show statistical difference.

Similarly, Pino et al. [13] tested 15 PhACs on *Lactuca sativa* L. germination and found that even at the highest concentration tested (3000 mg L⁻¹), seed germination was not affected. Hillis et al. [30] tested 10 antibiotics in carrots, lettuce, and alfa alfa and found that none of the antibiotics caused a significant decrease in seed germination. This result can be attributed to the seed coat, which is a protective barrier for the plant embryo against environmental contaminants [30–32].

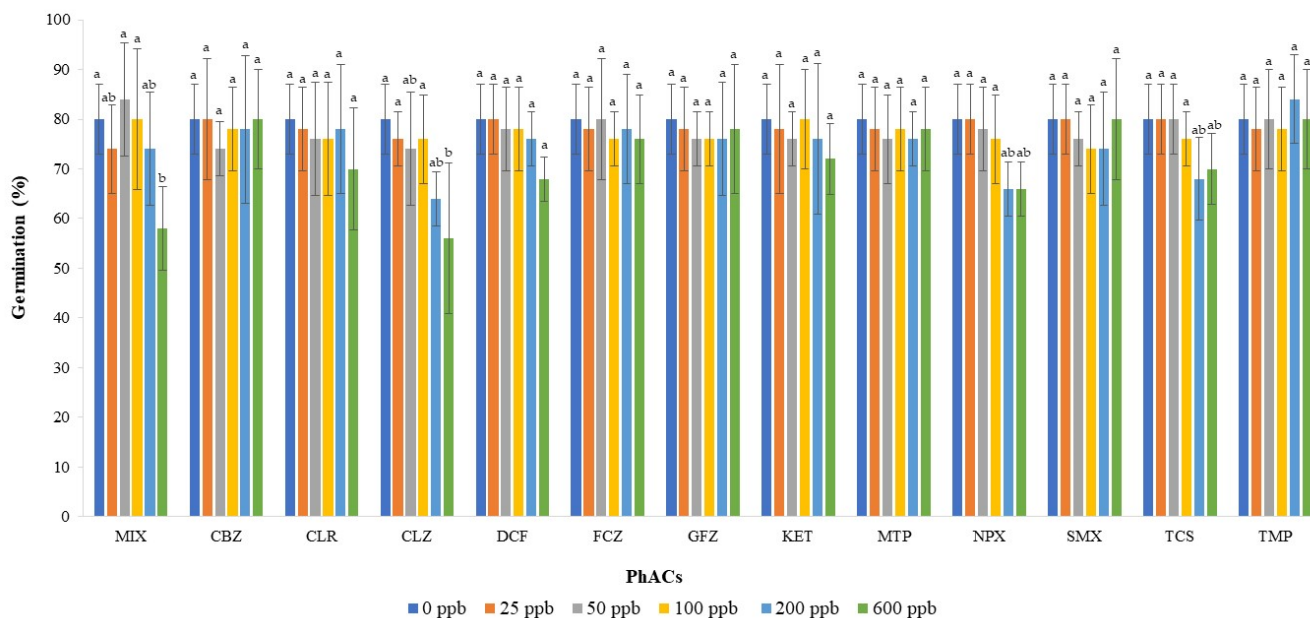


Figure 1. Effect of mixture and individual pharmaceuticals on seed germination in basil. Each bar represents the mean value of five independent replicates, and the error bar shows the standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$).

Table 3 shows the values of the germination indexes. GE was statistically the highest for 100 and 200 ppb solutions and the lowest for the control. With respect to the influence of PhACs, MIX and CBZ showed higher values of GE compared to those of other contaminants, whose GEs were quite comparable. On the FGP, this parameter was inversely proportional to the concentration of the solution applied, and the control showed the highest values. On the contrary, no significant differences were recorded when PhACs were considered individually or in combination. Therefore, in the first 4 days of the trial, the presence of PhACs was more helpful for the germination of the seeds, especially the PhACs mixture, possibly because of a short-term effect of chemical treatment on seeds. This was confirmed by the MGT values that resembled the GE trend. On the other hand, over the entire period of the trial, the PhACs solutions resulted in a lower inhibition of seed germination than the control. As the germinability varies in a seed population, it could be possible that seeds with the lowest intrinsic germinability, i.e., the ones that germinated later, were most affected by the addition of PhACs.

Table 3. Effects of pharmaceuticals and different concentrations on germination indexes.

	GE	FGP	MGT	SVI-I	SVI-II
Concentration	**	***	***	***	***
PhACs	***	n.s.	***	***	***
Concentration					
0 ppm	54.00 b	80.00 a	3.60 a	448.66 ab	83.78 b
25 ppm	57.84 ab	78.30 ab	3.36 ab	467.74 a	137.15 a

Table 3. Cont.

	GE	FGP	MGT	SVI-I	SVI-II
50 ppm	58.46 ab	77.53 ab	3.28 bc	453.41 a	128.14 a
100 ppm	60.76 a	77.07 ab	3.16 bc	406.53 b	120.42 a
200 ppm	60.92 a	74.46 bc	3.05 c	331.23 c	76.13 b
600 ppm	58.30 ab	71.53 c	3.05 c	266.45 d	63.28 b
PhACs					
Mix	69.00 a	75.00 ab	2.69 d	311.10 b	71.52 b
CBZ	67.00 ab	78.33 ab	2.85 cd	462.89 a	85.26 b
CLR	54.00 c	76.33 ab	3.48 ab	430.71 a	76.06 b
CLZ	53.33 c	71.00 b	3.26 abc	308.86 b	236.02 a
DCF	57.33 c	76.66 ab	3.26 abc	415.41 a	81.72 b
FCZ	56.33 c	78.00 ab	3.39 ab	438.23 a	80.90 b
GFZ	57.33 c	77.33 ab	3.37 ab	444.01 a	81.46 b
KET	58.00 c	76.66 ab	3.23 abc	452.97 a	83.94 b
MTP	54.00 c	77.66 ab	3.57 a	409.31 a	76.70 b
NPX	57.66 c	74.33 ab	3.26 abc	318.06 b	217.81 a
SMX	56.00 c	77.33 ab	3.40 ab	416.82 a	77.39 b
TCS	59.66 bc	75.66 ab	3.09 bcd	304.01 b	71.01 b
TMP	59.33 bc	80.00 a	3.39 ab	431.34 a	79.49 b

The values in each column (mean of five replications) followed by a different letter are significantly different. **: significant at $p < 0.01$; ***: significant at $p < 0.001$; n.s.: non-significant. GE, Germination Energy; FGP, Final Germination Percentage; MGT, Mean Germination Time; SVI-I, Seed vigor index-I; SVI-II, Seed vigor index-II.

3.2. Early Growth Parameters

Figure 2 shows the length of the roots (A) and shoots (B) of the basil seedlings affected by each pharmaceutical or their mixture, added at different doses, with respect to the control. CLR, CLZ, DCF, and GFZ showed better values than the control when applied at 25 and 50 ppb doses, while FCZ and KET showed better values up to 200 ppb dose. Anyway, previous PhACs showed the hormesis phenomenon, that is, a biphasic response, favorable and inhibitory effect at low and high doses, respectively [33]. Similarly, other authors reported a greater growth of roots in some plants with the application of low doses of pharmaceuticals and a growth inhibition with the application of high doses of pharmaceuticals [34–37]. Finally, CBZ, SMX, and TMP did not cause modification in root length at any concentration of applied dose, while MTP, NPX, TCS, and MIX induced a significant reduction in root length only when higher doses were applied.

No PhACs studied showed hormesis, and none resulted in longer shoots than the control, except CBZ, which showed slightly higher values when applied at the highest doses. FCZ and GFZ did not cause any significant variation in the length of the shoot, while the remaining PhACs showed a similar trend to one observed for the corresponding root length.

The VI-I index confirmed that doses 25 and 50 ppb resulted in the best vigor, while the same index decreased with increasing concentration of solutions (Table 2). In addition, the PhACs studied were segregated by the VI-I index in two groups: one composed of MIX, CLZ, NPX, and TCS, characterized by the lowest vigor, and the other one, represented by the remaining PhACs, showing the best vigor.

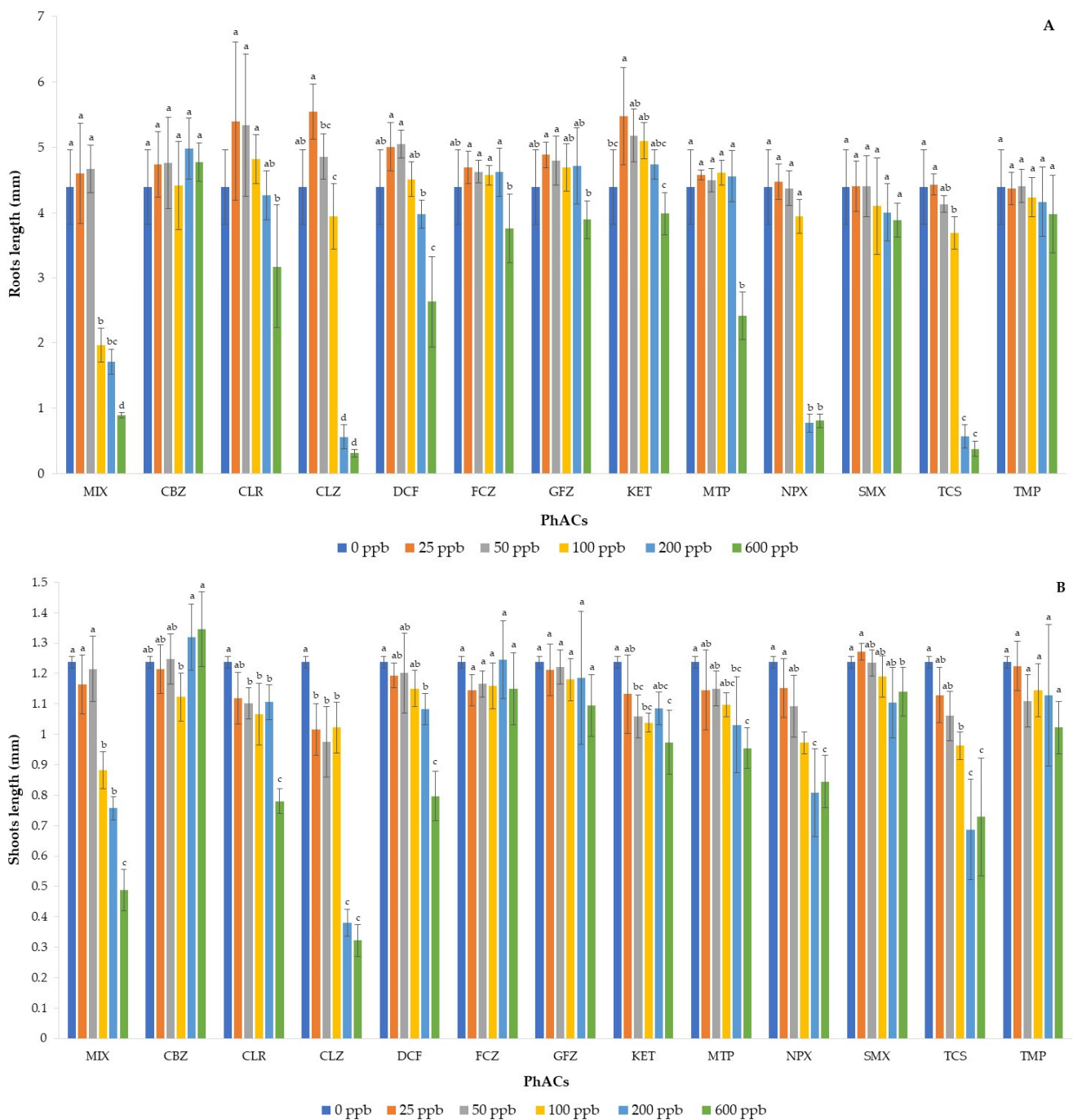


Figure 2. Effect of the mixture and individual pharmaceuticals on the length of the root (A) and shoot (B) in basil seedlings. Each bar represents the mean value of five independent replicates, and the error bar shows the standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$).

Figure 3 shows the dry weight of the roots (A) and the shoots (B). CBZ, CLZ, DCF, NPX, and TCS showed hormesis, that is, a significant increase in the dry weight of the root when applied at 100 ppb concentration (200 ppb for CBZ) compared to the control, while they decreased the same parameter at higher solution concentrations. KET and MIX did not influence the dry weight of the root at any concentration, while the remaining PhACs resulted in a weight reduction with the highest doses. Regarding shoot weight, all PhACs showed the same trend as that for the corresponding root dry weight, except for CBZ and NPX that did not show hormesis as observed for the roots. It was evident that

doses of 200 and especially 600 ppb drastically reduced the dry weights of the seedlings, highlighting the toxicity of the various compounds with respect to the applied dose. In general, the effects of PhACs on plants depend on the class of pharmaceuticals and its concentration and on the plant species [38]. Therefore, it is possible that some of the PhACs studied can stimulate basil growth in low doses due to possible hormone-like effects and/or seed protection against seedling damping.

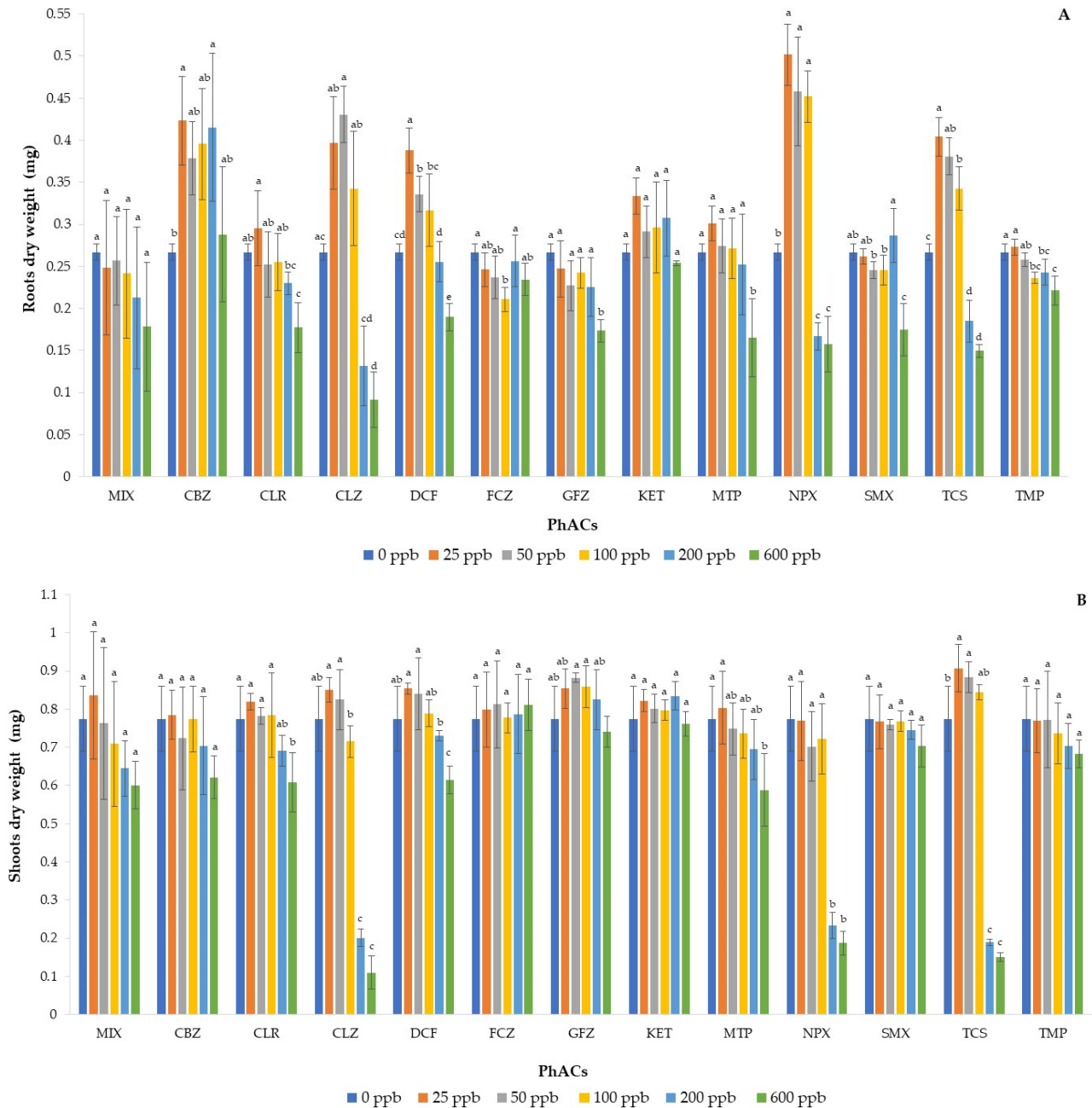


Figure 3. Effect of the mixture and individual pharmaceuticals on the dry weight of the root (A) and shoot (B) in basil seedlings. Each bar represents the mean value of five independent replicates, and the error bar shows the standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$).

The VI-II index confirmed that doses of 25 to 100 ppb resulted in better vigor compared to control and doses of higher concentrations (Table 2). Among the PhACs studied, CLZ and NPX showed the highest VI-II index due to their larger dry weight, especially at 25 to

100 ppb; however, significant differences in FGP values for each PhAC were not observed. In general, CLZ, NPX, and TCS showed the worst results for the aforementioned parameters at the highest dose. Regarding CLZ, Richter et al. [39] found that this compound negatively influenced seedling emergence, shoot length, and biomass of *Brassica napus* L. and *Avena sativa* L. In general, demethylase inhibitor fungicides, such as climbazole, can act as plant growth inhibitors that interact with sterol biosynthesis and inhibit gibberellin biosynthesis. Climbazole is an imidazole and has a higher toxicity than triazoles, such as FCZ, and isoxazoles, such as SMX [40]. In fact, in our study, FCZ and SMX showed slight toxicity only at the highest applied dose for some parameters. In particular, FCZ did not affect basil growth as its behavior is similar to that of other triazoles used as plant fungicides and growth promoters [41,42]. However, García-Valcárcel et al. [43] tested different azoles on lamb lettuce and found higher weights for plants grown in the presence of azoles compared to the control in the initial days of the experiment, and the same was observed in the present study for CLZ applied at lower doses.

The worst results of TCS at higher doses were possibly due to blockage of fatty acid synthesis in plants by inhibiting a reductase implicated in various metabolic pathways [44]. As a result, plants exposed to high concentrations of triclosan soon died. Regarding NPX, Pawłowska et al. [34] conducted a study on the effects of NPX and DCF on growth parameters of spring barley and found that NPX had the greatest negative effect on the plant. High doses of NPX can induce the formation of reactive oxygen species (ROS), lipid peroxidation, reduction of membrane integrity, and change in the antioxidant and redox system that, finally, negatively influence the root apparatus in plants [36].

With respect to MIX, the length of roots and shoots was significantly lower than the control at a dose of 100 ppb, showing greater toxicity of this dose than that of a single contaminant. However, at doses of 200 and 600 ppb, the toxicity of the mixture was in any case lower than that of CLZ, NPX, and TCS considered individually. Furthermore, the dry weight of the roots and shoots did not show significant differences compared to the control, even at the highest dose applied. These results could be derived from interactions among the different contaminants that have mitigated the effect of individual PhACs, lowering their toxic effect.

3.3. Photosynthetic Pigments

Figure 4 shows the concentration of CHA (A), CHB (B), and TCA (C) from basil seedlings as a function of each PhAC at different doses and of MIX at same doses. The trend of CHA content was quite similar among the studied PhACs, and from 100 ppb to the highest dose, its content decreased in all treatments, although to a different extent, with CLZ, NPX, TCS, and MIX resulting in the largest reduction in CHA concentration. Finally, CBZ, KET, and MTP showed slightly higher content at the single dose of 25 ppb. Instead, all PhACs resulted in the highest CHB concentration when applied at a dose of 25 ppb, while its concentration decreased from 100 ppb with increasing concentration of the dose.

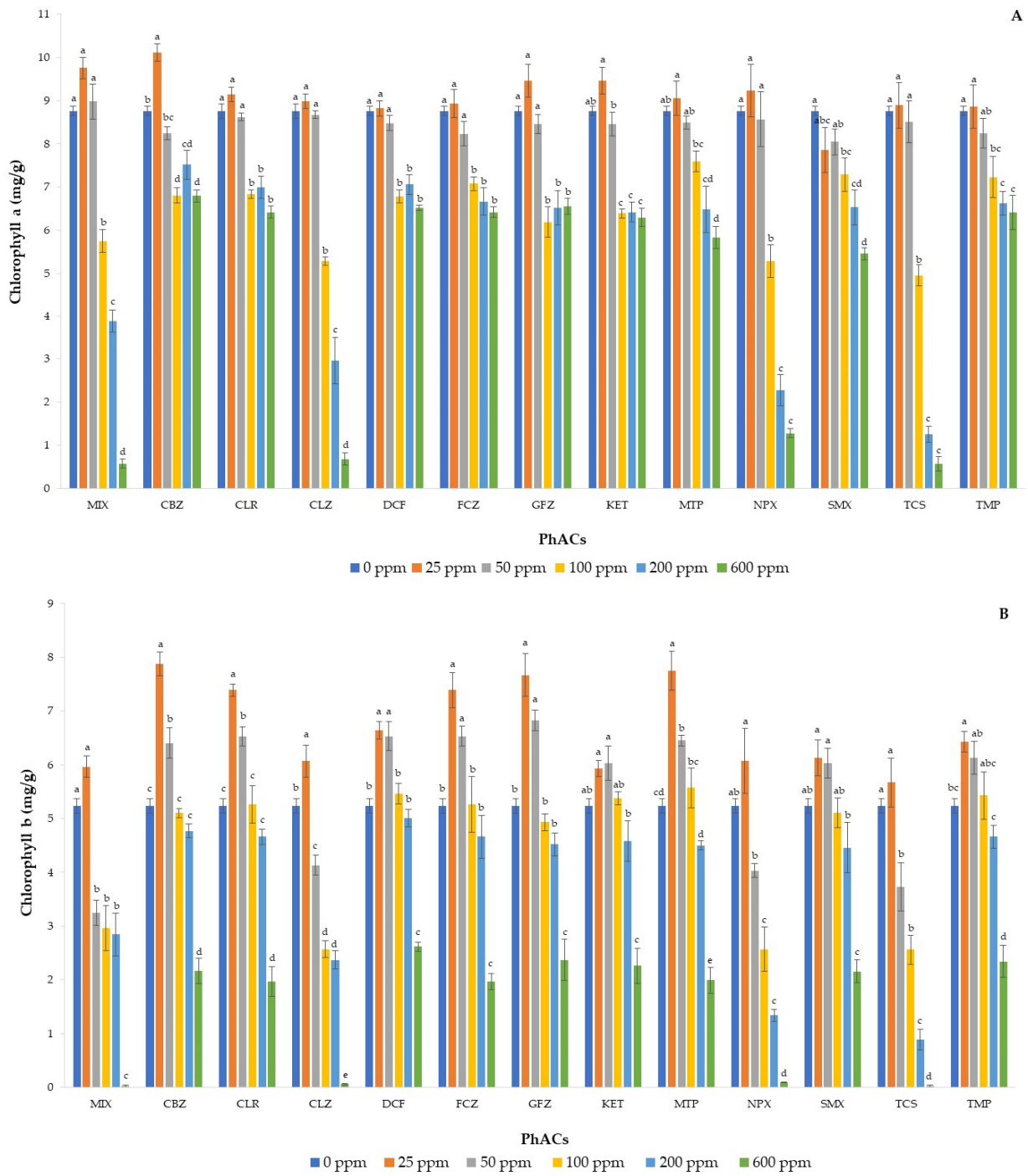


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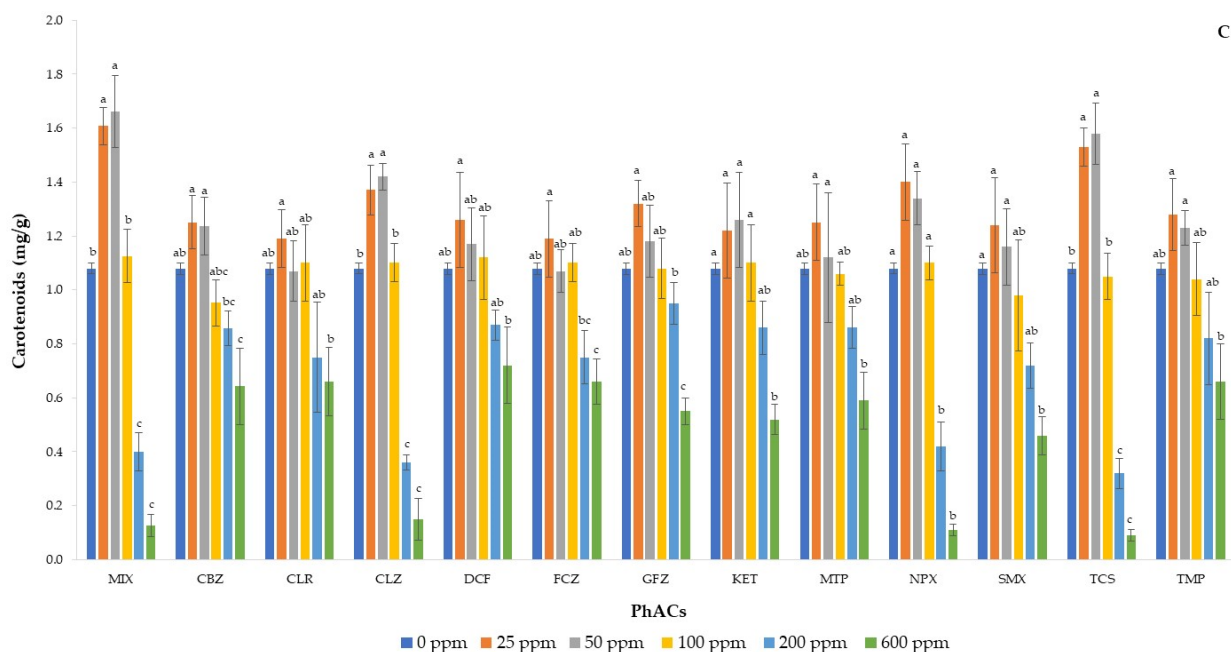


Figure 4. Concentrations of chlorophyll a (A), chlorophyll b (B), and total carotenoids (C) in basil plants after exposure to mixture and individual pharmaceuticals. Each bar represents the mean of five independent replicates, and the error bar shows the standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$).

The worst results were recorded with the application of 600 ppb of MIX, CLZ, NPX, and TCS, which resulted in an almost zero concentration of both chlorophylls. In fact, the latter are pigments involved in photosynthesis and, therefore, responsible for plant growth, with CHA being more abundant because it is the principal pigment involved in photosynthesis, while CHB is an accessory pigment [45]. Changes in their concentration are indicative of the cumulative impact of environmental stress [46] and mainly attributed to metabolic disorders or ROS accumulation in chloroplasts [47]. Therefore, all contaminants tested, at doses higher than 50 ppb, induced a stress condition in basil seedlings as the reduction in chlorophyll content is a typical stress response [48]. Similarly, Opreş et al. [49] tested the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on green leafy vegetables and found a reduction in chlorophylls, carotenoids, polyphenols, and flavonoids as a function of the dose applied. Wang et al. [50] also confirmed the toxicity of NSAIDs in green alga with a reduction in the concentration of photosynthetic pigments.

A significant reduction in the content of TCA was observed mainly with the application of the highest dose of contaminants, that is, 600 ppb and, only in some cases, 200 ppb. Therefore, carotenoids appeared to be less sensitive than chlorophylls to the addition of different contaminants in the basil growth medium. Carotenoids are secondary metabolites that help absorb light and protect elements against photodamage and ROS-induced oxidative stress [51,52]. Therefore, the reduction in carotenoids occurred only in the case of severe stress [49] and, in general, their trend resembled those of chlorophylls, confirming the highest toxic effect of CLZ, NPX, TCS, and MIX on basil seedlings.

4. Conclusions

The results of this study suggest that PhACs doses between 25 and 50 ppb do not interfere with basil germination and early growth and, rather, some PhACs show positive effects on these parameters as if they were a chemical treatment developed for seeds. Therefore, considering the doses of contaminants normally present in the soil or added with wastewater, it can be stated that basil does not suffer from the current environmental contamination of the PhACs studied. However, if PhACs somehow reach doses of 200 to

600 ppb, the effects on germination and early growth are hazardous, especially when basil is treated with CLZ, NPX, TCS, and MIX.

Among the germination parameters considered in the present study, the percentage of germination is the parameter least sensitive to contaminants, while a higher germination speed is not necessarily indicative of better development of future seedlings. In fact, MIX caused faster germination compared to other PhACs but later showed higher toxicity, resulting in less vigorous seedlings. More studies are needed to evaluate the long-term growth of basil and the possible translocation of PhACs to its edible parts.

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