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The Effects of Picloram and Clopyralid on Ocimum Basilicum (Basil)—A Study of the Uptake, Distribution and Translocation of Synthetic Auxins from Soil to Plant

Roxana Elena Scutariu, Valentina Andreea Petre *, Alina Tatarus, Diana Maria Puiu and Florentina Laura Chiriac *

National Research and Development Institute for Industrial Ecology—ECOIND, Drumul Podu Dambovitei 51-73, Sector 6, 060652 Bucharest, Romania; roxgrigoras@gmail.com (R.E.S.); alina.tatarus@yahoo.com (A.T.); diana_puiu@ymail.com (D.M.P.)

* Correspondence: petrevalentinaandreea@gmail.com (V.A.P.); laura.chiriac@incdecoind.ro (F.L.C.)

Abstract: The current study monitored the degree of uptake, distribution, bioaccumulation, and translocation of synthetic auxins (Picloram and Clopyralid) in medicinal plants (Basil). The study's main objectives were the development and optimization of an analytical method for the identification and quantification of auxins, an optimized method of auxins extraction from soil and plant samples, and, based on the analytical results obtained, the evaluation of bioaccumulation and translocation capacity. To evaluate the effects produced by synthetic auxins on the Basil plant, three experiments were carried out in parallel (Basil-Clopyralid, Basil-Picloram, and Basil-Clopyralid-Picloram) for 15 days, where the plant was permanently exposed to a constant concentration of auxins. The study results showed that in the individual tests and the test carried out in the mixture, the highest concentration was recorded for Clopyralid in the Basil leaves, 16 µg/kg d.w., respectively, 22 µg/kg d.w. The antagonist, Picloram, was primarily detected in the plant's roots, up to 7.2 µg/kg d.w. Therefore, Picloram favors the accumulation of Clopyralid in high percentages in all plant organs. The bioconcentration factors (BCF) and translocation factors (TF) calculation showed values lower than 1, indicating that Basil is an excluder and has no potential for phytoremediation.

Keywords: synthetic auxin herbicides; basil; LC-MS/MS detection; bioaccumulation and translocation capacity

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1. Introduction

Increasing global population demands have intensified the pressure on agricultural systems to produce sufficient food. This has led to an increased reliance on synthetic herbicides, particularly auxins, which are employed to control weed growth and enhance crop yields effectively [1]. Among the various synthetic auxins, chlorophenoxycarboxylic acids such as Clopyralid, Picloram, Triclopyr, Aminopyralid, Fluroxypyr, and Dicamba are commonly used in agricultural practices due to their selective herbicidal action [2–4].

Herbicides represented 44% of all pesticides used in Europe and the USA in 2012, highlighting their significance in modern agriculture [1]. While auxins can promote plant growth by altering cell wall plasticity, there are growing concerns regarding their environmental impact. These compounds have been detected in surface water samples from numerous regions, including the USA [5], Europe [6], and China [7]. Their presence poses

potential risks to aquatic ecosystems, as studies have revealed that synthetic auxins can exert toxic effects on phytoplankton populations, leading to declines in diatoms, green algae, and cyanobacteria [8]. Reported maximum concentrations in surface waters for compounds such as Clopyralid and Picloram can reach levels of 4 µg/L, with 2,4-D concentrations reaching 10 µg/L [5,7,9–11]. Additionally, investigations have shown that as much as 38% of wells in agricultural regions may be contaminated with these herbicides [12].

Synthetic auxins complicate plant dynamics: while they can inhibit the growth of unwanted weeds, low concentrations can promote the growth of desirable crops. However, these compounds can generate cyanide ions at high concentrations, leading to cell death and diminishing plant health [13]. In the European Union, regulatory standards exist for Clopyralid application, permitting dosages of up to 127 g/hectare for cereal crops and up to 300 g/hectare for rapeseed and sugar beet [14]. The selective effects of herbicides across different plant categories, such as monocots and dicots, influence their accumulation profiles in soil and how they may percolate to surface water and groundwater [15,16].

Understanding the uptake mechanisms of herbicides by plant roots is critical due to the potential for bioaccumulation. Various metrics, including the bioconcentration factor (BCF), root concentration factor (RCF), and translocation factor (TF), are utilized to assess the degree of herbicide accumulation in plants (Table S1). Compounds are deemed bioaccumulative when their concentration factor exceeds 5000 L/kg, indicating significant ecological risks [17–21].

Despite extensive research on the uptake and accumulation of organic pollutants in edible vegetables, there is a notable gap in studies focused on medicinal plants. This lack of information prompts the need for investigation into the bioaccumulation and translocation of specific herbicides within medicinal species. This study specifically focuses on the herbicides Clopyralid and Picloram due to their persistence in soils and potential risks to human health. To fill this research gap, we selected basil—a common medicinal plant in Romania known for its therapeutic and culinary uses—because of its resilience across various soil conditions and susceptibility to diffuse pollution.

The primary objectives of this research are: (a) to develop and optimize an analytical method for identifying and quantifying synthetic auxins in soil and vegetation samples; (b) to monitor the concentration dynamics of these synthetic auxins over time in soil and basil plants; and (c) to establish the bioaccumulation and translocation factors of these organic compounds in basil.

Through this investigation, we aim to provide clearer insights into the environmental and health implications of synthetic auxin usage in agriculture, particularly regarding their effects on medicinal plants that are commonly consumed or used for therapeutic purposes. Understanding these dynamics will contribute to a more comprehensive assessment of the sustainability and safety of herbicide applications in agricultural systems.

2. Materials and Methods

2.1. Chemicals and Reagents

Two of the most well-known synthetic auxins were chosen to evaluate the transfer of synthetic auxins between the soil and the plant: Clopyralid and Picloram. The molecular structure and physico-chemical properties of the two organic compounds are presented in Table S3. Analytical purity standards (≥98%) were purchased from Sigma Aldrich (Darmstadt, Germany). The solvents and reagents used for the preparation of the mobile phase and the preparation of the standard working solutions, namely acetonitrile (HPLC grade), glacial acetic acid, and methanol (HPLC grade) were also provided by Sigma Aldrich (Darmstadt, Germany). Ultra-purified water was prepared in-house, using a Merck milli-Q ultrapure water system (Merck Millipore, Burlington, MA, USA).

2.2. Plant Material and Growth

Basil was chosen as the vegetable component, an aromatic plant common in Romania. The basil was purchased from a local producer and distributor of aromatic plants. A universal substrate composed of peat and humus (without auxin content) was purchased for growing garden and balcony plants. According to the data provided by the manufacturer, the substrate is enriched with nutrients and has the following characteristics: pH-value 5.0–7.0, total nitrogen 1.9%, total potassium 0.5%, total phosphate 0.9%, conductivity of 1.2 μS/cm, and organic matter 35 weight % (dry). The substrate used in the experiment was manually cleaned of solid materials and left in the air for 24 h.

2.3. Experimental Procedure Conditions

Three experiments (performed in triplicate) were carried out in parallel: Basil-Clopyralid, Basil-Picloram, and Basil-Mix (Clopyralid and Picloram). For each experiment, 500 g of soil was weighed, with an initial humidity of approximately 45%. Soil contamination with auxin solutions was carried out in layers, using a volume of 250 mL of water, the final auxin concentration in the soil being 100 μg/kg d.w. In each pot, seven individual strands of basil were planted. In addition, the same number of Basil threads were also planted in pots with uncontaminated substrates with the tested solutions. Moreover, to observe the behavior of the two organic compounds in the absence of plants, three pots were prepared with a substrate containing: Clopyralid solution, Picloram solution, and Mix solution, the concentration being also 100 μg/kg. Plants (test and control) and substrate taken both from the root of the sampled plant and from the control pot were taken immediately after planting (0), and at day 1, 5, 8, 13, and 15 after the experiment began.

2.4. LC-MS/MS Instrument

Samples were analyzed using an Agilent 1260 liquid chromatograph, while for the analyte detection, an Agilent 6410B triple quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany) was used. The electrospray ionization source (ESI) was set up in positive mode. A Zorbax Eclipse type C18 chromatographic column (2.1 × 100 mm, 3.5 μm) was used to separate Picloram and Clopyralid. The mobile phase used was composed of 0.1% acetic acid (A) and acetonitrile (B), with a flow rate of 0.2 mL/min and elution in isocratic mode (10%B). The chromatographic column temperature was set at 20 °C. The injection volume was 5 μL. Analysis was performed in positive mode, using two MRM (multiple reaction monitoring) transitions for each compound, namely: for clopyralid, the precursor ion m/z 194.0 → product ions m/z 148.0 and m/z 146 (Collision energy, CE = 24 V; Fragmentor voltage, FV = 85V; and Cell accelerated voltage, CAV = 6/5 V), while for picloram, m/z 243 → m/z 197, and m/z 241 → m/z 195 (CE = 20 V; FV = 100/95 V; and CAV = 3 V). Product ions 146 and 197, respectively, were used as quantifier ions. The dwell time was set at 250 ms for each MRM transition. The ionization parameters were as follows: gas temperature 300 °C, gas flow 8 L/min, nebulizer 40 psi, and capillary voltage (CV) 4000 V. The chromatographic run time was 8 min (Figure S1).

2.5. Extraction of Synthetic Auxins from Soil and Plants

The soil and the plants (divided in advance into roots, stems, and leaves) were dried by lyophilization at −110 °C for 48 h. After drying, they were mortared, homogenized, and kept dry until pretreatment. The ultrasound-assisted extraction (EAU) process was chosen as the extraction method. In 20 mL vials, 0.5 g of each sample (soil/root/stem/leaves) was weighed. An amount of 10 mL of methanol was added to the samples and then they were ultrasonicated for 30 min at 30 °C. The organic extract was purified on silica gel, and the remaining solid was again subjected to ultrasound-assisted extraction. The organic extracts

were combined and evaporated almost dry, on a water bath, at 50 °C, using a weak stream of nitrogen. The residues were resuspended in water and centrifuged at 10,000 rpm for 10 min to separate the formed precipitate. An aliquot of 0.400 mL was transferred to a vial and kept at 4 °C until analysis by LC-MS/MS.

2.6. QA&QC

All analyses have been performed in triplicate. For each batch sample, a blank and a standard solution were used. The intra-day and inter-day precision was evaluated for both soil and vegetal samples. The RSD values for intra-day precision for clopyralid were 3.23% for soil and 4.43% for vegetal samples, while for inter-day precision, the RSD values were 8.42% and 9.22% for soil and vegetal samples, respectively. For Picloram, the intra-day and inter-day RSD values were 4.18% and 5.28% for soil and 8.96% and 9.05% for vegetal samples. The recovery values after the UAE procedure were 88% and 84% for Clopyralid in soil and vegetal samples, and 80% and 77% for Picloram in soil and vegetal samples, respectively. The quantification limits (LOQ) were measured as 0.13 ng/g and 0.22 ng/g for Clopyralid in soil and vegetal samples, and 0.51 ng/g and 0.74 ng/g for Picloram in soil and vegetal samples. The matrix effects were evaluated to 91% and 95% for Clopyralid in soil and vegetable samples and 84% and 91% for Picloram in soil and vegetal samples (Table S6). Linearity was confirmed from 0.1 to 100 µg/L (0.2–200 µg/kg), the obtained correlation coefficients being 0.998 for Clopyralid and 0.990 for Picloram (Figure S7).

2.7. Bioconcentration and Translocation Factors

The bioconcentration factor (*BCF*) is an essential quantitative parameter in assessing environmental risks because it provides information related to the ability of a contaminant to be absorbed by plants. *BCF* varies according to many factors, such as plant species, diffusion through its membranes, hydrophobicity, and others [18]. The ratio between the contaminant concentration in an organism and the contaminant concentration in the environment is called the bioconcentration factor (*BCF*). The measurements of the concentrations in the plant tissues and the concentrations in the soil will lead to the determination of the plant–soil BCF, calculated with Equation (1) [22–24]:

$$BCF = \frac{Cplant}{Csoil}, \quad (1)$$

where *Cplant* is the organic contaminant concentration determined in the plant tissues, and *Csoil* is the concentration determined in the soil (ideally at equilibrium, but practically at harvest).

Root concentration factor (*RCF*) is the ratio between the chemical concentration determined in the root (*Croot*) and the soil (*Csoil*) surrounding the root. These values are usually used to evaluate the bioaccumulation abilities of organic compounds by plant roots [25]. The *RCF* calculation was made using Equation (2):

$$RCF = \frac{Croot}{Csoil}. \quad (2)$$

TF represents the determination of the translocation capacity of organic compounds from the root to the stem or from the stem to the leaves In addition to the uptake of pollutants by the root, plants can transport them to the above-ground tissues. Thus, TFs-r (the ratio between the chemical concentration in the stem and that in the root) and TFl-s (the ratio between the chemical concentration in the leaf and that in the stem) were determined, which represent the relative transport of pollutants from the root to the stem and, respectively, from the stem to the leaves [25].

3. Results

3.1. Optimization of LC-MS/MS Conditions

Current analytical practices for the detection of these compounds rely on chromatographic methods such as gas chromatography coupled with mass spectrometry (GC/MS or GC/MSD) and liquid chromatography in tandem with mass spectrometry (LC-MS/MS) [26,27]. As summarized in Table S2, these methods have been applied to a wide range of matrices, including food, feed, soil, water, and air. However, despite the availability of these techniques, challenges remain in detecting low concentrations of these herbicides, especially in complex matrices like soil and plant tissues, where matrix interferences can compromise sensitivity and accuracy. Moreover, existing methods are often matrix-specific or optimized for single-compound detection, limiting their applicability in comprehensive residue monitoring programs.

Therefore, there is a clear need to develop and validate a robust, highly sensitive, and selective method for the simultaneous quantification of picloram and clopyralid in environmental samples and edible plants. Such a method would not only enhance monitoring capabilities but also contribute to risk assessment efforts and regulatory compliance in the context of food safety and environmental protection.

To separate the two auxins, due to the pore retention of Clopyralid, a high percentage of the aqueous component was used, the ratio of the components in the mobile phase was 90% water containing 0.01% acetic acid (A): 10% acetonitrile (B). The flow rate was set to 0.2 mL/min, and the elution occurred in isocratic mode. At the same time, to increase the retention, the chromatographic column was thermostated at a temperature of 20 °C, and the injection volume was set at 5 µL.

Through the established liquid chromatographic method, the separation of the analytes, at least at the baseline, is attempted, which will allow the setting of the MRM transitions for the quantification of the analytes on individual segments of time. In conclusion, there is a significant increase in the sensitivity of the method. The acquisition of the triple quadrupole detector was set in MRM mode, using two transitions each (Picloram m/z 192→146, m/z 194→148; Clopyralid m/z 243→197, m/z 241→195). After establishing the individual time segments, the dwell time parameter was set to 250 msec, generating a lower noise and implicitly a higher signal/noise ratio, and the optimized conditions of the chromatographic parameters allowed the elution of the two analytes in less than 8 min (Figure S1 and Table S4).

During the development of the LC-MS/MS method, an attempt was made to obtain the lowest detection and quantification limits that would allow the determination of low levels (below ng/g) at which these compounds should be identified in plant components. Thus, an analyte mixture solution with a concentration of 10 mg/L and an injection volume of 5 µL was used. Further, all the detection parameters related to the MS detector (QQQ) were optimized—fragmentor voltage, collision energy (CE), acceleration voltage in the collision cell, quadrupole resolution (MS1, MS2 Res), acquisition time per MRM transition (dwell time), and the voltage on the capillary. Once the mass spectrometric detection parameters were changed, their effect on the peak area and the signal/noise ratio (S/N) was observed. The values of the MS parameters that generated the best sensitivity were chosen. The fragmentor voltage values varied between 70 and 130 volts for Clopyralid and between 80 and 105 volts for Picloram. The collision energy varied between 22 and 40 V for Clopyralid and 10–30 V for Picloram. The optimal values are presented in Figure S3. The following optimized parameters were the acceleration voltage in the collision cell, the capillary voltage, and the nebulizer's pressure. The acceleration voltage in the collision cell varied between 3000 and 4500 V (Figure S4). The voltage on the capillary was varied between 3 and 6 for Clopyralid and between 1 and 4 V for Picloram (Figure S5) and the

pressure on the nebulizer was between 30 and 50 psi (Figure S6). The drying gas flow rate was 8 mL/min, and the drying temperature was maintained at 300 °C. The values of the MS parameters that generated maximum sensitivity are presented in Table S5.

3.2. Optimization of UEA Procedure

The optimization of the extraction parameters involved changing the amount of sample used, testing methanol and acetonitrile as solvents for the extraction of the two auxins, the amount of solvent used, and the temperature and the ultrasonication time. The tests were carried out on soil and vegetation samples without auxin content, contaminated in a controlled manner with a concentration of 50 µg/kg. To evaluate the optimal amount of sample, aliquots of 0.1, 0.25, 0.5, 0.75, and 1 g were analyzed. The results showed that above the amount of 0.5 g, the extraction yield does not change considerably (Table S7). To test the extraction solvent, quantities of 0.5 g of soil and vegetation were subjected to the UAE procedure using 10 mL each of methanol and acetonitrile. It was observed that methanol is a much more suitable solvent for the extraction of the target auxins from the two solid matrices (Table S8). The next step involved testing the volume of methanol used in the preceding UAE (5, 10, and 15 mL), the volume of 10 mL being sufficient to obtain maximum extraction yields (Table S9). The temperature of the water in the ultrasound bath was also tested (25, 30, and 35 °C), with 30 °C being the optimal value, favoring the extraction of the two compounds (Table S10). The ultrasonication time was tested between 10 and 40 min, and the results indicate that 30 min is sufficient to obtain maximum recovery yields (Table S11).

3.3. The Uptake of Clopyralid and Picloram in Plant Organs

Throughout the entire study, the concentrations of the organic compounds Clopyralid and Picloram were monitored in the control samples (soil samples and plants without auxin content) and the test samples (soil samples and plants contaminated with auxins). During the experiment, no visible morphological effects were observed in the basil plants. Upon harvest, evaluations revealed no significant differences in leaf shape, color, turgidity, or overall appearance between the treated and control groups. Measurements taken at harvest further indicated no substantial differences in size or development between the treated and untreated plants. These findings suggest that exposure to the herbicides under specific experimental conditions did not have a measurable impact on the vegetative growth of basil within the 15-day assessment period [4].

In the control samples, the presence of the studied organic compounds was not identified, the results being represented in Figure S8. The stability of Picloram and Clopyralid in the control soil samples was monitored, and their good stability over time was observed (Figure 1). Their concentration varied within the range of analytical errors, remaining almost constant throughout the 15-day test. Thus, the probability of abiotic degradation during the experiments was removed.

In the substrate of the test experiments, a more pronounced decrease in the concentration of Clopyralid compared to that of Picloram was observed, both in the individual and in the mixture tests (Figure 2). In the individual experiment, the concentration of Clopyralid decreases slowly in the first eight days, after which it reaches 56 µg/kg d.w. at the end of the experiment. In the soil where the two auxins are used in the mixture, the decrease in the concentration of Clopyralid starts to be observed after five days and continues to decrease until it reaches a value of 50 µg/kg d.w. at the end of the test. Regarding the behavior of Picloram in the test samples, a much slower decrease in its concentration was observed in both experiments. In the individual test, the concentration of Picloram reaches

a value of 87.6 µg/kg d.w., while in the Picloram–Clopyralid test, it reaches a value of 89.1 µg/kg d.w.

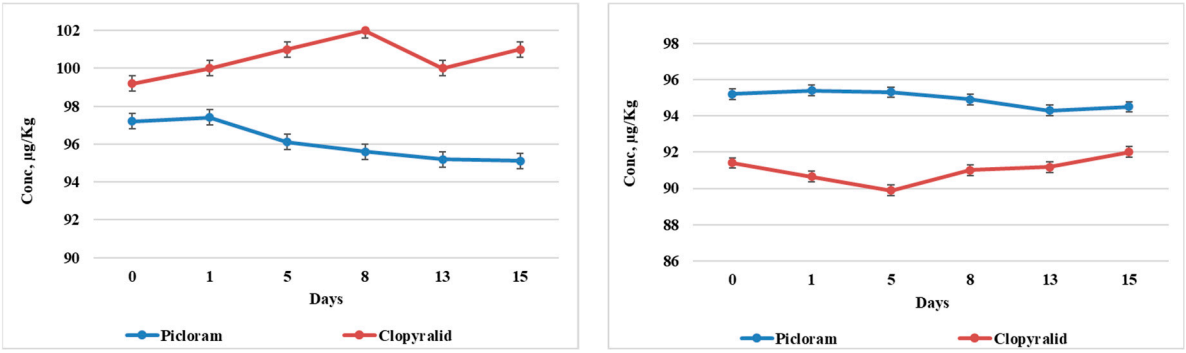

Figure 1. Stability of the organic compounds Picloram and Clopyralid individually (**a**) or in mixture (**b**) in control samples (soil without plants). Values are mean ± SE (n = 3).

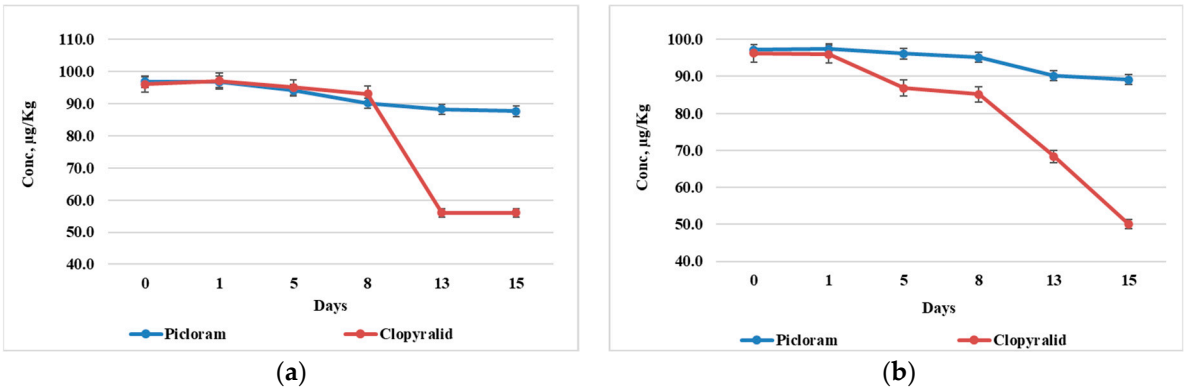

Figure 2. Variation in time of concentration of Picloram and Clopyralid in individual experiments (**a**) and used in a mixture (**b**). Values are mean ± SE (n = 3).

In individual experiments, both compounds could be identified and quantified in all plant organs except Picloram in basil leaves (Figure 3). The presence of Clopyralid in the root was observed from the fifth day, and its concentration increased to a value of 14 µg/kg d.w. Clopyralid was also detected in the basil stem, with its concentration constantly increasing throughout the experiment, with a maximum on the 15th day of 8 µg/kg d.w. The highest concentration of Clopyralid was accumulated in the leaves, reaching up to 16 µg/kg d.w. Regarding Picloram, its presence was observed in the root, at a value of up to 6 µg/kg d.w. In the basil stem, the concentration of Picloram could only be determined at the last sampling, having a value of only 2 µg/kg d.w. In the leaves, Picloram was not determined in any of the samples.

In the experiment in which the simultaneous transfer of the two synthetic auxins between the soil and the plant was monitored, a more significant accumulation in the plant organs of Clopyralid compared to Picloram was again observed (Figure 4). The presence of Clopyralid was observed, starting with the fifth day in all the organs of the plant. In the roots, the minimum concentration found was 5 µg/kg d.w., with a constant increase until the 13th day, when a maximum of 16 µg/kg d.w. was reached; at the end of the test, the recorded concentration was 12 µg/kg d.w. In the case of the Clopyralid concentration recorded for the stem and leaves, they increased throughout the experiment. The values determined in these organs of the plant at the end of the test were 10 µg/kg d.w. in the stem and 22 µg/kg d.w. in the leaves. Moreover, in this case, the highest accumulated concentration of the organic compound was in the leaves. Picloram could only be determined

in the roots after the eighth day of the test. The concentration increased over time, reaching 7.2 µg/kg d.w.

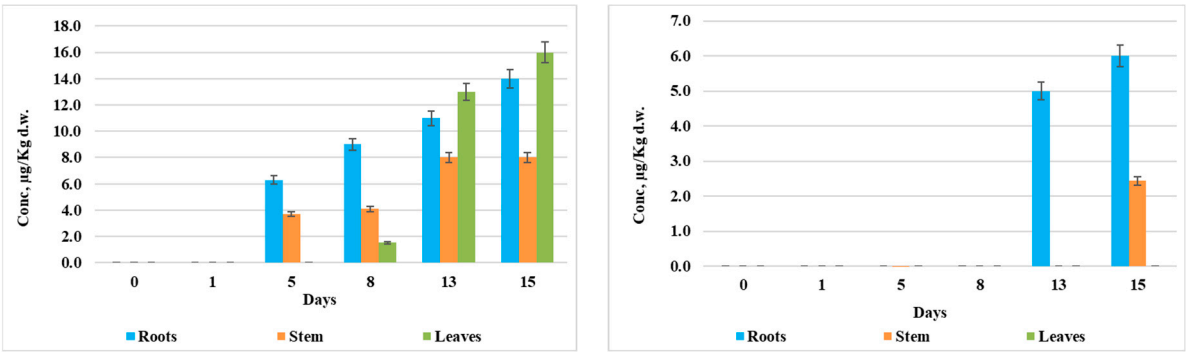

Figure 3. The presence of Clopyralid (**a**) and Picloram (**b**) in the plant organs in individual studies. Values are mean ± SE (n = 3).

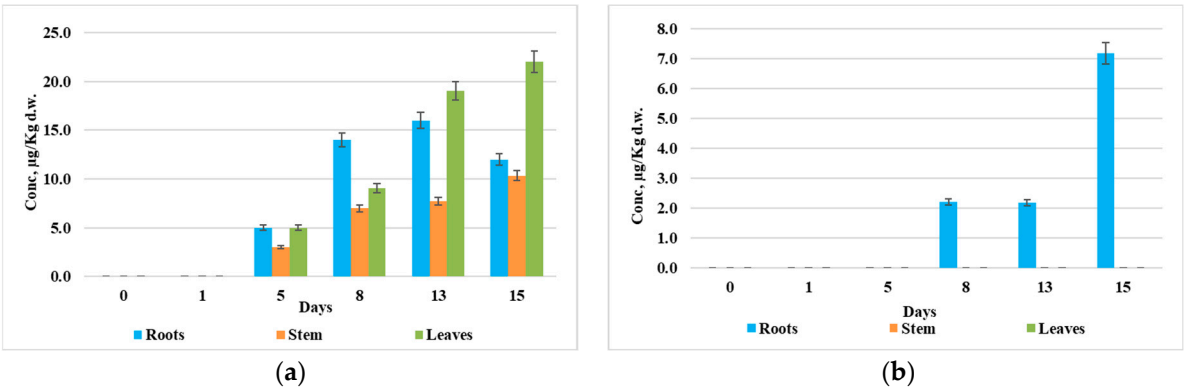

Figure 4. The presence of Clopyralid (**a**) and Picloram (**b**) in the plant organs in the test experiment was carried out in a mixture of synthetic auxins. Values are mean ± SE (n = 3).

By comparing the results obtained for the individual tests with those of the experiments carried out in the mixture, it can be seen that the presence of Picloram favors the accumulation of Clopyralid in a higher percentage of the plant organs.

3.4. Distribution of Synthetic Auxins in Plant Organs

According to the data presented in Figure S9a, in the individual experiment, Clopyralid accumulates in the roots starting with day 5, at a percentage of 63%. Clopyralid migrates from the roots through the stem to the leaves, and at the end of the experiment, the distribution among the plant organs is 36.8% in the roots, 21.1% in the stem, and 42.1% in the leaves.

The distribution of Clopyralid in the tests carried out in the presence of the synthetic auxin mixture does not register significant changes, being uniform throughout the experiment (Figure S9b). At the end of the test, the distribution between the plant organs is 27.27% in the roots, 22.72% in the stem, and 50% in the leaves. In both experiments, the highest concentrations of Clopyralid were determined in the leaves at the end of the investigation. In contrast, Picloram was mainly determined in the root, the migration in the basil stem being observed only in the experiment in which it was the only organic compound added (Figure S10a). In the presence of Clopyralid, Picloram was identified at a percentage of 100% in the root (Figure S10b).

3.5. Bioconcentration and Translocation Capacity of Organic Compounds to Plants

BCF and TF were further employed to evaluate the ability of basil plants to accumulate and translocate both synthetic auxins (Figure 5). The BCF values determined for Clopyralid increased from 0.11 to 0.68 in the individual experiment, and from 0.26 to 0.89 in the mix test (Figure 5a). For Picloram, the BCF values were lower than for Clopyralid, ranging between 0.06 and 0.1 in the individual test and from 0.02 to 0.08 in the mix experiment (Figure 5a). A similar trend was observed for the bioconcentration factors determined in the roots; values increased along the experiment period (Figure 5b). The only exception was observed for RCF of Clopyralid in the mix experiment, where the value determined at the end of the period was lower than the precedent sample. All RCF values were below the reference value of 1.

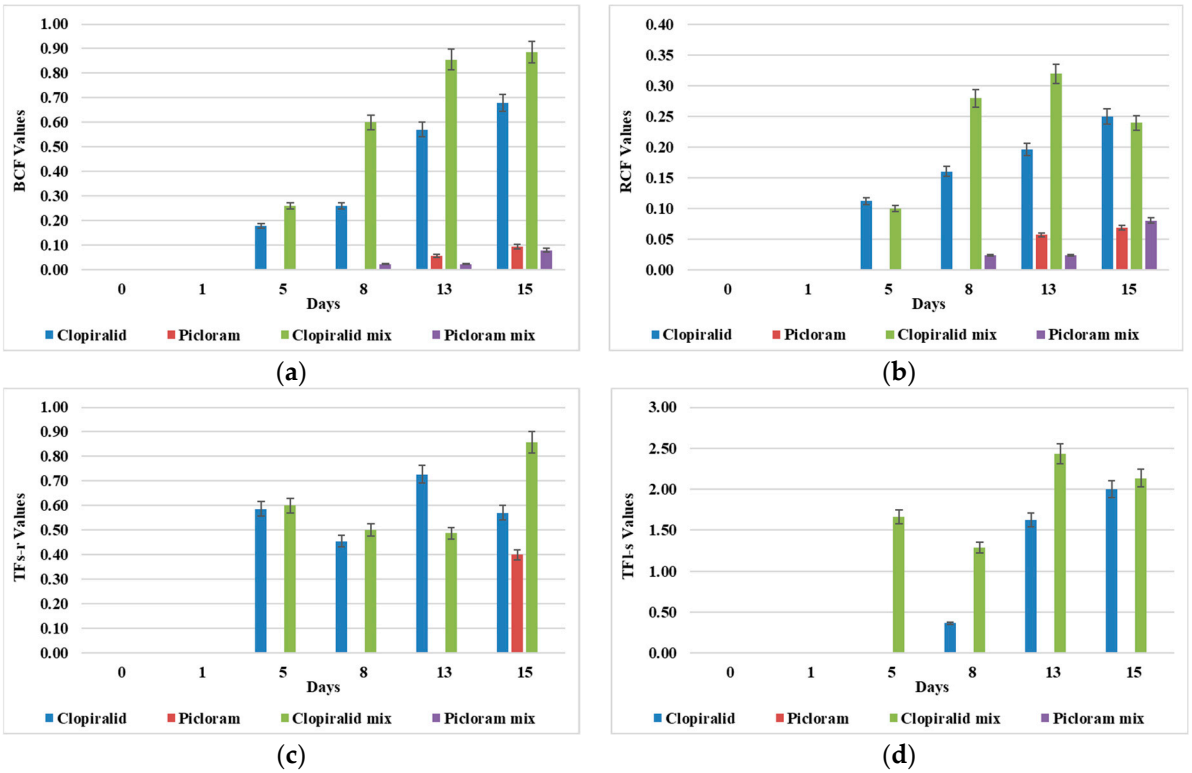

Figure 5. Effects of Clopyralid and Picloram treatments on (**a**) bioconcentration factor in throughout plant (BCF), (**b**) bioconcentration factor in roots (RCF), (**c**) translocation factor from root to stem (TFs-r), and (**d**) translocation factor from stem to leaf (TFl-s). Values are mean ± SE (n = 3).

The translocation factors from roots to stems varied in a random order for Clopyralid, while for Picloram, the only value could be calculated at the end of the experiments (Figure 5c). A similar pattern was also observed for the translocation factors from stem to leaf (Figure 5d). The final values of the BCF are between 0.68 and 0.89 for Clopyralid and between 0.08 and 0.1 for Picloram. Also, the final values of the RCF are in the range of 0.24–0.25 for Clopyralid and between 0.07 and 0.08 for Picloram (Table 1). Values lower than 1 for the BCF and RCF indicate that basil is an excluder, so it has no potential for phytoremediation [28].

Table 1. Final bioaccumulation and translocation factors in plant organs.

Test Samples	BCF	RCF	TFs-r	TFl-s
Clopyralid	0.68 ± 0.022	0.25 ± 0.008	0.57 ± 0.018	2.02 ± 0.064
Picloram	0.11 ± 0.003	0.07 ± 0.002	0.41 ± 0.013	-
Clopyralid—Mix	0.89 ± 0.028	0.24 ± 0.008	0.86 ± 0.027	2.13 ± 0.068
Picloram—Mix	0.08 ± 0.003	0.08 ± 0.003	-	-

However, it is important to note that clopyralid showed a tendency to accumulate preferentially in leaves, indicating partial upward translocation. This behavior raises potential food safety concerns, as leaves are the primary edible parts of basil. This dual role—being ineffective for phytoremediation yet capable of accumulating certain herbicides in edible tissues—warrants further attention in the context of herbicide residue risk in food crops. The differing behaviors of clopyralid and picloram may be explained by variations in their physicochemical properties and their interaction with plant transport systems. Clopyralid, a more mobile and water-soluble compound, may be more readily absorbed and translocated via xylem pathways, while picloram's lower translocation could be due to stronger root binding or limited mobility within plant tissues. These differences are consistent with known patterns of auxin-like herbicide transport, which can involve specific carrier-mediated mechanisms and passive movement depending on the compound's structure and ionization state. In particular, studies have shown that clopyralid is rapidly taken up by plant roots and accumulates in tissues through passive diffusion, influenced by pH-dependent ion trapping mechanisms. This accumulation behavior supports its potential for translocation to aerial parts of the plant, including leaves [29].

The final TFs-r values determined for Clopyralid were 0.57 in the case of the experiment in which it was the only contaminant and 0.86 in the case of the experiment in which it was used simultaneously with Picloram. For Picloram, the final TFs-r values were 0.41 in the case of the experiment in which it was the only contaminant. All TFs-r values were lower than 1, suggesting the ineffective translocation of Clopyralid and Picloram from the root to the stem and the tendency to accumulate in the root [30]. The final TFl-s values determined for Clopyralid were 2.00 and 2.13, respectively, when used individually and in the mixture. In the current experiments, basil showed a preferential accumulation of clopyralid in the leaves rather than the stem, the TFl-s value being more significant than 1.

4. Discussion

The mobile phase composition plays an important role in selectivity, peak shape, and retention time in liquid chromatographic separations in LC-MS/MS analysis. In this study, 90% water containing 0.01% acetic acid and 10% acetonitrile was used as a mobile phase for clopyralid and Picloram separation, and there were no interfering peaks. The chromatographic run time was less than 8 min. For MS detection, the precursor ion and the two most abundant product ions were chosen to establish MRM transitions for Clopyralid and Picloram LC-MS/MS analysis. To achieve high sensitivity, the MS parameters were optimized for each specific transition. The suitable MS conditions for Clopyralid and Picloram are shown in Table S5. The developed and validated LC-MS/MS method was used to quantify both target synthetic auxins from soil and plant tissue, in concentrations up to 0.13 and 0.22 ng/g for Clopyralid and up to 0.51 and 0.74 ng/g for Picloram.

Synthetic auxins are some of the most widely used herbicides all over the world [31]. Clopyralid and Picloram are effective at controlling broadleaf weeds in various plant crops. They selectively target broadleaf weeds while having minimal impact on grasses [32]. Both target auxins can have residual effects in the soil, meaning they can persist and remain active for a certain period after application. This can affect subsequent crops planted in the treated area [33]. As it was well documented, most plants are not damaged by clopyralid

or picloram. However, plants in the sunflower family (Compositae), the potato family (Solanaceae), the bean family (Leguminosae), and the sugar beet crop are very sensitive to these herbicides. Clopyralid can restrict clover, tomato, pea, lentil, lettuce, bean plants, sunflower, and pepper at extremely low concentrations in soil (up to 10 pg/kg). The susceptibility of these crops can vary, and certain crops may exhibit phytotoxic symptoms such as stunted growth, leaf curling, chlorosis, or necrosis [34,35]. In the present study, no injury was observed in the basil plant with the application of clopyralid and/or picloram auxins.

To evaluate and find possible explanations regarding the auxin's uptake and translocation in plant organs, it is important to take a look at the basic parameters of active molecules, namely: water solubility, pKa-value, octanol–water partition coefficient (log Kow), and binding to soil particles (log Koc) [36]. Based on the log Koc values, both auxin compounds are expected to have very high mobility (1.59 for clopyralid and 1.26 for picloram). The pKa values of clopyralid and picloram are almost identical (2.32 and 2.3, respectively), indicating that these compounds will exist almost entirely as anions in the environment and anions generally do not adsorb stronger in the soil.

The bioaccumulation of Clopyralid and Picloram in plant organs can vary depending on several factors such as plant species, application rates, soil type, and environmental conditions. Generally, research indicates that these herbicides have the potential to accumulate in certain plant tissues, such as roots, stems, leaves, and fruits. A study conducted in 2021 investigated the distribution of Clopyralid in lawns [37]. The study found that target auxin accumulated mainly in the foliage of treated plants, with limited translocation to other plant tissues. Another study examined the potential of bioaccumulation of clopyralid in different plant species. The study observed varying levels of bioaccumulation in different plant organs, with higher concentrations found in roots compared to other plant organs [38,39]. The bioconcentration factors determined in the basil plant were lower than 1, suggesting that both Clopyralid and Picloram do not tend to accumulate or concentrate in the body plant. This is generally considered a desirable characteristic as it indicates a lower potential for the auxin molecules to cause adverse effects on the plant in terms of bioaccumulation. RCF values lower than 1 could indicate a lower tendency for the auxin's accumulation in roots, and the potential for transformation or degradation of the target compounds. Correlating the RCF values with the translocation coefficients, a higher tendency for the organic molecules to migrate from roots to stem and to the leaf. The steam-to-leaf translocation values higher than 1 sustained the above affirmation.

Our data revealed that clopyralid was more quickly taken up and had higher translocation to both roots and shoots than picloram. Although clopyralid and picloram have very similar parameters, such as log Kow and pKa values, the differences in uptake and translocation were significant, behavior was very difficult to explain. A similar case was also reported in a study regarding the absorption and translocation of Clopyralid and aminopyralid in Canada Thistle (Cirsium arvense) [40]. Picloram showed contrasting absorption and translocation patterns in Canada thistle, also compared with clopyralid, in which only 12% of applied picloram was absorbed and only 2.2% was translocated [41]. Kleier's mathematical model claims that both compounds have limited translocation, based on intermediates' permeability and weak theories [42]. The results discussed in the model claim that the log Kow values for these molecules are too low and the pKa to acidic to balance for low membrane permeability. In these circumstances, clopyralid's level of uptake and translocation is possibly uncommonly high due to its log Kow and pKa values. Future studies regarding the biological activity of both auxin herbicides may help elucidate these differences, including identifying the place of action for clopyralid and picloram and determining the binding kinetics of the two auxins.

The concentrations used in this study were intentionally high compared to typical agricultural applications. This was performed to simulate a worst-case exposure scenario and to assess the behavior of these herbicides in plant tissues within a short experimental timeframe. While such concentrations are unlikely to occur under standard agricultural practices, the potential for accumulation in edible plant parts—even at high doses—does underline the importance of continued monitoring from a food safety perspective [33].

In this context, the absence of visible phytotoxic effects under elevated concentrations suggests that basil may tolerate short-term exposure to clopyralid and picloram without visible impairment. However, due to the observed accumulation of clopyralid in edible tissues, it is recommended that producers remain cautious when using herbicides near aromatic or leafy edible plants. Adhering strictly to approved application rates and observing appropriate pre-harvest intervals can help mitigate any potential risk.

5. Conclusions

In this study, the identification of synthetic auxins from soil and vegetation samples was pursued. From an experimental point of view, three experiments were carried out, which consisted of exposing an aromatic plant (basil) to a known concentration of synthetic auxins (Clopyralid, Picloram, and a binary mixture) in the soil (100 μg/L), for 15 days. It was observed that basil was able to accumulate approximately half of the amount of Clopyralid existing in the soil. In contrast, the amount of Picloram determined was less than 10 μg/kg. Clopyralid was determined in all plant tissues, while Picloram was primarily determined in the root and only in one sample, in the stem.

By calculating the bioconcentration factors, the determined values were lower than 1, which means neither of the two compounds is bioaccumulative. The values of the concentration factor in the roots were also less than 1 in all cases. The translocation capacity of organic compounds from root to stem was calculated, and values lower than 1 were obtained, suggesting inefficient translocation of Clopyralid and Picloram from root to stem and the tendency to accumulate in the root. In the current experiments, basil showed a preferential accumulation of Clopyralid in the leaves rather than in the stem; the value of its translocation capacity from the stem to the leaves was greater than 1.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments12050144/s1, Table S1: Representative indices of the transfer processes of contaminants from the soil to the root and then to the plant parts, stem and leaves; Table S2: Chromatographic techniques used in the detection of Picloram and Clopyralid in food, water, soil and air; Table S3: Chemical properties of Clopyralid and Picloram; Figure S1: MRM chromatogram corresponding to a concentration of 1 mg/L; Table S4: The acquisition windows set to increase the sensitivity of the method; Figure S2: The variation in the peak areas for Clopyralid and Picloram during the fragmetor voltage optimization; Figure S3: The variation in the peak areas during collision energy optimization; Figure S4: The variation in the peak area during the acceleration voltage optimization in the collision cell; Figure S5: The variation in the peak area of the analytes during the optimization of the voltage on the capillary; Figure S6: The variation in the peak area of the analytes during the optimization of the pressure on the nebulizer; Table S5: MRM transitions and optimal values of MS parameters for the simultaneous detection of Picloram and Clopyralid; Figure S7: Calibration curves obtained for Clopyralid and Picloram; Table S6: Performance parameters obtained for the determination of Picloram and Clopyralid in soil and plant; Table S7: Recoveries (% ± RSD) measured during the samples amount optimization; Table S8: Recoveries (% ± RSD) measured using methanol and acetonitrile as extraction solvent; Table S9: Recoveries (% ± RSD) measured using different volumes of methanol; Table S10: Recoveries (% ± RSD) measured using different water temperatures of the ultrasonication bath; Table S11: Recoveries (% ± RSD) measured using different sonication times; Figure S8: MRM chromatograms recorded for: a. Clopyralid and picloram mixture

standard solution 100 µg/L and samples without auxin content; b. soil; c. roots; d. stem; e. leaves; Figure S9: The distribution of Clopyralid among the plant components in an individual experiment (a) and in the mixture (b); Figure S10. The distribution of Picloram among the plant components in an individual experiment (a) and in the mixture (b).

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