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Riparian strips as attenuation zones for the toxicity of pesticides in agricultural surface runoff: Relative influence of herbaceous vegetation and terrain slope on toxicity attenuation of 2,4-D

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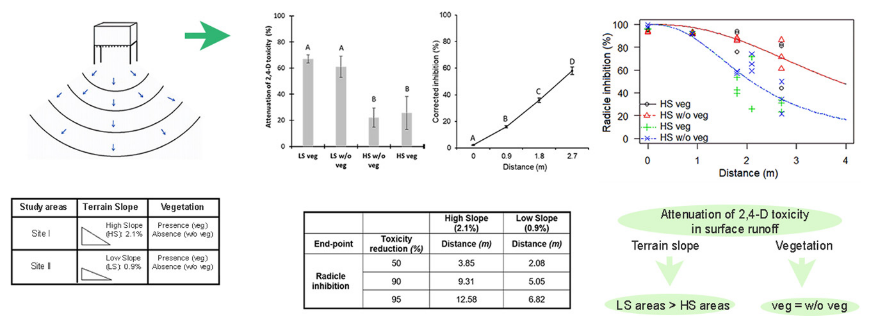
HIGHLIGHTS

- Riparian vegetation and slope roles were studied on 2,4-D toxicity attenuation.
- Differential effects of 2,4-D with standardized and natural water were examined.
- Lower 2,4-D toxicity in natural water than standardized medium was observed.
- Terrain slope was principal factor on the mitigation of runoff toxicity.
- Safety widths are provided to reduce the toxicity of 2,4-D associated with runoff.

GRAPHICAL ABSTRACT



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ABSTRACT

Pesticides reach aquatic ecosystems via surface runoff becoming one of the main contributors to their deterioration. Among the strategies to mitigate these impacts, the use of riparian strips is recommended, but the knowledge of how much each ecosystemic variable contributes to the process is still incipient. We analyzed the influence of terrain slope and vegetation in the attenuation of 2,4-Dichlorophenoxyacetic acid (2,4-D) toxicity in surface runoff using *Lactuca sativa* as a diagnostic organism. In addition, the differential effects of this herbicide were examined under laboratory conditions, with standardized water and ambient water as a dilution medium. The study was conducted in plots with different terrain slopes and presence/absence of vegetation. The herbicide was applied to each plot and rain was subsequently simulated. The runoff was collected at regular distances and the toxicity was measured. The runoff toxicity decreased with the distance from the application area in all plots, this reduction being greater in low-slope plots. No differences in attenuation of runoff toxicity were found between plots with and without vegetation. The data were incorporated into models to estimate the minimum widths of safety to reduce the toxicity of 2,4-D by 90% under these conditions, suggesting distances of 5 and 20 m for low-slope and high-slope zones, respectively. In laboratory experiments, lower relative toxicity of 2,4-D was detected when natural water was used as solvent. These results contribute to the design of sustainable agricultural practices.

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1. Introduction

Currently, the dominant agricultural production model has a strong dependence on biotechnology (genetically modified organisms) and agrochemical inputs (fertilizers and pesticides). While they have largely allowed an increase in productive capacities, agrochemicals have caused many environmental problems with impacts on public health and ecosystems. These include the expansion of the agricultural frontier, the homogenization of landscapes and an increase in the amount of pesticides used (Davari et al., 2010), whose effects on non-target biota have been indicated as one of the main problems associated with this activity (Scanlon et al., 2007; Stehle and Schulz, 2015).

Following application, pesticides can interact with several environmental components, volatilize, degrade, be carried in surface and groundwater through runoff and leaching, be retained by soil particles or be absorbed/adsorbed by non-target organisms (Potter et al., 2014; Remucal, 2014; Knauer et al., 2017). It is estimated that only 0.1% of pesticides reach their target organisms and the remaining 99.9% disperse into the environment negatively affecting biota, contaminating soil, water and air (Pimentel, 1995). The distribution of pesticides in the environment and their bioavailability, which ultimately determine their toxicity and impact on non-target biota, depends on the physicochemical characteristics of the substance itself and the physical, chemical and biological properties of the environment. Thus, the extent to which associated natural ecosystems can be reached by residues from agricultural activity is site-specific and depends on many interrelated factors. Among these factors, those that condition the rate of ground runoff such as relief, slope, vegetation cover and density stand out (González, 2007).

Surface water bodies are particularly vulnerable to the effects of pesticides, since they act as sinks for the practices carried out in the basin, receiving agrochemical residues from the fields through drift and runoff (Laterra et al., 2011). Although drift control is not simple, some tools have been developed to avoid product losses, making the application techniques more effective (e.g., low drift application equipment, products with low volatility, adjustment of application parameters and consideration of meteorological conditions at the time of application). On the other hand, few technological advances have been developed to control pesticide loss due to runoff. The use of safety zones (riparian strips) that reduce the free diffusive movement of substances of agricultural origin into surface waters (Stutter et al., 2012) is one of the main recommendations for mitigating the impacts of pesticides associated with agricultural surface runoff (Zaccagnini et al., 2014). Riparian zones are one of the most complex systems in the biosphere, due to them being transition zones between the terrestrial and aquatic environments that vary widely in their physical, morphological and ecological characteristics (Robert et al., 2000). Thus, although their role as attenuation sectors has been recognized, few studies focus on the degree of influence that each variable plays in this process. Furthermore, published papers often compare levels of attenuation between highly contrasting systems such as herbaceous versus forest riparian systems, considering the decrease in the concentration of pesticides as a parameter of the attenuation rather than the resulting toxicity, which is what matters most when it comes to protecting biota (Giaccio et al., 2017a; Klapproth and Johnson, 2009; Lowrance et al., 1997).

This study was carried out on the main agricultural production zone in Argentina, which is characterized by large extensions of land with low slopes that are irrigated by rivers and meandering streams typically of alluvial plains (Laterra et al., 2011). Although these conditions favor infiltration processes, surface runoff is also important. Among the range of pesticides used in the country, herbicides make up the larger portion in tons of active ingredient applied; and 2,4-Dichlorophenoxyacetic acid (2,4-D) stands out as one of the most frequently used, together with glyphosate and atrazine (CASAFE-Cámara Argentina de Sanidad Agropecuaria y Fertilizantes, 2012; PBA-DP, 2013). However, the use of 2,4-D is being reviewed due to the bibliographic background show lethal and sublethal effects (e.g. endocrine disruption, neurotoxicity, adverse effects on growth and reproduction) on different components of the biota such as vascular plants, aquatic invertebrates, fish, amphibians, among others (Neumeister, 2014).

In this framework, the aim of this research was to evaluate the influence of terrain slope and presence/absence of herbaceous vegetation on the attenuation of the toxicity of surface runoff of agricultural origin, taking as a model a post-application study scenario of 2,4-D. In addition, the differential effects of this herbicide were analyzed under laboratory conditions, using standardized water and natural water as solvent.

2. Materials and methods

2.1. Assayed pesticide

The experiments were carried out using the commercial formulation of 2,4-D Asi Max® 50 (Chemotecnica S.A.), which active ingredient (a.i.) is the dimethylamine salt of 2,4-Dichlorophenoxyacetic acid with a purity of 60.2% (g mL^{-1}) and a 2,4-D acid equivalent of 50% (g mL^{-1}). This herbicide is a weak acidic (pKa 2.6) with a molar mass of 221 g mol^{-1}, a water solubility of 400 g m^{-3} and a log Kow of 2.81 at 25 °C (Mackay, 2001). The 2,4-D molecule is available for rapid advective-dispersive transport due to its relative high solubility, limited volatilization rate and negative charge at low soil pH (Boivin et al., 2005). The dissipation of 2,4-D depends on microbial-degradation and photo-degradation in water and soils. Data indicates that this herbicide degrades rapidly in soils (half-life = 6.2 days) and in aerobic aquatic environments (half-life = 15 days); and it is relatively persistent in anaerobic aquatic environments (half-life ranges from 41 to 333 days) (USEPA, 2005).

2.2. Toxicity analysis. Bioanalytical tools assessment

The herbicide toxicity in the field and laboratory samples was evaluated through two standardized bioassays widely used worldwide to evaluate toxicity in the aqueous phase of both pure substances and environmental samples, and with relatively high sensitivity to different pollutants (particularly pesticides) respect to other components of the biota (Miller et al., 1985; Wang and Freemark, 1995; Ronco et al., 2000: (a) acute toxicity test with *Daphnia magna*, a static test performed under controlled conditions with 48 h of exposure was used according to USEPA (2002). The field samples were evaluated with and without previous agitation to corroborate if there were differences in toxicity under these conditions; (b) acute toxicity test with *Lactuca sativa* seeds, a standardized static test performed under controlled laboratory conditions during 120 h of exposure was used, evaluating percentage of seed germination, and radicle, hypocotyl and total length, according to USEPA (1996). For each sample, four replicates of 20 seeds were used.

The *D. magna* organisms were acquired from cultures kept in the laboratory under controlled conditions. *L. sativa* seeds were obtained from a selected batch. In all bioassays, the laboratory conditions (e.g., photoperiod, temperature) were monitored and kept constant and the requirements of quality control and quality assurance (control charts) along with the criteria for the acceptability of results—i.e., an effect <10% in the negative control and a coefficient of variation <20% in the negative control of the *L. sativa* test— were rigorously followed.

2.3. Laboratory experimental methodology

Before the field experiments, the relative toxicity of the 2,4-D formulated was evaluated under controlled laboratory conditions on the selected diagnostic species. For each species, six concentrations of the herbicide were tested in a range from 0.016 to 6.8 mg a.i. L^{-1} for *L. sativa*, and 18 to 301 mg a.i. L^{-1} for *D. magna*. Three different types of solvents were used: Dechlorinated tap water (standardized water), and ambient water from two streams, Juan Blanco (35°08′7.90"S; 57°26′16.79"W) and Punta Indio (35°08′7.57"S; 57°23′59.56"W). These streams are located within a Biosphere reserve, have very little influence from anthropogenic activities, and are routinely taken as reference streams in environmental quality studies of surface waters (Solis et al., 2017). The LC50 at 48 h of exposure was estimated for *D. magna*, while the IC50 (root elongation inhibition) at 120 h was estimated for *L. sativa*.

2.4. Field study

2.4.1. Characterization of experimental sites

Field experiments were carried out in two geographically close sites (Site 1: 34°55′32.34"S, 58°6′44.57"W; Site 2: 34°57′47.48"S, 58°04′ 44.91"W) located in NE Buenos Aires Province, in the district of La Plata (Argentina) (Fig. SM1). These soils are characterized by being silty loam or silty clay loam. The surface horizon (A) contains less than 30% clay and thicknesses of 20 to 30 cm. At the headwaters of the streams the soils are susceptible to flooding, which implies limitations for crops in times of high rainfall (Hurtado et al., 2004). Both sites have marked similarities in their soil and vegetation cover characteristics, but present differences in terrain slope (Terrain slope of site 1 = 2.1%; Terrain slope of site 2 = 0.9%). In each area, a general characterization was made, determining: main floristic composition, by observation; mean vegetation density, through a simple random sampling (Mostacedo and Fredericksen, 2000) using a 0.23 m^2 grid quantifying the number of individuals manually; vegetation cover, calculated using the software CobCal v1.0 (INTA, Entre Ríos, Argentina. 2007. www.cobcal.com.ar) (methodological error < 5%); soil infiltration, using a disc infiltrometer (Eq. (1)); and soil apparent density by the cylinder method, in this case, samples were taken using a specific sampler brand (Tonomar S.A.), and dried in an oven (105 °C) for 24 h to obtain the dry weight, then the parameter was calculated (Eq. (2)).

$$I\left(mm\ h^{-1}\right) = \frac{dh(cm)}{dt(min)} \times 600 \quad (1)$$

where *I* is soil infiltration, *dh* is the decrease in the water column height, *dt* is the elapsed time interval.

$$ASD = \frac{Soil\ dry\ weight}{Cylinder\ volume} \quad (2)$$

where *ASD* is the apparent soil density.

In addition, the terrain slopes were determined in the sectors where the experimental plots were located. This parameter was calculated based on the difference in height between different points. For this, a KOLIDA optical level (model KL24) was used and this information together with the distance between the points, allowed the calculation of the slopes in the following way (Eq. (3)):

$$Slope\ (\%) = \frac{Height\ difference\ between\ 2\ points}{Horizontal\ distance\ between\ points} \times 100 \quad (3)$$

2.4.2. Field experimental methodology. Runoff toxicity mitigation

At each site, two types of 2.7-m plots were established: ones with not-perturbed vegetation ("with vegetation"), and others where the entire vegetation cover was eliminated, leaving the soil bare ("without vegetation").

Prior to the experiments, the plots were irrigated to the saturation point, in order to equalize the conditions related to the percentage of soil moisture among plots and sites. A solution of 2,4-D at 58.3 mL L^{-1} was applied at the head of each plot over an area of 0.64 m^2 (80 × 80 cm) for 80 s at 0.2 m from the ground. The application concentration matched the maximum concentration recommended for field use (CASAFE-Cámara Argentina de Sanidad Agropecuaria y Fertilizantes, 2015). A Micron Herbi 4 manual sprayer was used for herbicide application, which allows regulation of drop size, set at 250 μm-diameter drops. After 20 min of herbicide application, a rain event was simulated on the application surface using a rain simulator similar to the one designed by Weber et al. (2009) with minor modifications that simulates homogeneous rainfall with a flow rate of 1.24 ± 0.72 L min^{-1}. At 0, 0.9, 1.8 and 2.7 m from the area where the rain was simulated, runoff traps consisting in 100-mL amber glass bottles, were placed in order to collect samples by triplicate. The apparent density sampler was used to bury the bottles in order to minimize the deterioration of the soil structure in the plots. Whenever a bottle filled up, it was immediately capped with the aim of collecting the runoff front. The rain simulation event was stopped once all the bottles were filled up. Control plots in which the pesticide was not added, were considered to determine if there was any basal toxicity at the study sites.

Runoff samples collected during the experiments were transported to the laboratory and stored at 4 °C in the case of the analyses being performed within 48 h after treatment, or at -20 °C when it took longer. In all cases, undiluted samples were evaluated (direct samples), and in cases where the lethal effects reached 100% the 5% dilution was also evaluated (high-slope site).

Net decay of 2,4-D toxicity was calculated for each treatment as the difference in toxicity between the sample closest to the emission source and the one furthest away. In order to evaluate the attenuation of the 2,4-D toxicity present in the surface runoff across the distance from the emitting source, the values of inhibition percentages of each distance were corrected for inhibition percentage of the samples at distance 0, so the values were standardized (equalizing the initial conditions in all the plots) to make comparisons.

2.5. Selection and adjustment of mathematical models

To predict the minimum width of riparian strips needed to reduce the 2,4-D toxicity present in surface runoff by a desired percentage, the fit of the experimental results to three mathematical models was evaluated; namely:

Linear model ($y = b\,x$), selected for being a simple model with high parameter interpretability.

Exponential model ($y = a\ exp^{-\frac{x}{b}}$), selected by analogy with the model decay of pesticides.

Logistic model ($y = \frac{1}{1+\ exp^{b(\ \log(x)-a)}}$), selected for being the most used in ecotoxicology for the evaluation of endpoints, expressed as percentage or limited in the interval [0,1].

For each of the endpoints, the maximum model was obtained, considering the independent variables (slope, presence/absence of vegetation and distance) and their interactions. From the same, a sequential elimination test was carried out to find the minimum adequate model. The best model was selected according to the principle of parsimony and minimizing the Akaike information criterion (AIC) (Crawley, 2013).

2.6. Statistical analysis

Parametric techniques were used (Student test, one or two way-ANOVA, repeated measures ANOVA) after evaluating the assumptions for these analyses. For pairwise comparisons, the Fisher test (LSD) was used a posteriori.

Lethal and inhibitory concentrations median (LC50 and IC50) and 95% confidence limits were obtained by Probit analysis version 1.5 and following the method of linear intersection to estimate concentration-response curves (USEPA, 1989). All statistical analyses were performed with a significance level of 0.05, using the R software (R Core Team, 2020) and drc package (Ritz et al., 2015).

3. Results

3.1. Laboratory experiments

Fig. 1 illustrates the concentration-response curves exhibited by *D. magna* and *L. sativa* when subject to the herbicide 2,4-D. The

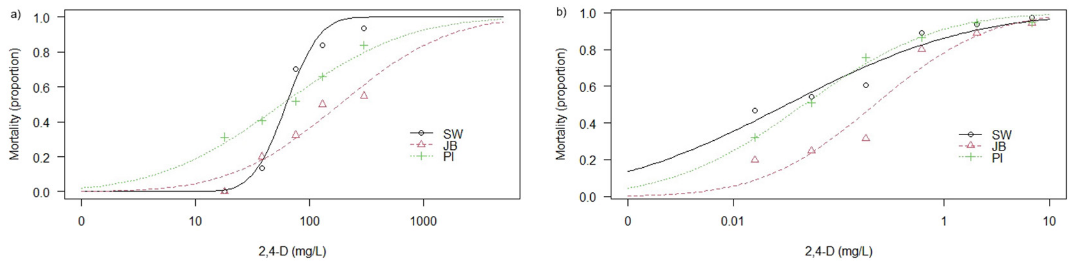

Fig. 1. Concentration-response curves for a) *D. magna* and b) *L. sativa* induced by the herbicide 2,4-D. Reference: SW, standardized water medium; JB, water from Juan Blanco stream; PI, water from Punta Indio stream.

concentrations evaluated did not produce significant effects on *L. sativa* germination, but they did inhibit radicle elongation with IC50 values of 0.03 ($\pm$0.01) mg a.i. L^{-1}. On the other hand, *D. magna* was significantly less sensitive than *L. sativa* with LC50 values near four orders of magnitude above the IC50 values obtained for vascular plants (Table 1). Significant differential toxicity was only observed with respect to the reference dilution medium (standardized water) when Juan Blanco stream water was used as a solvent (*L. sativa* $t = 16.93$, $p \leq 0.00001$; *D. magna* $t = 14.41$, $p \leq 0.00001$) where the effects on both species were minor. The concentration-response curves for *L. sativa* were similar in shape, showing that the reduction in toxicity observed when using Juan Blanco stream water occurs independently of the concentration. Conversely, the curve for *D. magna* for standardized water crosses with those corresponding to the natural water batch, so the protective effect (understood as significantly lower toxicity than reference water) of Juan Blanco stream water would only become apparent at high concentrations.

3.2. Field experiments

3.2.1. Site characterization

The floristic composition at the sites where the experiments were conducted was dominated by a few herbaceous species such as *Trifolium repens* (Leguminosae), *Anthemis cotula* (Compositae) and *Echinochloa* sp. (Gramineae). The average vegetation density recorded was 86.1 $\pm$ 25.5 individuals m^{-2}, while the average coverage was 91.95 $\pm$ 1.15% for the plots with vegetation.

Regarding the general characteristics of the soil, the apparent density that describes the compaction of the soil from the relationship between solids and pore space reached values of 0.96 $\pm$ 0.05 g cm^{-3}, while the infiltration rates were 331.58 $\pm$ 70.12 mm h^{-1} to high slope site and 669.6 $\pm$ 162.99 mm h^{-1} to low slope site.

3.2.2. Experimental results

The differences in the sensitivity of *L. sativa* and *D. magna* observed during the laboratory assay were also reflected in the field experiments since *D. magna* did not show significant effects in any of the samples (even when the direct samples were tested with- and without agitation). Therefore, this diagnostic organism did not turn out to be a good tool to evaluate the attenuation of the 2,4-D toxicity present in the surface runoff.

Table 1
Lethal Concentrations 50 (LC50) for *Daphnia magna* and Inhibitory Concentrations 50 (IC50) for *Lactuca sativa* of 2,4-D (mg L^{-1}). Measured effect: elongation of the *Lactuca sativa* root, in standardized medium, water from Juan Blanco stream and water from Punta Indio stream. Standard errors in parentheses. The different letters indicate significant differences ($\alpha = 0.05$) between the LC50 or IC50 of each matrix for each species.

2, 4-D (mg L^{-1})	Standardized medium	Juan Blanco	Punta Indio
	LC50	LC50	LC50
D. magna	62.5($\pm$3.0)a	183.7($\pm$22.9)b	57.3($\pm$6.1)a
	IC50	IC50	IC50
L. sativa	0.03($\pm$0.01)a	0.20($\pm$0.07)b	0.04($\pm$0.009)a

On the other hand, among the different endpoints evaluated on *L. sativa*, in general, no differences were observed in terms of response sensitivity. In this sense, the analysis of the differences between its standard deviations did not show statistical significance ($F = 0.48$; $p = 0.621$), so the use of any of these to measure toxicity according to this criterion, is acceptable.

None of the field control samples induced significant inhibition of radicle ($t = 2108$; p = 0,103), hypocotyl ($t = 2576$; p = 0,062) or total elongation ($t = 2569$; p = 0,062) in the exposed seeds with respect to the bioassay control, evidencing the absence of background contaminants in the sectors where the experiments were developed.

In the case of the plots exposed to the herbicide, all the samples coming from the "high-slope" plots induced 100% inhibition in germination, while in those coming from the "low-slope" plots, only sublethal effects were observed.

Fig. 2 shows the net decay of 2,4-D toxicity according to each of the categorical variables analyzed (slope and vegetation). Decrease in the surface runoff toxicity was observed in all plots. Regarding each one of the variables, only significant differences for terrain slope were observed (radicle inhibition: $F = 12.253$, $p < 0.0001$; hypocotyl inhibition: $F = 5.567$, $p < 0.0001$; total inhibition: $F = 12.995$, $p < 0.0001$). Consequently, pairwise comparisons showed no significant differences between plots with- and without vegetation for the same slope for any of the endpoints evaluated. Furthermore, some similarity in the attenuation of toxicity was observed in the plots without vegetation between the different terrain slopes (especially when evaluating total and hypocotyl inhibition), while the difference in attenuation as a function of slopes was well marked among the vegetated plots. In percentage

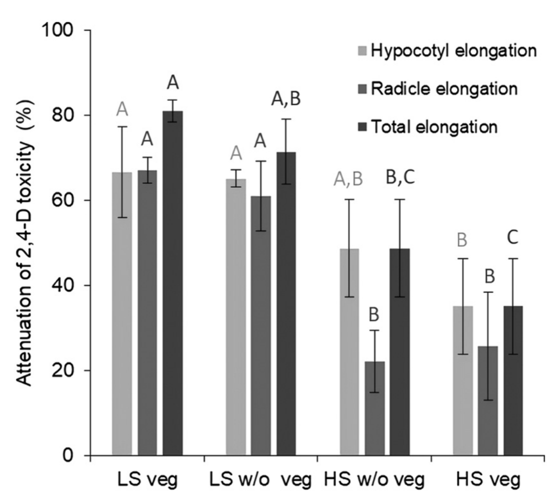

Fig. 2. Net attenuation of herbicide (2,4-D) toxicity according to the study variables: terrain slope: low slope (LS) and high slope (HS), and presence of vegetation: with vegetation (veg) and without vegetation (w/o veg); evaluated in hypocotyl, radicle and total elongation of *L. sativa* plantlings. Toxicity attenuation in samples from the high slope site was calculated with 5% dilution with respect to the direct samples. The values correspond to the mean $\pm$ error standard. The different letters of the same color denote statistical differences among the analyzed variables.

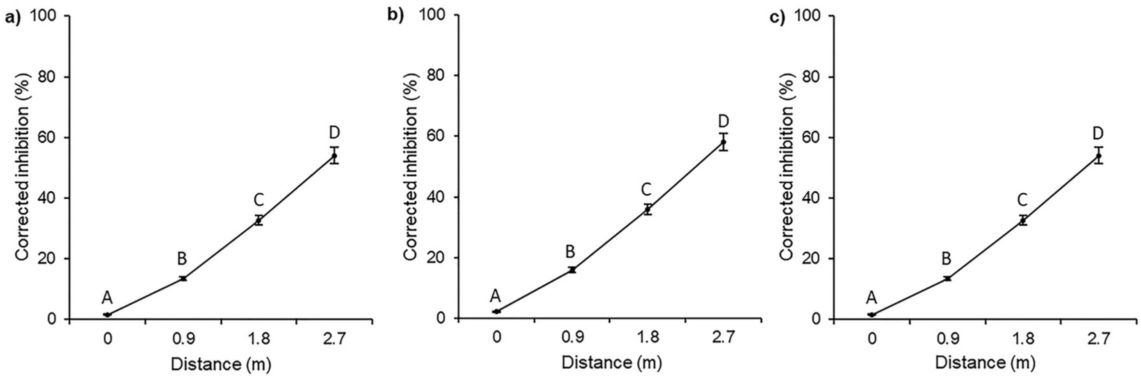

Fig. 3. Attenuation of runoff water toxicity according to the distance from the point-source of herbicide application in: a) hypocotyl; b) radicle; c) total elongation of *L. sativa* plantlings. Toxicity attenuation in samples from the high slope site was calculated with 5% dilution with respect to the direct samples. The values correspond to the mean ± error standard. Different letters refer to significant differences among analyzed distances.

Table 2
AIC (Akaike information criterion) values and number of parameters for each of the models evaluated.

Model	Radicle inhibition (%)		Hypocotyl inhibition (%)		Total inhibition (%)	
	AIC	*#p*	*AIC*	*#p*	*AIC*	*#p*
Linear	410.24	4	422.21	4	417.48	4
Exponential	419.04	4	437.31	4	429.47	4
Logistic	401.77	4	418.77	5	412.76	5

terms, the toxicity in the plots was reduced between 20 and 50% in the site of greater slope, whereas in the site of lesser slope the toxicity was reduced between 60 and 80%. Note that the attenuation of 2,4-D toxicity in the high slope site samples was estimated from the sublethal effects induced by dilution to 5%, because all direct samples caused 100% inhibition of germination.

Considering the attenuation of 2,4-D toxicity present in the surface runoff across the distance to the emitting source, significant differences were recorded in the toxicity of the samples corresponding to different distances, highlighting the existence of a progressive increase in the "protective" effect on which it depends (Fig. 3). In accordance with this, in general, along with the distance to the pollutant source, a greater dispersion of the effect values was also observed between replicates. On the other hand, as we observed for the toxicity net decay, when we evaluated the decay across the distance we also did not observe significant differences according to the presence or absence of vegetation, but rather between the different evaluated terrain slopes.

3.2.3. Theoretical modelling of experimental results

Among the evaluated adjustment models, the logistic model presents more parsimony (lower value of AIC -Table 2) and therefore is the "best model" to establish the minimum safety widths under these conditions (Supplementary material). The estimates of the selected model for each of the evaluated endpoints are summarized in Table 3.

From these estimates the following models were generated for each treatment and endpoint evaluated (Fig. 4 and Eqs. (4)–(9)):

$$\textit{Radicle inhibition}\ (\%) = \left(\frac{1}{1+\ exp^{2.49*(Distance-3.85)}}\right)*100\ \mid\ \text{High slope} \tag{4}$$

$$\textit{Radicle inhibition}\ (\%) = \left(\frac{1}{1+\ exp^{2.49*(Distance-2,09)}}\right)*100\ \mid\ \text{Low slope} \tag{5}$$

$$\textit{Hypocotyl inhibition}\ (\%) = \left(\frac{1}{1+\ exp^{1.24*(Distance-3.54)}}\right)*100\ \mid\ \text{High slope} \tag{6}$$

$$\textit{Hypocotyl inhibition}\ (\%) = \left(\frac{1}{1+\ exp^{3.06*(Distance-1.53)}}\right)*100\ \mid\ \text{Low slope} \tag{7}$$

$$\textit{Total inhibition}\ (\%) = \left(\frac{1}{1+\ exp^{1.24*(Distance-3.54)}}\right)*100\ \mid\ \text{High slope} \tag{8}$$

$$\textit{Total inhibition}\ (\%) = \left(\frac{1}{1+\ exp^{2.81*(Distance-1.72)}}\right)*100\ \mid\ \text{Low slope} \tag{9}$$

From these models, inverse regressions were made to estimate the distances that would be necessary to reduce the 50, 90, 95 and 99% of the 2,4-D toxicity present in the surface runoff in each of the cases (Table 4).

Thus, taking as reference riparian strip widths of 2.7 m, which were the maximum lengths of the plots used here, under high slope conditions (2.1%) the reduction in toxicity would be less than 50%, while at low slopes (0.9%) the attenuation of 2,4-D toxicity would be greater.

Table 3
Estimates describing the decay of each end-point (radicle inhibition, hypocotyl inhibition and total inhibition) as a function of distance to the point-source application area, adjusted to a logistic model.

Endpoint	Parameter	Estimate	Standard error	*t* value	*p* value
Radicle inhibition	b	2.49	0.35	7.10	0.0000
	Distance:High Slope	3.85	0.36	10.62	0.0000
	Distance:Low Slope	2.09	0.08	24.60	0.0000
Hypocotyl inhibition	b: High Slope	1.24	0.40	3.07	0.0035
	b: Low Slope	3.06	0.43	7.07	0.0000
	Distance:High Slope	3.54	0.76	4.67	0.0000
	Distance:Low Slope	1.53	0.08	19.92	0.0000
Total inhibition	b: High Slope	1.24	0.38	3.24	0.0021
	b: Low Slope	2.81	0.41	6.92	0.0000
	Distance:High Slope	3.54	0.72	4.93	0.0000
	Distance:Low Slope	1.72	0.08	22.43	0.0000

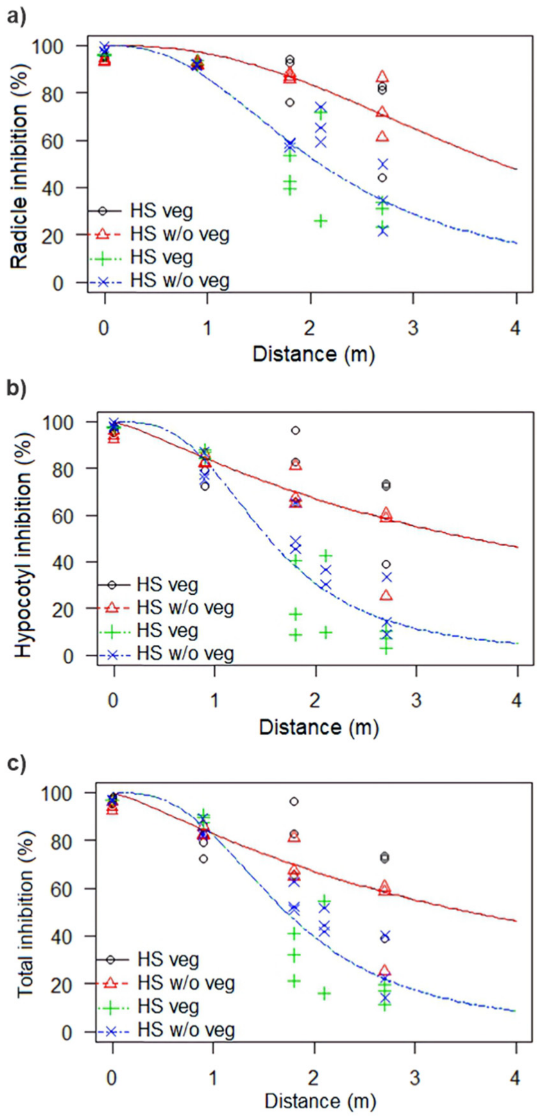

Fig. 4. Toxicity decay curves with respect to the distance according to the study variables: land slope: low slope (LS) and high slope (HS), and vegetation: with vegetation (veg) and without vegetation (w/o veg); evaluated in a) radicle, b) hypocotyl and c) total elongation of *L. sativa* plantlings. Toxicity decay for the high slope site was estimated from the toxicity values of 5% dilutions with respect to the direct samples.

4. Discussion

Riparian vegetation strips have been recommended as a strategy for mitigating the adverse effects of agrochemicals on aquatic ecosystems (Lin et al., 2011), but their use in some countries is still not a common practice. Despite knowing how some characteristics of riparian vegetation strips reduce pesticide arrival to surface water bodies, to our knowledge, this is the first study to assess the relative influence of herbaceous vegetation cover and terrain slope on the ability of riparian strips to mitigate the toxicity of a herbicide in surface runoff through a controlled experiment.

Initially, we proposed the standardized bioassays of *D. magna* and *L. sativa* as bioanalytical tools to evaluate toxicity, which have been widely used to evaluate toxicity of pesticides and environmental samples of agricultural origin (Priac et al., 2017; Rimoldi et al., 2018). However, only the bioassay with *L. sativa* proved to be adequate to address the overall objective of the study; conversely, the freshwater cladoceran *D. magna* did not show significant effects in any of the field samples analyzed. Similarly, the laboratory studies also showed higher sensitivity of *L. sativa* to the herbicide 2,4-D with respect to *D. magna* (Fig. 1), in both cases when standardized and natural water batches were used as the solvent for the herbicide. These results are consistent with the precedents in the literature, where the vascular plants exhibit a greater intrinsic sensitivity to herbicides than *D. magna* (Miller et al., 1985). Although these diagnostic organisms were selected with the aim of being used al bioanalytical tools, the information generated from these toxicity tests provides data about the possible effects of 2,4-D on other biota components. Thus, the effects of the 2,4-D on the germination or elongation of the radicle or hypocotyl of *L. sativa*, are very sensitive indicators for the evaluation of biological effects on plants and could influence the establishment of future seedlings in the riparian vegetation. In addition, the greater sensitivity of this species compared to other autotrophic organisms would allow early detection of toxic effects that could alter the plant communities associated with these environments and consequently the entire ecosystem. Miller et al. (1985), for example obtained EC50s value <0.1 mg L^{-1} for seed germination/root elongation of various species including lettuce, while algae were about 500 times less sensitive. These authors attributed these differential effects to 2,4-D is an auxin analog herbicide, and algae do not have auxin growth regulators, therefore they lack action target-site. Furthermore, *L. sativa* is also an interesting species to consider due to its importance from the horticultural point of view because the area where the experiments were conducted is one of the most productive horticultural belts in Argentina (SCI, 2012). The low slopes existing in the study area configure frequent flooding scenarios, which is a problem not only for the receiving water bodies but also for the neighboring untreated fields.

Table 4
Distances estimated by the best logistic model according to the desired reduction in toxicity at each end-point, for radicle, hypocotyl and total growth inhibition of *L. sativa* plantlings, for sites with high- and low terrain slope.

End-point	*Toxicity reduction (%)*	High Slope (2.1%)				Low Slope (0.9%)			
		Distance	*Standard error*	*LI IC 95%*	*LS IC 95%*	*Distance*	*Standard error*	*LI IC 95%*	*LS IC 95%*
Radicle inhibition	50	3.85	0.36	3.12	4.58	2.08	0.08	1.91	2.25
	90	9.31	1.86	5.59	13.05	5.05	0.68	3.69	6.41
	95	12.58	3.01	6.54	18.63	6.82	1.19	4.42	9.22
	99	24.43	8.05	8.26	40.59	13.24	3.53	6.14	20.33
Hypocotyl inhibition	50	3.54	0.76	2.02	5.07	1.53	0.08	1.38	1.69
	90	20.73	15.88	−11.17	52.64	3.14	0.29	2.56	3.71
	95	37.82	36.29	−35.11	110.74	4.01	0.49	3.02	4.99
	99	142.64	198.20	−255.66	540.94	6.87	1.34	4.17	9.56
Total inhibition	50	3.54	0.72	2.10	4.99	1.72	0.08	1.57	1.88
	90	20.73	15.04	−9.49	50.96	3.76	0.40	2.95	4.57
	95	37.81	34.37	−31.26	106.89	4.91	0.70	3.50	6.32
	99	142.62	187.72	−234.62	519.86	8.83	1.99	4.83	12.83

Likewise, the absence of effects on *D. magna* is also informative because its relative high sensibility respect to another species, would allow us to think that other native aquatic crustaceans may not be susceptible to these environmental concentrations of 2,4-D.

Considering that the effects were significantly lower in both organisms when natural waters collected from a stream was used as a solvent, there seems to be a "protective effect" associated with the environment. Similarly, Knauer et al. (2017) also found a reduction of pesticide toxicity when comparing the toxicity of the natural water batch with standardized mediums. Although there are many factors that condition the differential effects of pesticides on biota depending on the environment in which they are dissolved, one of the main factors that determine the bioavailability of pesticides in natural waters is attributed to their sorption to suspended solids (Laskowski et al., 2010). Environmentally, these results are a step forward in the extrapolation of laboratory results to field conditions and could also show how the toxicity of 2,4-D behaves once it reaches surface water bodies. In addition, with regard to the main objective of this study, changes in the intensity of the effects associated with the dissolving medium should be taken into account when analyzing the results of the field tests; in our experimental rain simulation, runoff dragged soil particles from the field, which may ultimate affect herbicide bioavailability (Yang et al., 2006).

The distribution of a pollutant in the environment as well as its bioavailability are conditioned by the physicochemical characteristics of the compound and by those of the environment itself (Knauer et al., 2017; Lin et al., 2011; Zhang et al., 2010). Therefore, in order to make generalizations of experimental results such as presented in this work, it is desirable to know the general characteristics of the area of experimentation. According to our determinations, both sites used are comparable, with soils suitable for cultivation equivalent to those found throughout the study area (Alcalde et al., 2019).

The search for tools to minimize the negative impacts of pesticides on the environment is a challenge for environmental science. Previous studies have examined the efficiency of riparian strips in retaining sediments and pesticides transported by runoff water, as well as the main environmental variables that influence these processes. The consensus indicates that riparian strips are sometimes effective systems for reducing nutrient- and pesticide losses by adsorption to soil and vegetation (Boyd et al., 2003; Klapproth and Johnson, 2009; Giaccio, 2011; Stutter et al., 2012; Lerch et al., 2017). Vegetation strips improve infiltration and impose resistance to runoff flow by reducing its speed. Sediment deposition increases when the velocity of runoff water decreases, helping to reduce the passage of pesticides sorbed to soil particles into aquatic ecosystems (Boyd et al., 2003; Liu et al., 2008; Giaccio et al., 2017a, 2017b, 2019). In the present study, the efficiency of these areas in reducing the 2,4-D toxicity could also be verified.

Studies related to the buffer capacity of the riparian strips focus mainly on their capacity to reduce the compounds concentration, however, this is not necessarily linearly correlated with a decrease in toxicity, since the latter depends not only on the concentration of the herbicide, but also on its bioavailability. The main physicochemical characteristics that determine the bioavailability and partitioning of a compound in runoff are its solubility in water and its capacity to interact with ground particles. Many herbicides, including 2,4-D, are considered moderately sorbed to soil particles (USDA, 2000). Adsorption occurs when the negatively-charged 2,4-D molecule interacts with the iron and aluminum oxides present in the soil through ion exchange, Van der Waals forces and charge transfer (Barriuso et al., 1992). In the present work, a commercial formulation of the dimethyl amine salt of 2,4-D acid was used, which has a much higher solubility in water than 2,4-D acid, so it is reasonable to assume that a higher concentration of the applied herbicide remained in the aqueous phase. This could be one of the causes, together with the compound's own mode of action, of the high phytotoxicity observed in field experiments, since only the aqueous phase may permeate into seeds. Considering this high solubility in water, the first rain events following the field applications would play a fundamental role in the transport of the herbicide (Vellidis et al., 2002).

In this research, we experimented with the worst conditions that would favor the loss of pesticide, simulating a rain event shortly after its application. The use of rain simulators is quite widespread to evaluate water erosion in soils (Sanguesa et al., 2010; Aksoy et al., 2012), but records of their use to study environmental dynamics of pesticide toxicity are scarce. The use of the equipment to simulate rainfall allowed us to move towards more realistic results than those obtained only with laboratory studies, avoiding, for example, the dependence of natural rainfall of sufficient duration and intensity prior to sample collection.

Based on a review of more than 100 scientific papers, Arora (2014) concluded that water infiltration and sediment retention are critical factors that determine the carrying capacity of pesticides in surface runoff. However, the role of sediment deposition seems to attenuate as the sorption of the compound decreases. According to Pikul and Allmaras (1986), infiltration processes are favored in reliefs with lower slopes since the water remains in contact with the pores and fissures of the soil for longer times, while on steep-slope terrains the velocity of surface runoff is greater and infiltration decreases. On the other hand, Zhang et al. (2010) highlight the role of slopes on riparian strips as a relevant factor for the removal of pollutants and point that a 30-m buffer zone under favorable slope conditions ($\approx$ 10%) removes more than 85% of all pollutants. We observed that in the sectors with low slopes (where infiltration processes predominate), the reduction in herbicide toxicity was greater than that registered in the sectors with high relative slopes. In addition, lethal effects reached 100% in all samples of the high-slope plots when they were tested directly (undiluted sample), which may be interpreted as a lower retention of the herbicide in the area of application and, consequently, a greater input of the herbicide into the plot.

Lerch et al. (2017) concluded that the condition of vegetation and soil in buffer zones are critical to reduce herbicides losses from agroecosystems both in the aqueous phase and adsorbed to sediment particles, with soil moisture, surface porosity and the growth and density of vegetation being the most important factors. Accordingly, the type of vegetation that should be grown in the buffer zone is one of the most discussed topics. Some authors indicate that herbaceous vegetation presents greater density of stems to decrease the speed of water flow and greater surface area for the deposition of sediments; in addition herbaceous plants get established quickly, which may also be advantageous (Klapproth and Johnson, 2009; Giaccio et al., 2017a). Conversely, other authors point out that the woody stems of trees offer greater resistance to water flow, greater stability against possible flooding and higher denitrification rates in forested environments (Lowrance et al., 1997). Giaccio et al. (2017b) studied the relationship between different floristic associations of riparian vegetation strips and infiltration processes, and observed that soils with floristic associations including trees and native herbaceous plants without trees had greater hydraulic conductivity than soils with exotic herbaceous plants without trees. We did not find significant differences in the attenuation of toxicity in relation to the presence or absence of (herbaceous) vegetation with our experimental setup; but it is worth highlighting that extreme conditions were tested (e.g. saturated soils, rain simulated shortly after application, and 2.7 m-long plots of riparian vegetation), so our results must be interpreted in this context. Reichenberger et al. (2007) analyzed roughly 180 publications related to mitigation of pesticide inputs into water bodies and concluded that the efficiency of vegetation strips seems to be higher for strongly sorbed pesticides due to the effect of sediment retention. Considering that 2,4-D is a moderately sorbed compound, much of it is expected to be dissolved and mobilized mainly in the aqueous phase, so in this case the efficiency of the vegetation to retain the herbicide could be reduced. Similarly, Schmitt et al. (1999) and Spatz (1999) could not demonstrate greater efficiency of grassed strips with respect to strips with crops or bare soil in reducing runoff volumes and dissolved pesticide loads in contrast to the observed for strongly sorbing pesticides. In addition, we observed that the reduction in toxicity of this herbicide, and the dispersion of effect values between replicates, increased with distance from the emission source. Other authors studied the influence of microtopography on glyphosate retention (and its main metabolite, aminomethylphosphonic acid - AMPA), within riparian vegetation strips, having found that this herbicide was more readily retained within flow paths than outside them, and that in the presence of preferential flow paths, vegetation characteristics were of minor importance for its retention (Giaccio et al., 2019). All the above leads us to think that, for other studies of this type involving greater distances, a greater number of replicates and the evaluation of their successive rainfall events, may be necessary.

In Argentina the use of butyl and isobutyl esters of active compound 2,4-D were prohibited since 2021 due its high toxicity and volatility, whereas the use and commercialization of 2,4-D salt are still approved. The European Union has evaluated and included the 2,4-D in its list of pesticides approved, renewing its permit in 2016. These regulations are based on physicochemical and toxicological characteristics of the compounds, and even though in many countries riparian strip use is considered a good agricultural practice to reduce the input of pesticides to water bodies, the widths of buffer strips are not always regulated. The riparian habitat comprises areas within at least 10 m on either side of a watercourse (Lewin, 2001). In agroecosystems, this delimitation is rarely met and riparian zones are planted as part of the crop area. The width of the riparian strips necessary to achieve a certain reduction in the runoff water toxicity is site-specific and depends on the characteristics of the compounds in solution. However, several authors point out that the removal of various contaminants (suspended solids, nutrients, organic matter, heavy metals and pesticides) is more important in the first 0–5 m of the buffer zone where the greatest retention occurs (20–60%) (Doyle et al., 1977; Knauer and Mander, 1989, 1990; Vought et al., 1994). On the other hand, Asmussen et al. (1977) found that in a 24.4 m-wide riparian strip of herbaceous vegetation, approximately 30% of the concentration of 2,4-D in the runoff was retained, and 94 to 98% of the suspended sediment was retained in humid and dry soil conditions, respectively. The adjustment of the experimental results from our study to a logistic model indicate that the minimum safety widths to reduce the toxicity of the herbicide by 90% in areas of high relative slope (where less reduction was observed), are in the order of those mentioned by these authors. It is also worth pointing that the experimental design in our work considered a ratio of drainage area to buffer strips in experimental plots that is inverse to that assayed by Arora et al. (1996), who tested two different relationships between drainage areas and buffer strips (15:1 and 30:1), and found no significant differences in the percentage of retention. This lack of differences contributes to the validation of our experimental design, since it could indicate little influence of the drainage area on the buffer effect. However, further studies are needed to validate this hypothesis, as the spatial scale is one of the most discussed points in relation to the extrapolation of results obtained in simulations.

5. Conclusions

The experimental methodology used to evaluate the reduction of the 2,4-D toxicity present in agricultural runoff is innovative and allows obtaining quite realistic results by controlling variables that usually have a strong influence on final results.

Under the experimental conditions considered, in high-slope (2.1%) areas, the 2,4-D toxicity present in surface runoff that reaches riparian areas in a post-application scenario is higher, and the attenuation along these areas is lower than in areas with a low slope (0.9%). On the other hand, the presence of herbaceous vegetation does not seem to influence the attenuation of the toxicity of this herbicide. If a 90% reduction in toxicity is considered acceptable, in sectors of terrain slopes of approximately 0.9% it is recommended to leave an average riparian strip width of 5 m, which can be extended to 6.5 m in more conservative cases. Following the same criterium, if the terrain slope is close to 2.1%, the recommended strip length is 20 m, with 50 m as the upper limit. Although the results obtained are site-specific and our experiments considered worst-case scenarios, the factors evaluated in this study (terrain slope and presence/absence of herbaceous vegetation) are representative of agroecosystem conditions, and therefore constitute a fundamental input for making decisions within the framework of sustainable agricultural management.

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CRediT authorship contribution statement

María Florencia Yorlano: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Pablo Martín Demetrio:** Conceptualization, Methodology, Formal analysis, Writing – original draft. **Federico Rimoldi:** Conceptualization, Methodology, Formal analysis, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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