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Effect of Aquatic Vegetation on the Persistence of Cypermethrin Toxicity in Water

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Abstract Soybean production in Argentina comprises 15 million ha. Cypermethrin is the main insecticide applied amounting 150 g of active ingredient per hectare, thus representing roughly 2.3 thousand tons yearly released to the environment. Toxicity pulses have been observed in small streams draining agricultural basins, most of them sustaining macrophyte growth. Cypermethrin concentrations and its toxicity to the amphipod *Hyalella curvispina* was compared following an addition to laboratory mesocosms with and without a vegetation cover of the floating macrophyte *Lemna* sp. Both concentrations and toxicity decreased faster in the treatments covered with *Lemna*. Fast adsorption of the hydrophobic pesticide to the roots and fronds of *Lemna* was suggested.

Keywords Cypermethrin · Macrophyte · Toxicity persistence · *Hyalella curvispina*

Agricultural production in Argentina has recorded large changes in the last decade. For a long time farmers employed a mixed system of livestock and crops, mainly wheat and corn. Soybean was not a traditional crop. The genetically modified soybean tolerant to the widespread herbicide glyphosate was released to the market in 1996, and quickly adopted by farmers. It was not until the soybean resistant to glyphosate became available that the no-tillage managerial practice was massively adopted. Several applications of the cheap and efficient glyphosate herbicide are needed to avoid weeds during the fallow period. Wheat varieties with a short growing period allowed two harvests per year: wheat followed by soybean. Livestock was moved to marginal areas or concentrated in feedlots. At present, soybean represents roughly one-half of both the total harvest and Argentina's cultivated area (50 million tons and 15 million ha, respectively). Almost all of it belongs to the genetically modified soybean resistant to glyphosate, making Argentina one of the three largest transgenic producers with the United States and Brazil (CASAFE 2008). Enhanced agricultural production increased the amount of agrochemicals consumed. The main insecticides used in soybean cultivation are the pyrethroid cypermethrin, the organophosphate chlorpyrifos, and the organochlorine endosulfan. Cypermethrin represents over 50% of all insecticides currently used (Jergentz et al. 2004a). Cypermethrin is applied at a rate of about 50 g of active ingredient/ha, and is commonly applied 2 or 3 times per growing season, thus representing 2,250 tons yearly released into the environment in the main soybean district.

The application of pesticide mixtures is also a common practice. Whenever glyphosate is applied, the farmers add cypermethrin in the belief that the residual action will prevent insect attacks and thus save the cost of gasoline by avoiding a later application. When the crop reaches a certain size, aerial applications are commonly used to avoid crop damage caused by terrestrial vehicles.

The progressive increase in pesticide consumption in rural basins represents an environmental risk because a variable portion of the applied pesticide is transported to

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the surface watercourses. Marino and Ronco (2005) detected pesticides in rivers going through the main agricultural districts in the Argentine Pampa. Jergentz et al. (2004a, b) showed the occurrence of toxicity pulses in streams draining intensively cultivated basins. Jergentz et al. (2005) detected cypermethrin, chlorpyrifos and endosulfan in a first order stream originating in a soybean cultivated plot in the main soybean district of Argentina. Mugni (2009) emphasized the ephemeral nature of the toxicity pulses and observed that runoff contamination was more important than aerial applications within the regional environment. The smooth relief and the parental soil material rich in fine grained textures determine the abundance of muddy bottoms providing luxuriant macrophyte growth. Macrophytes are known to reduce pesticide concentrations (Moore et al. 2001; Cooper et al. 2004). It might therefore, be hypothesized that macrophytes contribute to the observed rapid dissipation of cypermethrin toxicity in the regional streams.

The present contribution assesses the effect of the common floating macrophyte *Lemna* sp. on the toxicity persistence of cypermethrin to *Hyalella curvispina,* a freshwater amphipod, very common and often dominant in benthic and epiphitic communities of shallow environments in southern South America (García et al. 2010). The purpose of this study was to compare the toxicity persistence in laboratory aquaria containing water and sediments from a typical Pampasic stream with and without a vegetation cover treated with cypermethrin at realistic environmental concentrations.

Materials and Methods

The persistence of cypermethrin toxicity was compared in two treatments conducted in triplicate, one containing only sediments and water, the other with the water surface completely covered by the locally common *Lemna* sp. A negative control treatment was conducted with water and sediments without cypermethrin addition. Water, sediments and macrophytes were sampled from the Sauce Stream, located 15 km southwest of La Plata city, Buenos Aires, Argentina (35° 01′ S, 57° 59′ W). Water and sediments were previously tested for toxicity to *H. curvispina* and did not show toxicity (% mortality $= 0 \pm 0$, $n = 3$). Laboratory microcosms consisted of 60 cm long, 20 cm wide and 20 cm deep glass aquaria. A bottom sediment layer approximately 2 cm thick containing 2.2 kg of wet sediment and 20 L of water were placed in each aquarium. The surface cover of *Lemna* sp. in the macrophyte containing treatment represented a biomass of 150 g dry weight (dw)/m^2. Dissolved oxygen and temperature in the aquaria were measured daily with a Yellow Springs Instrument (YSI 51B), pH with an Orion 250A meter and conductivity with a Hanna 8,733 meter. All meters were calibrated prior to each use utilizing appropriate standards.

The commonly available commercial product Galgotrin, containing 25 g active ingredient/100 mL, was used. A diluted solution was made and added to each aquarium in order to attain an initial nominal concentration of 10 μg/L. The water was gently stirred soon after cypermethrin addition to attain a homogeneous distribution. Water samples were taken after 2 h and 3, 7, 12 and 17 days after pesticide addition from each treatment and the negative control.

Laboratory toxicity tests with *H. curvispina* were performed following USEPA (2000). Ten *H. curvispina* of 10–15 mm length were exposed to 100 mL water in 250 beakers, in triplicate. Tests were conducted without feeding, at 22 ± 2°C and natural photoperiod (15:9 light: dark photoperiod conditions), assessing mortality after 48 h exposure. As a validity criterion for the negative control, less than 10% mortality was considered as no effect (USEPA 2000). Additionally, as a standard laboratory quality control practice, a reference test with copper sulfate (SO_4Cu5H_2O, 99.9% Merck®) was performed. The 48 h LC50 positive control was 265 μg CuII/L. This value lies within the acceptable range in the control chart (225 ± 79 μg CuII/L) conducted by Mugni (2009).

The LC50 of cypermethrin to *H. curvispina* under standardized conditions was determined following USEPA (2000). Tests were performed under static conditions, without feeding, and assessing mortality after a 48 h exposure. Water temperature was 22 ± 2°C, and the photoperiod 16 h light/8 h darkness. The dilution medium was a moderately hard synthetic water prepared following APHA AWWA (1995). Ten *H. curvispina* of 10–15 mm length were exposed to 100 mL water in 250 mL beakers, in triplicate. Different cypermethrin concentrations assayed were prepared using dilution series from a stock solution of 1 mg/L in deionized water. Nominal concentrations assayed were 0.3, 0.2, 0.1, 0.05, 0.025, 0.01 and 0.005 μg/L. The stock solution was prepared using a certified reactive standard (Accustandard®). Cypermethrin concentrations at the three highest doses were determined after 2 h exposure; concentrations were 0.295, 0.236 and 0.09 μg/L, respectively. No mortality in the controls was registered. The LC 50 was estimated by probit analysis.

Pesticide concentration in the water samples was determined after a liquid-liquid extraction in NaCl saturated water enriched with methylene chloride, at pH < 4, followed by roto-evaporation (vacuum: 600 mmHg; bath temperature: 40°C) and a solvent change to n-hexane, in order to reach a final concentration factor of 1,000. Pesticide analysis was carried out by GC-ECD (Carlo Erba, 6,000), equipped with a HP5 column, 15 m and 0.53 ID, N_2

carrier, ramp and detector temperatures: 190–250°C and 320°C respectively (Marino and Ronco 2005). Recovery was tested by spiking environmental samples with known concentrations of the assayed pesticides (72% recovery of cypermethrin). Sample storage at −20°C was also tested to assess holding time of the tested pesticides. J. T. Baker solvents were used for pesticide analysis. A standard of cypermethrin was provided by SENASA (Argentine National Agriculture and Food Sanitation and Quality Service). Detection limits were 0.025 µg/L cypermethrin.

The non parametric Wilcoxon sign test was used to compare *H. curvispina* mortalities and cypermethrin concentrations between treatments (with and without *Lemna* sp.) throughout the experiment. This method was selected because of the heterogeneity of variances between treatments in the mortality data. Cypermethrin disappearance from water in each treatment was fitted to a potential equation using iterative non-linear estimation methodology.

The half-life of cypermethrin in each treatment was calculated by solving the equation for the log transformed linear regression. The LC50 estimation for the unvegetated treatment and the standardized test were conducted following probit analysis. The LC50 for the vegetated treatment was estimated by the Spearman Karber method because the available data were inappropriate for probit analysis.

Results and Discussion

The Sauce Stream, from which the water was taken to perform the experiment, has high organic and suspended matter content: mean TOC amounted to 17 mg/L and suspended solids to 86 mg/L (Mugni 2009). In the sampling taken one week before the experiment, bicarbonate amounted to 78 mg/L and hardness to 49 mg $CaCO_3$/L (Mugni 2009).

Table 1 shows the measurements performed in the water in both treatments throughout the experiment. There were no significant differences between treatments. The aquaria were kept adjacent to a window, maintaining the natural photoperiod of roughly 15/9 h, but without receiving direct sunlight. Phytoplankton was not abundant and oxygen concentrations were rather low.

Cypermethrin concentrations were significantly lower ($p < 0.05$) in the aquaria covered with *Lemna* sp. throughout the experiment (Fig. 1). Estimated half-lives were 37 and 46 h in the vegetated and unvegetated treatment, respectively. Twelve days after cypermethrin application, concentrations were 0.09 and 0.2 µg/L, respectively. Seventeen days after addition, the concentration in the *Lemna* sp. treatment fell below detection limits, but was measured in the *Lemna*-free water treatment (0.09 µg/L). The rapid disappearance of cypermethrin from the water observed in the present study was in accordance with previously reported half-lives of 1 (Farmer et al. 1995) to 2 days (Friberg-Jensen et al. 2003) for unfiltered water and from 1 (Friberg-Jensen et al. 2003) to less than a day (Crossland et al. 1982; Muir et al. 1985) for filtered water.

Figure 2 shows the *H. curvispina* mortality in exposures with water sampled at different times throughout the experiment. The water samples from the aquaria covered with *Lemna* sp. showed a significantly lower mortality ($p < 0.05$) than the aquarium with free water surface. In both treatments mortality attained 100% up to a week following cypermethrin application. Twelve days after application mortality decreased in both treatments. Mortality in the controls was always below threshold level for no effect concentration (USEPA 2000). Estimated LC50 were 0.13 and 0.09 µg/L for the unvegetated and vegetated treatments, respectively. Mortalities of 100% occurred in 3 of 5 samplings in both treatments, and 0% in the last sampling of the vegetated treatment, without attaining differences among replicates. No confidence limits could be ascertained for the vegetated treatment.

The 48 h LC50 of cypermethrin to *H. curvispina* assayed in an independent experiment carried out with laboratory synthetic water of intermediate alkalinity, following USEPA 2000, was calculated to be 0.038 µg/L (95% confidence limits: 0.026–0.072). Higher toxicity in laboratory synthetic water than in stream water has been reported (Carriquiriborde et al. 2007), and was interpreted to be caused by the organic matter content of the latter. Brander et al. (2009) reported a 96 h LC50 of permethrin to *H. azteca* of 0.05 µg/L measured in synthetic water. Adam et al. (2009) reported a 96 h LC50 of cypermethrin to *Gammarus pulex* of 0.09 µg/L also measured in synthetic water. Friberg-Jensen et al. (2003) estimated a 48 h LC50 of cypermethrin for the total abundance of cladocera and copepoda of 0.06 and 0.11 µg/L in mescocosms suspended in a eutrophic shallow lake. Present results suggest that *H. curvispina* appears to be among the most sensitive aquatic organisms to cypermethrin (Crossland et al. 1982; Friberg-Jensen et al. 2003).

Table 1 Means and ranges for water characteristics in toxicity tests with and without *Lemna* present

	Temp. (°C)	pH	Conductivity (µS/cm)	Dissolved oxygen (mg/L)
Unvegetated	18.7 (17.5–19.0)	7.4 (7.1–7.7)	203 (168–230)	3.5 (2.0–4.8)
Vegetated	18.7 (17.5–19.0)	7.4 (7.0–7.5)	209 (165–242)	4.8 (2.8–6.5)

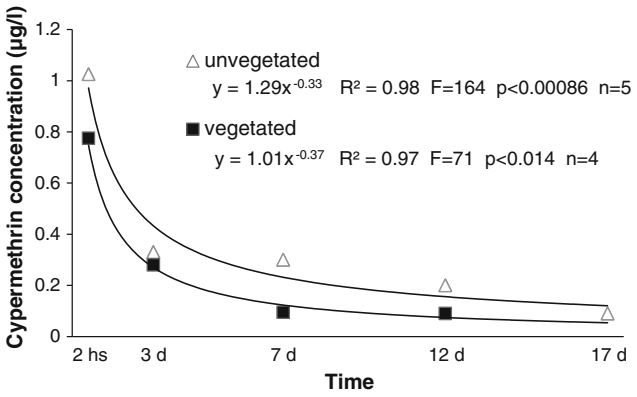

Fig. 1 Cypermethrin concentrations in water of aquaria with and without *Lemna* cover. Reported values are the means of two replicates

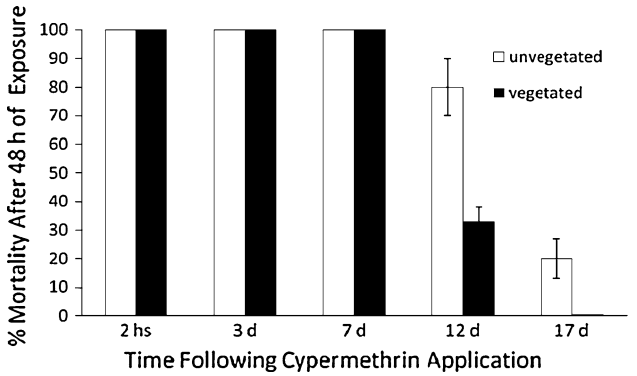

Fig. 2 Percentages of *H. curvispina* mortality in 48-h exposures to aquaria water at various times following cypermethrin application

Both exposure assays and chemical analysis consistently showed decreased toxicity and faster cypermethrin dissipation in the *Lemna* sp. treatments. Because cypermethrin is strongly hydrophobic (log Kow = 6.6, Friberg-Jensen et al. 2003), differences in concentrations between treatments suggest fast adsorption to biological surfaces.

Several authors have studied the effect of macrophytes on pesticide fate. Leistra et al. (2003) studied the fate of the pyrethroid lambda-cyhalothrin in enclosures with different densities of the submerged macrophyte *Myriophyllum spicatum.* Fast dissipation from the water was reported, with only 24–40% of the applied dose remaining on the first day after application. Dissipation was faster at the treatments with the highest plant density, attaining 150 g dw/m^2, where lambda-cyhalothrin content in the macrophytes decreased by 50% of the applied dose on the first day after application. Cooper et al. (2004) studied the fate of the pyrethroid esfenvalerate in a simulated runoff event on a vegetated ditch. Concentrations in water fell below the detection limit the first day after application; 86% of the added pesticide was retained in the vegetation, while 14% was present in the bottom sediments. Moore et al. (2001) showed in a similar experiment that 87% of the added lambda cyalothrin was stored in the vegetation in the first hour following the application. Bouldin et al. 2005 showed faster lambda cyhalothrin toxicity mitigation in microcosms containing the macrophytes *Ludwigia peploides* (water primrose) and *Juncus effusus* than in unvegetated microcosms.

The above mentioned studies determined pesticide retention by macrophytes. Decreased pesticide concentration in water resulted in lower exposures for aquatic organisms and therefore, shorter toxicity persistence. Macrophytes not only provide biological surfaces to bind hydrophobic compounds but also sediment detritus, contributing additional binding sites for insoluble compounds. Moreover, macrophytes sustain a community including periphytic algae and bacterial assemblages.

In our experiment, there was a large difference between the nominal initial concentration and the first cypermethrin determination performed 2 h later, which is consistent with the exponential shape of the dissipation fitted function, suggesting a fast and large initial adsorption to biological surfaces later followed by slower uptake and degradation. Our research, performed at realistic field macrophyte biomass and pesticide concentrations, demonstrated the significance of vegetative attributes in the mitigation of the toxicity persistence of cypermethrin to an important representative of the resident freshwater community.

Hyalella curvispina is a widely distributed organism attaining high densities in shallow waterbodies of southern South America and ranks among the species most sensitive to pyrethroids; it could therefore, be proposed as a sentinel species for regional environmental risk assessment.

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