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Leaching of Glyphosate and AMPA from Field Lysimeters

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Abstract: Leaching of glyphosate and AMPA as affected by the time elapsed between the spraying and first leaching event was studied on large-scale lysimeters in a two-year study. The leaching events were induced by irrigation interventions able to deliver 336 L, equivalent to a rainfall of 40 mm. Four groups of three lysimeters were randomly selected between the 12 lysimeters available. They were irrigated on either one day after herbicide treatment (1 DAT), 7 DAT, 14 DAT or 28 DAT. The same group of lysimeters were irrigated a second time 14 days after the first irrigation, corresponding to a period of time of 15 DAT (1 + 14), 21 DAT (7 + 14), 28 DAT (14 + 14) and 42 DAT (28 + 14). In both years, lysimeters were sprayed with glyphosate (360 L ha⁻¹) at a rate of 12 L ha⁻¹, the maximum field rate allowed on the label. Our results pointed out that the leaching of glyphosate and AMPA is effectively event-driven and highlighted the importance of the first rainfall event in moving glyphosate through the soil, increasing the potential risk of water contamination. Overall, both chemicals showed a risk of water contamination. Glyphosate may persist more than usually considered, and its residues were found in leached waters from lysimeters treated 30 days before the leaching event. Other factors may affect the movement of these two compounds through the soil profile after spraying: temperature pattern and soil moisture. Finally, the results of this study refer to a very high application rate of glyphosate. Hence, at lower field rates, observed concentrations can likely be minor.



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1. Introduction

The concern about the environmental and health effects of glyphosate and AMPA arising in the last years is likely related to the increased use of the parent compound due to the introduction of genetically modified glyphosate-resistant crops and to the possibility to use the herbicide in pre-harvest [1,2].

Glyphosate is considered a non-mobile herbicide, being well-retained by clay particles, organic matter and iron hydroxides, save in light soil, where preferential flows may occur. The transportation of glyphosate is also facilitated by a water soluble colloidal fraction [1]. AMPA is extremely more mobile than the parent compound [1,2]. Starting from spraying, glyphosate undergoes microbial degradation through two pathways: the formation of sarcosine and glycine and the formation of AMPA [3]. The mobility of both chemicals in the soil depends on their persistence and their rate of adsorption [4].

Glyphosate average soil half-life is generally less than 30 days [5,6]; however, different soil characteristics may greatly affect its soil residence time [1,7,8]. Chemical or physical degradation is considered negligible [1,9]. The movement of glyphosate and AMPA in the soil is also affected by the presence of iron-oxides, which may retain both chemicals in the soil matrix diminishing their transport through the soil profile [10]. The risk of leaching of both glyphosate and AMPA is affected by soil characteristics. Soil characteristics affect the residence time of the chemicals in the soil, hence their proneness to be degraded or transported along the soil profile [11]. For instance, on non-structured soils without

macropores, leaching is limited. However, glyphosate leaching might be expected in coarse and oxide-poor soils, as well as on soils with a low glyphosate sorption capacity [1,3]. Furthermore, the adsorption of glyphosate on soil particles can be affected by the presence of phosphate, which competes for sorption sites [9], even though this phenomenon may be limited to a few soils [12]. Both chemicals have a low leaching potential according to the GUS index, but AMPA, compared to glyphosate, has a higher water solubility, being 140-fold more soluble in water than glyphosate [5].

Glyphosate and AMPA, as other commonly used pesticides, may move into the water through many different phenomena such as runoff, leaching and drift [13]. Despite residues of glyphosate and AMPA in surface waters being widely reported worldwide [14–16], their presence in groundwaters is significantly lower. For instance, in Italy, AMPA and glyphosate residues were detected in 43% and 66% of the surface-water monitoring points, respectively (22% and 52% of them exceeded the law limit), but only in 5% and 8% in groundwaters, respectively (about 2% of them exceeded the law limit). Similar data are reported in France [14]. Other studies reported the presence of glyphosate and AMPA in groundwaters. Rendon-Von Osten and Dzul-Caamal [17] monitored the presence of glyphosate in groundwaters in the agricultural areas of Yucatan, Mexico. They found glyphosate in all monitored wells, with concentrations up to $1.41 \mu\text{g L}^{-1}$. However, there is no indication about the depth of monitored wells.

The presence of glyphosate and AMPA in groundwaters has also been reported by Scribner et al. [18] in a monitoring study carried out in the USA. Van Stempvoort et al. [3], based on previous studies and their findings, postulated that glyphosate migration to deep groundwaters is limited due to microbial degradation and sorption processes. In an agricultural area of India characterized by years of glyphosate application, shallow groundwaters showed contamination, with concentrations ranging from 1 to $4 \mu\text{g L}^{-1}$ for glyphosate and up to $11 \mu\text{g L}^{-1}$ for AMPA. Similar levels of concentrations were found in Sri Lanka (from 0.7 to $3.5 \mu\text{g L}^{-1}$) in abandoned wells close to fields [19]. Leaching of glyphosate and AMPA from 1 m depth lysimeters was monitored in a 3-year study in Central Italy. Both chemicals were found in leachates, AMPA with a more pronounced frequency compared to the parental compound. The concentrations ranged from 0.5 to $13.5 \mu\text{g L}^{-1}$ for glyphosate, while AMPA from 1 to $24.9 \mu\text{g L}^{-1}$ (maximum peak) [20]. Previous studies had highlighted the importance of the time interval elapsed from the spraying and first leaching event as well as the intensity of the first rainfall [3,21,22]. Similar findings were also obtained by Kjær et al. and Norgaard et al. [11,23].

The risk of water contamination by pesticides can be derived considering the physical and chemical properties of the chemicals (e.g., GUS index, solubility) and the properties of the soil (texture) [24,25]. Field studies can be carried out using specific devices such as lysimeters. Lysimeters are devices commonly used to estimate the leaching of pesticides in agricultural soils [26,27]. While several studies focused on the presence of glyphosate and AMPA in surface waters, less information is available regarding their risk of leaching, particularly at significant soil depths.

The aim of this study was to evaluate the leaching potential of glyphosate and AMPA on agricultural soil as affected by the time elapsed between spraying and the first leaching event. Moreover, the relationship between the time of herbicide application, the first event of leaching and the entity of residues transported in leached waters was assessed. The study was carried out on large-scale lysimeters under field-like conditions.

2. Materials and Methods

The study was hosted at the experimental station of the Dipartimento di Scienze Agrarie, Forestali e Alimentari of the University of Torino, Italy, in 2013 and 2014. The experimental station is located in the municipality of Carmagnola (NW Italy, $44^{\circ}53'08.99''$ N, $7^{\circ}41'11.33''$ E; WGS84), about 40 km far from the city of Turin.

A group of 12 lysimeters built in 1991 was used during the two-year trial. Lysimeters are disposed of two adjacent rows (Figure 1). Each lysimeter has a rectangular shape made

of high-density polyethylene with a surface area of 8.4 m^2 ($2.8 \times 3 \text{ m}$) and a depth of 1.8 m. To facilitate water discharge, a series of polyethylene tubes arranged horizontally were laid at the bottom part of the lysimeters. Soil was separated from the tubes by three layers of gravel (30 cm), sand (30 cm), and non-woven polypropylene fiber, respectively. These layers constituted the drain component for each lysimeter. At the time of installation, lysimeters were filled with disturbed soil taken from the surrounding experimental station soil.



Figure 1. The lysimeters used during the two-year trial are included in the red box.

The main characteristics of the soil, which is classified as typic udifluent, are reported in Table 1.

Table 1. Main physical characteristics of the soil.

Soil Component	%
Sand	34.8
Silt	59.8
Clay	5.4
Organic matter *	0.44

* average in the 0–2.2 m depth.

In order to ensure field-like conditions, the lysimeters were buried, and the adjacent area followed the same agronomic practices adopted in the lysimeters. At the experimental fields, the groundwater level was about 6 m deep with negligible seasonal variation. In the 0–0.5 m soil depth, the soil bulk density was 1.30 mg m^{-3} , and the water content at saturation averaged $0.56 \text{ mm}^3 \text{ mm}^{-1}$ [28,29].

Zavattaro et al. [30] performed a physical and hydrological characterization of the lysimeters soil eight years after their installation. The main soil hydrological parameters and the soil bulk density, evaluated at 0–20 cm and 20–50 cm soil depth, resulted in very similar between lysimeters and the undisturbed soil. The bulk density and water tension were measured at 0, 33, and 1500 kPa. The soil of lysimeters did not suffer from compaction due to machinery transit as the lysimeter soil was spade-tilled. Bulk density differences were encountered only in the plowed layer of the soil surrounding the lysimeters. No significant differences between the lysimeter soil and the undisturbed one were found in the deeper layers. The water storing capacity of the soil was evaluated at field capacity and at permanent wilting point. Only at field capacity, few differences were observed for

the volumetric water content [30,31]. According to a study carried out in 2007, the soil infiltration rate was 70 mm/h [32].

2.1. Agronomic Practices Adopted in the Previous Years and Lysimeter Preparation

Until 2012 lysimeters were cultivated with maize following the local agronomic practices in terms of fertilization and crop protection. In that period, tillage operations within the lysimeters were manually performed by using a spade until to a depth of 30 cm, while weed control in maize was carried out in pre-emergence with mixtures of different herbicides (see [21]). At that time, the crop was cultivated within both the lysimeter and all the surrounding surfaces to mimic field-like conditions. About a week prior to the start of each growing season trial, the lysimeters were left to fully discharge the percolating water, if present, to ensure the absence of gravitational water flows. Lysimeters were sprayed only after the end of percolation.

In both years (2013 and 2014), lysimeters were sprayed with Taifun MK® (Adama Italia srl, Grassobbio, Italy), a herbicide containing 360 g L⁻¹ of glyphosate, at rate of 12 L ha⁻¹. The chosen application rate was the maximum allowed on the label. Treatment was applied using a backpack sprayer (Bellspray Inc dba R&D Sprayers, Opelousas, LA, USA; model D-201-S), with 2-L bottle header, with an aluminum CO₂ cylinder, equipped with 4 nozzle spray boom. In the previous years, glyphosate was never applied on lysimeters to control weed infestation. In 2013, herbicide application occurred on 17 June, while in 2014, on 5 June. In case of unfavorable weather forecast, during lysimeter preparation and after herbicide application, temporary covers were set up on the lysimeters and removed immediately after rainfall.

Four groups of three lysimeters were randomly selected between the 12 lysimeters available. Each group was irrigated (first irrigation) at different times after treatment: at one day after herbicide treatment (1 DAT), 7 DAT, 14 DAT and 28 DAT. The same lysimeters were irrigated a second time (second irrigation) 14 days after the first irrigation, corresponding to a period of time from treatment of 15 DAT (1 + 14), 21 DAT (7 + 14), 28 DAT (14 + 14) and 42 DAT (28 + 14). Each lysimeter was irrigated using water withdrawn from a 30 m-deep well located 150 m far from the lysimeter facility. Three samples of irrigation water were analyzed to verify the absence of glyphosate and AMPA in the water. In addition, the experimental site hosted an official monitoring point of the groundwater monitoring network of the Piedmont region (Monitoring point: 00105910002—TF2 Tetto Frati—Carmagnola—GWB-S5a). The analysis carried out each year by the regional authority for environmental protection in the 13 m-deep aquifers did not find residues of both chemicals (<0.01 µg L⁻¹). The amount of water distributed on each lysimeter during a single irrigation was 336 L, a quantity corresponding to a 40 mm rainfall. This amount of rainfall per event was calculated considering the last ten years' meteorological pattern of the zone in the period of potential herbicide application. In a previous trial carried out on the same lysimeters in 2011 and 2012, this amount of water was able to produce important leaching. At the time of irrigation, each lysimeter was irrigated separately. The irrigation required about 30 min to deliver the selected quantity of water. Irrigation was carried out by means of a hose with a dispersion device attached to its end.

A 200 L collection tank placed 2.5 m deep into an inspection chamber was used to collect the percolated water drained by gravity into each lysimeter. The percolated water flowed from the lysimeter into the tank by means of a valve, with a manual regulation. After each percolation event, the water was withdrawn from the collection tank by electric pump. The total percolated volume was measured with an in-line flow meter (K24 Turbine meter, Piusi Instruments, Suzzara, Italy).

The presence of percolated water was monitored in the irrigated lysimeters starting from one day after irrigation. About a week after the irrigation, the water drained at the bottom of each lysimeter was collected and the full volume was measured. Three samples per lysimeter were collected from the entire volume of leached water. The total leached volume was collected with a submersible drainage pump (Calpeda, Montorso Vicentino,

Italy). The water samples were then put into 1 L graduated square polyethylene bottles (Kartell®, Noviglio, Italy) and immediately stored in a -25°C cold room until analysis. In order to assess the presence of background residues from the previous season, in 2014, before the starting of the new trial, three samples of leached water were collected from each lysimeter previously used in 2013 trial (blank samples).

2.2. Soil Moisture Measurements

Soil moisture was measured just before each planned irrigation in the upper soil layers (0–5 cm soil depth), taking the soil sample by means of a trowel. Soil samples were not collected using core samplers to avoid the creation of preferential ways. After collection, soil samples were immediately weighted, then let dry into laboratory stoves at 105°C for 24 h., hence re-weighted.

2.3. Glyphosate and AMPA Analysis

The analyses were performed using high-performance liquid chromatography (HPLC) using a Varian instrument (Agilent Technologies Italia, Cernusco sul Naviglio, Italy) equipped with a ternary pump (Pro Star mod. 230) and a fluorescence detector used at excitation and emission wavelengths of 266 nm and 305 nm, respectively. The column was a Supelco-sil® (Sigma Aldrich, Saint Louis, MO, USA) LC-NH2 (25 mm $\times$ 4.6 μm , 5 μm) with a mobile phase (75/25 *v/v*) composed by KH_2PO_4 0.1 N: acetonitrile (Carlo Erba® reagents, Cornaredo, Italy). The mobile phase was pumped at an isocratic rate flow rate of 1 mL min^{-1} . The retention time for glyphosate was 9.5 min, for AMPA 4.6 min. Before the analysis, each water sample was prepared to evaporate 100 mL of the initial sample until a final volume of 4 mL using a Rotavapor (Rotavapor® R-100, Buchi, Cornaredo, Italy) set at 50°C . The evaporation process was facilitated by adding small aliquots of acetone (Carlo Erba® reagents, Cornaredo, Italy). The reduced sample was introduced in a 5 mL flask and filled to the total volume by adding deionized water. The sample was finally filtered using a 0.45 μm nylon filter. Once filtered, each sample underwent derivatization. The derivatization was performed preparing a solution with 400 μL of borate buffer (0.05 M, pH 10), 200 μL of 9 fluorenyl-methyl chloroformates (FMOC-Cl) (Sigma-Aldrich®, Merck Group, Darmstadt, Germany) dissolved in acetonitrile, 200 μL of the sample within a test tube with screw cap. The solution was vortexed for one minute, then left undisturbed for 60 min. After this period, 600 μL of 2% concentrated H_3PO_4 was added to the solution that was vigorously shaken. After this step, 2 mL of ethyl ether (Carlo Erba® reagents, Cornaredo, Italy) were added, and the solution was shaken another time. Once the separation of the phases occurred, 1.6 mL of the aqueous phase were withdrawn and transferred in a vial for analytical determination. The limit of quantification (LOQ) was $0.1\text{ }\mu\text{g L}^{-1}$ for both chemicals.

2.4. AMPA/Glyphosate Ratio (AMPA/GLY Ratio)

The metabolite/parent compound ratio (MPR) has been considered a way to discriminate between diffuse and point pollution sources [33–35]. An MPR above 1 is an indicator of diffuse pollution: parent compounds are transported slowly along the soil profile, and they have time to be partially or totally degraded. By contrast, a low MPR value indicates rapid leaching of the parent compound. We use the AMPA/GLY ratio to explain the differences in AMPA and glyphosate concentrations observed at different time intervals from spraying and first leaching event. The AMPA/GLY ratio was calculated for all sampling dates when concentrations data were available. In case of concentrations below the quantification limit, the value of the correspondent limit of quantification was considered for the calculation of the ratio.

2.5. Statistical Analysis

The *t* test was used to individuate statistical differences ($\alpha \leq 0.05$) in glyphosate and AMPA concentrations between years at the same temporal interval from irrigation. A bi-

variate correlation analysis was performed to verify the existence of a linear relationship between the percolated volumes measured at the first and the second irrigation and corresponding glyphosate and AMPA concentrations. The software SPSS, version 27.00 (SPSS released 2020, IBM Corporation, Armonk, NY, USA), was used to perform the analysis.

2.6. Weather Conditions

In Figure 2, the meteorological data observed during the period of the study are reported. All the weather data were collected from the meteorological station located 150 m far from the lysimeters. The meteorological station is part of the regional weather network system. In 2013 two periods of high temperatures were recorded, during the days that preceded the spraying and in the last ten days of July (which corresponded to the end of the trial). In 2014, the average temperature was lower than in the previous year (more than 1 °C). During 2013, the good weather conditions during the weeks after herbicide application did not require the covering of lysimeters for rainfall events. In the night between 30 and 31 July 2013, just before the irrigation at 28 DAT (Days After Treatment) and 14 + 14 DAT, a sudden storm discharged 24 mm of rain on the uncovered lysimeters. The irrigation planned that day has considered the rainfall fallen during the night, and thus the amount of water distributed was 130 L. In 2014, we recorded two heatwaves, the former just in the days following herbicide application, the latter in the middle of July. Both periods, of about 7 days each, were characterized by max and min mean temperatures significantly higher than the average. For instance, during the first heatwave from 7 June to 13 the maximum average temperature was 33.5 °C and the minimum 16.5 °C, compared to the average temperature of the period June–July of 28.7 °C and 15 °C, respectively. These periods of high daily temperatures likely amplified the evaporation processes, influencing the total amount of water available for percolation.

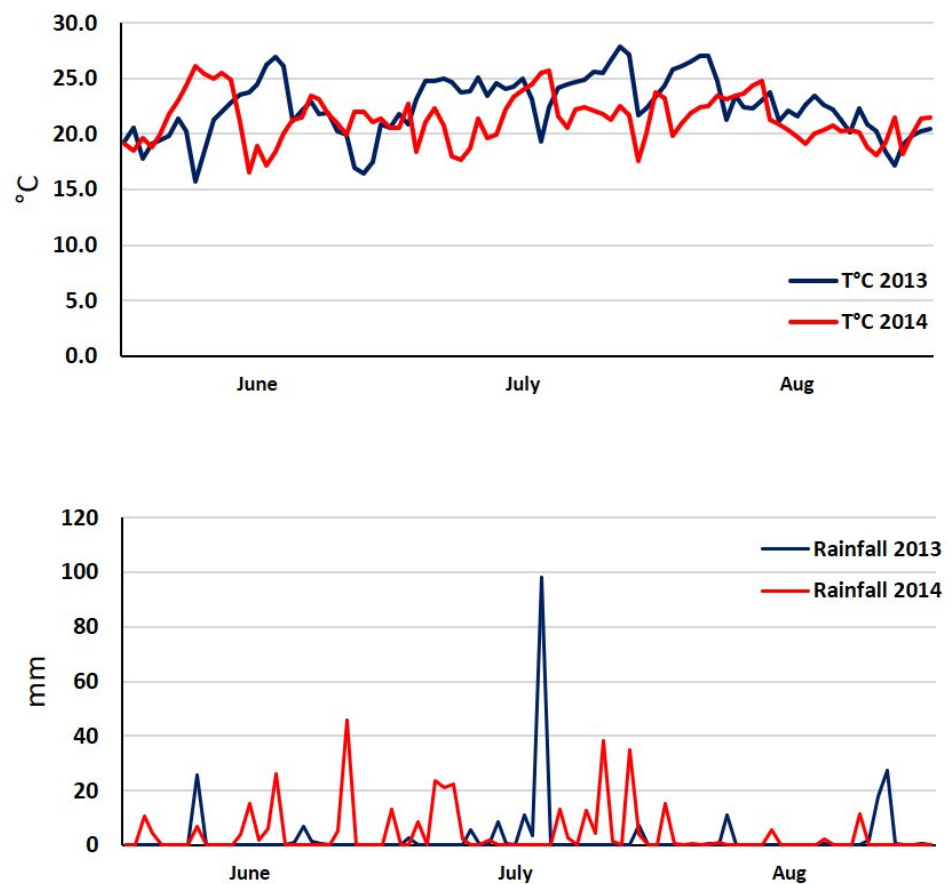


Figure 2. Meteorological trend observed in 2013 and 2014.

3. Results

3.1. Percolation Volumes

In both seasons, the total volumes percolated through the lysimeters after each planned leaching event were measured (Table 2). The amount of percolation water leached through the soil profile was likely affected by the soil conditions before and after the leaching event. In general, the highest volumes were observed after the second irrigation. In 2013 the lowest amount of leached waters was observed at the leaching event planned at 7 and 28 DAT and at 42 DAT. The repetition of irrigation caused a more pronounced movement of water along the soil profile. This was evident by the observation of the leached volumes. The percolated volumes monitored in 2014 resulted lower compared to those observed in 2013 due to the different meteorological conditions that occurred during the trial. The highest temperatures recorded during the 2014 trial have likely affected the evaporation rates of the soil. This is confirmed by the moisture data measured before each planned irrigation (Table 3). In the first four irrigation times (1, 7, 14, 28 DAT), soil moisture percentage was always about one point below the level observed in the previous season before the execution of the irrigation (Table 3). The average amount of percolated water resulting from the three lysimeters at 28 DAT (38 l) was unexpected considering the null or negligible percolation volumes observed in the previous sampling times. However, only two days before the irrigation (on 1 July), a severe storm occurred during the afternoon. For a while, part of the covering structure installed to protect the lysimeters was removed by the strength of the wind. The technicians were able to re-establish the impermeable cover quite soon, but an imprecise amount of rain has certainly reached the soil surface. To confirm this, the moisture level measured on the soil surface just before the irrigation was higher than that observed at the two previous sampling times.

Table 2. Volumes of percolated waters ($\pm$ SE) during the two-year study. Values are the arithmetic mean of three replications.

Days Elapsed between the Treatment and the First Irrigation	2013	2014
DAT (1° irrigation)	Percolated water (L)	
1	57 ($\pm$ 13)	NL
7	12 ($\pm$ 2)	7 ($\pm$ 2)
14	14 ($\pm$ 8)	NL
28	11 ($\pm$ 5)	38 ($\pm$ 23)
Days Elapsed between the treatment and the Second Irrigation	Percolated water (L)	
DAT (2° irrigation)		
(15) 1 + 14	53 ($\pm$ 10)	72 ($\pm$ 23)
(21) 7 + 14	21 ($\pm$ 5)	18 ($\pm$ 9)
(28) 14 + 14	146 ($\pm$ 45)	103 ($\pm$ 3)
(42) 28 + 14	3 ($\pm$ 2)	2 ($\pm$ 1)

Note: NL: no leaching.

Table 3. Soil moisture at the first 5 cm depth measured before the irrigation of lysimeters. Arithmetic mean of three replications $\pm$ SE.

DAT	2013	2014
	Soil moisture (%) $\pm$ SE	
1	19.6 ($\pm$ 0.29) a	18.9 ($\pm$ 0.09) b
7	18.4 ($\pm$ 0.82) a	17.3 ($\pm$ 0.25) b
14	17.9 ($\pm$ 0.79) a	17.0 ($\pm$ 0.16) b
28	20.8 ($\pm$ 0.53) a	18.3 ($\pm$ 0.14) b
(15) 1 + 14	18.1 ($\pm$ 0.38)	20.5 ($\pm$ 0.27)
(21) 7 + 14	20.2 ($\pm$ 1.15)	20.6 ($\pm$ 0.18)
(28) 14 + 14	20.2 ($\pm$ 0.28)	20.5 ($\pm$ 0.30)
(42) 28 + 14	20.2 ($\pm$ 0.35) a	19.5 ($\pm$ 0.03) b

Data values with different letters are statistically different (Students's *t*-test; $\alpha = 0.05$).

3.2. Glyphosate and AMPA Concentrations

3.2.1. Season 2013

During 2013 the highest concentration of glyphosate was recorded at the first leaching event (1 DAT) with a value of $1.39 \mu\text{g L}^{-1}$ (Table 4). A higher percolation volume, compared to the following leaching events, characterized this sampling. In the following weeks, glyphosate concentration remained quite stable, never exceeding $0.60 \mu\text{g L}^{-1}$. In water samples collected after the second round of irrigation, glyphosate residues still remain ten times higher than the LOQ ($0.1 \mu\text{g L}^{-1}$) at 15 and 21 DAT. The leached water collected from lysimeters irrigated a month after spraying still showed relevant traces of glyphosate ($0.27 \mu\text{g L}^{-1}$). AMPA had a similar trend in the first set of irrigation, even though with lower concentration values (Table 4). The concentration peak ($0.97 \mu\text{g L}^{-1}$) was measured in percolated waters collected from lysimeters irrigated at 1 DAT. On percolated waters collected after the repetition of irrigation, the highest AMPA concentration was reached at 15 DAT ($0.84 \mu\text{g L}^{-1}$). At 42 DAT, residues of AMPA were above $1 \mu\text{g L}^{-1}$. At the first leaching event, there was a significant correlation between percolated volumes and concentration values for both chemicals (Table 5). The analysis carried out on the blank samples collected in 2014, just before the beginning of the new experimental season, showed residues of glyphosate and AMPA below $0.1 \mu\text{g L}^{-1}$ in all the analyzed samples.

Table 4. Glyphosate and AMPA concentrations detected in percolated waters in 2013 and 2014. Arithmetic mean of three replications $\pm$ SE.

	2013	2014	2013	2014	2013	2014
DAT	GLY	GLY	AMPA	AMPA	AMPA	AMPA
1° Irrigation	$\mu\text{g/L}$	$\mu\text{g/L}$	$\mu\text{g/L}$	$\mu\text{g/L}$	GLY	GLY
1	1.39 (± 0.60)	-	0.97 (± 0.35)	-	0.7	-
7	0.59 (± 0.28) a	0.19 (± 0.09) b	0.22 (± 0.10)	<0.1	0.4	≤ 0.5
14	0.57 (± 0.22)	-	0.18 (± 0.05)	-	0.3	-
28	0.27 (± 0.13)	0.52 (± 0.40)	0.11 (± 0.01)	<0.1	0.4	≤ 0.2
DAT						
2° irrigation						
(15) 1 + 14	1.04 (± 0.45) a	0.13 (± 0.04) b	0.84 (± 0.28) A	0.22 (± 0.09) B	0.8	1.7
(21) 7 + 14	1.19 (± 0.70)	<0.1	0.11 (± 0.01)	<0.1	0.1	≤ 1
(28) 14 + 14	0.28 (± 0.10)	0.12 (± 0.02)	0.08 (± 0.01) B	0.52 (± 0.30) A	0.3	4.3
(42) 28 + 14	<0.1	<0.1	1.07 (± 0.25)	<0.1	≥ 1	≥ 1

GLY: glyphosate; Data values with different letters are statistically different (Students's *t*-test; $\alpha = 0.05$). Lowercase letters: differences in glyphosate concentrations between years; Capital letters: differences in AMPA concentrations between years.

Table 5. Correlation between percolated volumes and glyphosate and AMPA concentrations in 2013 and 2014 according to the two irrigations period.

	2013		2014	
	GLY	AMPA	GLY	AMPA
1° irrigation	0.725 **	0.665 *	0.850 *	-
2° irrigation	-0.462	-0.375	0.337	0.875 *

** ($\alpha \leq 0.01$); * ($\alpha \leq 0.05$).

3.2.2. Season 2014

In 2014, due to the unfavorable weather conditions, it was not possible to collect samples after the first (1 DAT) and the third (14 DAT) planned irrigation. Overall, the percolated volumes were greatly lower than in the previous season. Samples collected at 7 and 28 DAT both showed residues of glyphosate above the detection limit. On the contrary, AMPA residues resulted below the limit of quantification. The repetition of irrigation determined a flux of water along the soil profile, allowing the collection of water samples after each event. Glyphosate residues never exceeded $0.13 \mu\text{g L}^{-1}$, while in the

case of AMPA, a concentration peak of $0.52 \mu\text{g L}^{-1}$ was reached at 28 DAT, likely due to the important percolation flux (see Table 2). The high concentrations of AMPA recorded at 15 and 28 DAT are likely justified by the fact that after the first irrigations at 1 and 14 DAT, no percolation was detected. The repetition of the irrigations induced the leaching of AMPA residues formed until that moment. A positive correlation between percolated volumes and AMPA concentrations was found (Table 5).

3.2.3. AMPA/GLY Ratio

In 2013, the AMPA/GLY ratio was below 1 (on average 0.4) in almost all the sampling dates, with the exception of the last sampling date (Table 4). These values may reflect the rapid transport, due to the irrigations, of the parent compound through the soil, which may have delayed the formation of the metabolite. During 2014, in a few cases, it was not possible to calculate the MPR ratio due to the absence of leaching. The high AMPA/GLY values observed at 15 and 28 DAT (1.7 and 4.3 at 15 and 28 DAT in 2014), derived from the high concentrations of AMPA observed. The high AMPA/GLY ratio may be explained by both the rapid degradation of glyphosate due to the high temperature that occurred the day following herbicide application and by the high interaction of the parent compound residues with soil, which may bring faster degradation or higher absorption. The fact that, in the last sampling dates, the MPR ratio reached high values was quite unexpected considering that from the spraying to first irrigation, enough time elapsed to allow the parent compound to be degraded by microorganisms. During the permanence in the soil of the parent compound, biodegradation processes are favored, and metabolite formation occurs. At the moment of the first irrigations, no leached water was observed in the lysimeters due to the particular weather conditions as described in the previous paragraphs.

4. Discussion

The present study dealt with the mobility of glyphosate and AMPA in long-established field lysimeters. The information obtained may help to explain the behavior of these chemicals in field-like conditions under different scenarios in terms of the occurrence of percolation events. The lysimeters used in this study allowed the collection of percolated water to a high depth (1.8 m) compared to other studies carried out worldwide both on lysimeter and at field scale [1], giving the possibility to understand the mobility of these chemicals at uncommon depths.

According to our results, the mobility of glyphosate and AMPA seems to be related to the amount of percolation water involved and to the time elapsed from the spraying to the leaching event. Similar findings are reported by Giuliano et al. [36]. At a higher amount of percolation, volumes generally correspond to greater chemical residues in leached waters. In addition, the results demonstrated that glyphosate is more susceptible to leaching in case of important rainfall very close to spraying time; this behavior was previously seen by other authors [1,3,12,20]. According to Napoli et al., rainfall occurring within two weeks after spraying may lead to a leaching of glyphosate until a depth of at least 1 m [20]. Similarly, Al-Rajab et al. [37] found glyphosate residues only 18 days after the first percolation. Other authors found that in tile drains posed at 1 m depth, glyphosate and AMPA concentrations frequently exceeded $0.1 \mu\text{g L}^{-1}$. In this study, the concentration found at 1 m depth in one of the experimental sites were on average of $0.54 \mu\text{g L}^{-1}$ for glyphosate and $0.17 \mu\text{g L}^{-1}$ for AMPA, but they refer to an application field rate 3-fold lower (4 L ha^{-1}) than that used in the current study (12 L ha^{-1}). In the same study, it is reported that heavy rains fallen soon after herbicide application may carry to marked leaching with concentrations of up to $11 \mu\text{g L}^{-1}$ for glyphosate and $0.6 \mu\text{g L}^{-1}$ for AMPA at 19 days after application [15]. Giuliano et al. [36] found high percolation peaks of mesotrione and glyphosate in water samples collected during the season from tension plates lysimeters. All these data fit with our findings.

While the highest concentrations of glyphosate were detected at leaching events close to herbicide spraying, the presence of AMPA residues did not follow a regular pattern.

This is because AMPA is a metabolite and its formation depends on the availability and degradation of the parent compounds as well as by other concomitant factors. In addition, AMPA can be adsorbed by phosphonate groups [3], and its biodegradation is considered slower than that of its parent compound [38]. Furthermore, its release in the soil occurred over a longer period of time compared to glyphosate [11]. Our results pointed out that the leaching of glyphosate and AMPA is effectively event-driven and highlighted the importance of the first rainfall event in moving glyphosate through the soil, increasing the potential risk of water contamination. This was observed also by Kjær et al. and Rasmussen et al. [11,39].

However, our results showed that other factors could affect the movement of these two compounds through the soil profile after spraying: temperature pattern and soil moisture. Similar findings were observed by Al-Rajab et al. (2008) [37]. Even though high temperatures may increase glyphosate degradation, the rapid transport of the chemical in the deeper soil layers due to close heavy rainfall may diminish the formation of AMPA, favoring the sorption of glyphosate on the soil matrix. The dryer the soil, the higher the risk of easy transport of pesticides through the soil profile by rainfall events by means of macropore flow [39,40] and reduced microbial activity. In 2014, glyphosate and AMPA fates were likely influenced by the climatic conditions that occurred during the trial, and in particular by the two recorded heatwaves, the former only a few days after spraying. The high temperatures boosted the microbial activity and significantly increased the evaporation processes. The importance of the weather conditions before and after the pesticide application, as well as the initial moisture conditions of the soil, are highlighted by Rasmussen et al. [39]. Our results showed that residues of glyphosate could be found in leached waters from lysimeters treated 30 days before the leaching event. That means that glyphosate may persist more than usually considered [5,41]. A possible explanation derives from the results of Bento et al. [42], which reported a reduced degradation of glyphosate and AMPA at dryer conditions.

In our study, we used the AMPA/GLY ratio to assess the percolation dynamics of the two compounds in relation to the occurrence of leaching events. In our study, in both years, AMPA/GLY ratio was generally below one, indicating a common degradation of the parent compound. After herbicide application, the microbial activity starts to degrade the parent compound, generating the metabolite. However, there are certain conditions that can alter this natural trend; in high permeable soils, the transfer of the parent compound can be very rapid, and microbial activity has insufficient time to degrade the chemical. The high MPR values observed in 2014 are probably related to the specific weather conditions that occurred after herbicide spraying and the irrigation events. The weather conditions affected the percolation dynamics limiting or, in two cases, annulling the leaching phenomena. In particular, the highest AMPA/GLY ratio observed at 15 and 28 DAT indicates a significant prevalence of the metabolite over the parent compound. As a possible explanation of these findings, we may consider that even when a leaching event does not produce leached waters, it determines the movement of the chemical through the soil profile. When no significant leaching occurred, chemicals remained confined in the soil matrix; hence they are more available to microbial degradation.

5. Conclusions

Glyphosate and AMPA can be transferred to deeper soil layers at concentrations above the law limits ($0.1 \mu\text{g L}^{-1}$ is the maximum allowable concentration in the European Union for a generic pesticide in groundwater), even in case of leaching events far from spraying. Our results showed that AMPA might pose a risk of contamination of groundwaters as well as its parent compound. The weather and soil conditions can affect the dynamic of glyphosate movement and likely its degradation pattern. Both chemicals showed a potential risk of water contamination. Finally, we may consider that these results refer to a very high application rate of glyphosate. Hence, at lower field rates, concentrations can likely be minors.

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