MONITORING OF ATRAZINE IN SURFACE WATERS OF AN AGROECOSYSTEM IN THE PROVINCE OF CÓRDOBA (ARGENTINA) USING AN IMMUNOASSAY TECHNIQUE

Mariana García[1], Verónica Lutri[2], Mónica Blarasín[2], Edel Matteoda[2], Susana Bettera[1],

[1]Departamento de Microbiología e Inmunología. Universidad Nacional de Río Cuarto.
Ruta Nacional Nº36, Km 601. (5800) Río Cuarto, Argentina

[2]Departamento de Geología, Universidad nacional de Río Cuarto.
Ruta Nacional Nº36, Km 601. (5800) Río Cuarto, Argentina
E-mail: mcgarcia@exa.unrc.edu.ar

ABSTRACT

Although the use of atrazine has been banned and restricted in several parts of the world, in Argentina this herbicide is still being used. The atrazine detection was made with a fast and relatively cost-effective immunoassay test whose major advantage lied in its ability to quantify with high accuracy both atrazine and its main metabolite, a useful aspect for monitoring and regulatory programs. The atrazine values found in surface water (0.05– 15.6 µg/L) and rainwater (0.23 – 0.73 µg/L) is an undeniable indicator of the impact that agricultural activities generate on the water resource in the Aeolian fluvial plain of Argentina. The major values were detected in the humid periods which also coincides with the herbicide application time.

Key Words: atrazine, ELISA, surface waters, pesticides

INTRODUCTION

Water is of transcendental importance for the humanity's development since it is a vital resource for people activities and also is an important variable in diverse processes in all ecosystems (Blarasin *et al.*, 2005).The pollution of aquatic ecosystems is directly related to the land uses. In agricultural ecosystems, surface water and the unconfined aquifer can be impacted by the arrival of mobile and/or persistent contaminants from the application of agrochemicals (pesticides and/or fertilizers). Pesticides are substances or a mixture of substances that are intended to control the vectors of human and animal diseases, as well as unwanted species that cause harm or that interfere with agricultural and forestry production (USEPA 2010). Pesticides suffer photo-decomposition, chemical and biological degradation, adsorption-desorption and volatilization processes in the environment. In addition there are various mechanisms by which they can be transferred from one compartment of the environment to another, highlighting the atmospheric processes and all those hydrological phenomena (infiltration, runoff, percolation, etc.) that allow the advective/dispersive/reactive transport of these compounds in surface and groundwater (Lutri *et al.,* 2018). Water is the main diffusing medium of these compounds or their metabolites to other ecosystems where they have harmful effects (Singh and Walker, 2006). With the population growth in recent years and, consequently, the scarcity of farmland and the destruction of crops by pests, the use of pesticides such as atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), herbicide of the s-triazine type, increased significantly and is one of the most common in the world

(Derakhshan *et al.*, 2018). Atrazine is an herbicide used in pre and post-emergence for the weed control and broad-leaved herbs in rice, wheat, corn and sorghum crops. Many studies have determined that atrazine is an endocrine disruptor and gives rise to potential risks to human health. When applied to the soil, it can remain chemically intact or may be degraded. Three of its main metabolites are desethylatrazine (DEA), deisopropylatrazine (DIA) and hydroxytrazine (HA). DEA and DIA have been found in both groundwater and surface water (Yu *et al.*, 2017). The HA metabolite is more commonly detected in groundwater than in surface water. It is most likely that the presence of atrazine and its metabolites in surface waters will be intermittent, particularly in flowing waters, but groundwater contamination is relatively persistent. The rate of degradation normally decreases when depth increases and atrazine, therefore, can be quite stable in groundwater (WHO, 2011).

Regarding to toxicity, it has been reported that DEA and DIA have structural and toxicological similarity with ATZ. The metabolite HA does not have the same mode of action as ATZ, DEA or DIA, and its main effect is renal toxicity. Therefore, all three compounds have been regulated by the World Health Organization (WHO) (Yu *et al.*, 2017).

The guideline values for drinking water according to WHO for ATZ and its metabolites are 100 μg/L for ATZ and 200 μg/L for metabolites that include chlorine in its chemical composition (DIA and DEA) (WHO, 2011). In Europe, the concentrations of this pesticide in groundwater and in drinking water must not exceed 0.1 μg/L for the single compound and 0.5 μg/L for the sum of all pesticides according to the European Union Guidelines 98/83/EC (Commission, 1998; Yu *et al*., 2017; Bohn *et al*., 2011). In China, the maximum contaminant level of ATR in drinking water is 2 μg/L (Geng *et al*., 2013), but these standards do not include their metabolites (Yu *et al*., 2017). Although there are no regulations in the Argentine Republic, in the province of Córdoba it is regulated by the Provincial Drinking Water Quality and Control Standards that indicate that the limit of atrazine for drinking water is 3 μg/L (SSRH, 2016).

Atrazine is considered an environmental pollutant due to its slow biodegradation. In general, its average life is variable, but it can last up to 360 days and even longer (Derakhshan *et al*., 2018). In the soils it has a persistence in a range of 15 to more than 60 days, depending on the soil composition and the microbial activity. Also influence parameters such as pH, temperature, water content among others. Atrazine is moderately soluble in water (30 mg/L at 20 °C) and can be washed from soils during heavy rains that commonly occur during spring and autumn runoff in temperate climates. Also it was often observed in runoff water and groundwater in areas of high application rates (Byer *et al*., 2011; WHO, 2011; Bécher Quinodóz, 2013; Lutri *et al*., 2015; Lutri *et al*., 2018).

Most monitoring programs have economic constraints that limit the number of samples to be analysed, generating problems in either spatial or temporal resolution in research programs. The cost-effectiveness is important to optimize monitoring and maximize information as long as there would not a significant reduction in the accuracy and sensitivity of the analysis. Enzyme-linked immunosorbent assay (ELISA) is used as a fast, cost-effective methodology and does not require, in general, any pre-treatment of the sample (Byer *et al*., 2011). Immunoassays are analytical methods that are based on the interaction of an analyte with an antibody that is recognized with high affinity and specificity. They are simple trials and do not require sophisticated instrumentation. In addition, a large number of samples can be analysed simultaneously with accuracy and precision comparable to those achieved by chromatographic methods, even for drinking water (Abad *et al*., 2001; Toldrá and Reig, 2006). Previous studies have compared gas chromatography with ELISA and indicate that this is a highly reproducible methodology with great advantages in monitoring programs with the aim of doing complementary studies to increase both spatial and temporal resolution. One of the great advantages of the technique ELISA is the possible automation of reading and, therefore, its objectivity (Byer *et al.*, 2011). The immunoassays offer certain analytical advantages to the conventional instrumental methods (or chromatographic reference) and with comparable results for the analysis of pesticide residues. They are simple and offer quickness, high sensitivity and selectivity, also highly efficient and profitable, ideal for detecting low levels of a specific analyte in a large number of water samples and minor components, including organic contaminants in the environment and food (Feng *et al*., 1990; Garcés García, M. 2008).

The central-southern region of the province of Córdoba is characterized by concentrating productive activities (agricultural, livestock and dairy) that constitute an important base of its wealth. The livestock activity is mainly extensive (cattle) although the intensive breeding has increased for pigs, cattle and hens. Agriculture is extensive, being characterized by soybean and corn although subserviently wheat, sorghum and peanut may be found. The direct seeding or non-till farming technique is used which leads to a strong agrochemical use (Blarasin *et al*., 2005; Blarasin *et al*., 2014). Atrazine is an herbicide frequently used in Argentina, and its behaviour is strongly controlled by the characteristics of the soil and the system management (Hang *et al*., 2003; 2007; Becerra *et al*., 2013)

In this framework, the aim of this study was to carry out an expeditious water monitoring using immunoassay tests to elucidate how impacted or not with atrazine is the surface water in a typical agroecosystem of the south-central region of the Córdoba province.

MATERIALS AND METHODS

Location of the study area

The studied area corresponds to a sector of the Pampa plain of Córdoba, typical of the south-central region of Cordoba province taking into account its geomorphological, hydrological, lithological, edaphic and land use characteristics (Degiovanni, 2005; Blarasin *et al*., 2005; 2014). The area (3,500 km^2) is located between the coordinates 32 ° 30 - 32 ° 14 'S and 63 ° 14' -64 ° 14 'W. It is placed south of the Ctalamochita River in the eastern slope of the Las Peñas and the Los Cóndores Mountains, formed by a broad Aeolian fluvial plain, being Villa Maria city the most important urban area in this region.

From the hydrological point of view, the area is crossed by the Cabral stream, of permanent regime, and by other streams with headwaters in the mountainous area which carry waters mainly in rainy seasons and infiltrate them in the piedmont. There are also small lagoons located in low topographical areas and artificial drainage channels.

To perform the analysis of the month distribution of rainfalls, the Hernando city series covering the longest regional period (97 years) was chosen.

Collection of water samples

For the detection and quantification of atrazine, 6 sites or water monitoring stations corresponding to surface water samples from lagoons, channels and streams were established. The sampling campaign was carried out in the humid season (November 2017) and in the dry season (August 2018). Additionally, a sample of harvested rainwater (P59) from a farmer's storage tank was collected (Figure 1). The sampling stations were identified with coordinates of the global positioning system (GPS) Table 1. For all samples, 100 mL were collected in clean glass bottles, remaining at 4 ° C until they were analyzed by ELISA immediately when arrived at the Food Microbiology laboratory (UNRC).

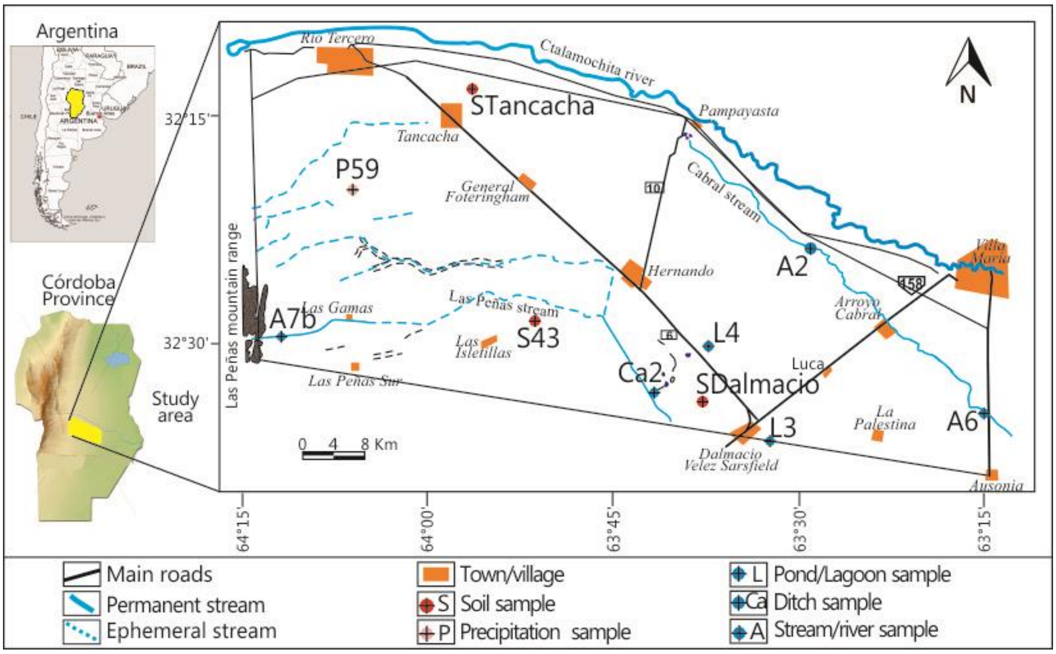

Figure 1: Location of the study area

Collection of soil samples and preparation of soil extracts

Taking into account that the soils are the bodies that receipt the pesticide application, and in order to compare with pesticide values in water, three soil extracts identified as: SDalmacio, S43 and STancacha were prepared and included in the analysis. The soils are located in different positions within the study area, and have different textural and compositional characteristics. They are named according to SAGyP–INTA (2009) as: STancacha: an undifferentiated soil complex in an alluvial plane (sandy-loam, NW sector of the Ctalamochita alluvial paleofan), S43: an Entic Haplustoll soil (silty loam, central sector) and SDalmacio: a Typical Natracualf soil (loam silty clay, SE sector).

The soil samples were collected in clean bags and then, once in the laboratory, the corresponding extracts were obtained at the UNRC Dept. of Geology Laboratory. Thus, 100 g of sediment were weighed to which 50 ml of distilled water were added (pH: 5.8 and CE: 5 µS/cm) and then, the pH of the saturated paste was measured (5.3). After that, it was underwent to mechanical agitation for 30 minutes. Then, it was centrifuged 15 minutes at 3,000 rpm, the supernatant liquid was extracted and finally it was filtered, keeping refrigerated until the atrazine determination.

To determine atrazine in soils, according to the bibliography (Olarte *et al.*, 1999; Acevedo Buitrago *et al.*, 2000; Fuentes *et al.*, 2003; Da Cunha *et al.*, 2012) some soil extracts are prepared with methanol, followed by an extraction in solid phase, cleaning and subsequent determination with UHPLC MS / MS with UV detection. Nevertheless, for this particular case, it was decided to carry out an experiment as close to reality as possible, with the objective of knowing the capacity of rainwater to extract atrazine from the soil without any additional chemical addition, knowing that in the field, the reactions are carried out only by the contact of the soils and the rainwater after infiltration processes.

The mass of the solute released (Sd) was determined by the difference between the initial concentration of the initial aqueous solution (Cci) and the concentration in the final aqueous solution (Ccf) after its interaction with the used soil mass, according to the following equation:

$$Sd = \frac{(Cc_f - Cc_i)\, V}{M}$$

The concentration of atrazine obtained in soil extracts would indicate the amount of atrazine per kilogram of soil that is available to be mobilized from the soil solution, by rainwater, by vertical transport to the unconfined aquifer, or lateral transport to the surface water bodies.

Thereby, the total sampling in the humid season consisted of 6 surface water samples, 3 soil extracts and 1 sample of harvested rainwater. The sampling in the dry season comprised 4 surface water samples (for the permanent water bodies), 3 soil extracts and 1 sample of harvested rainwater.

Atrazine analysis by ELISA technique

The atrazine detection was performed by competitive enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's specifications. The commercial kit Abraxis Atrazine ELISA (Abraxis, Inc. Warminster, PA) was used for this purpose. The colour intensity was determined by reading the optical density at 450 nm using a Labsystems Multiskan MS plate reader. The value of the optical density obtained is inversely proportional to the analyte concentration in the sample. The concentrations were expressed as μg/L. The detection limit established by the manufacturer of the test kit is 0.04 μg/L of atrazine.

RESULTS AND DISCUSSION

Analysis of the temporal distribution of rainfall

The average monthly rainfall distribution curve obtained for the Hernando series (Figure 2) shows, in agreement with that obtained by Capitanelli (1979), Blarasin (2003) and Maldonado (2014). The 79.3% of the rainfall concentrates from October to April, registering the maximum values in December and January with 124.4 and 110.6 mm respectively. The average precipitation value for the dry season is 26 mm.

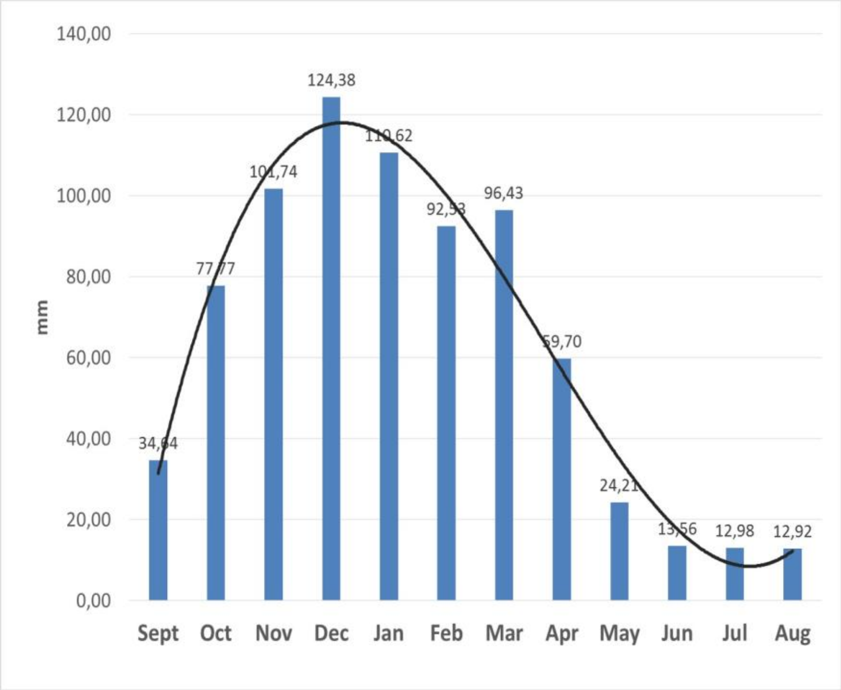

Figure 2: Distribution of average monthly rainfall. Hernando series 1921-2018

Surface water and rainwater

During the monitoring carried out in the humid season, atrazine residues were detected in 86% of the analysed water samples, including the surface water samples and the rainwater sample (n=7). The mean concentrations detected ranged from 0.22 (A6) to 15.6 µg/L (Ca2) (Table 1). The highest value corresponded to a channel that receives water by surface runoff from the surrounding farmlands after the rains.

The analysed stream samples correspond to different situations: A6 pertains to the Cabral stream, a permanent water course located in the fluvio-aeolian plain and A7b belongs to the Las Peñas stream, in the piedmont zone, just 2 km from the Sierra de Las Peñas, corresponding to a site where the water course still has permanent regime. The values found for atrazine are 0.22 and 0.62 µg/L for A6 and A7b, respectively. The found atrazine value for A2, located in the Cabral stream, is very close to the detection limit of the technique, reason why it was considered negative. The values found in the lagoons L3 and L4 were 0.24 and 0.52 µg/L, respectively. The channel (Ca2) has the highest measured atrazine value (15.6 µg/L). The high value in Ca2 is linked to the fact that it is located in hydrological discharge area, where very low topographical slopes and low water velocity (0.09 m/s) predominate, so the dilution processes caused by hydrodynamic dispersion are diminished.

The lentic aquatic ecosystems present a greater contamination degree than the lotic ecosystems. This is interpreted as the result of the greater water flow velocity in the lotic systems and the consequent possibility of greater dilution, by hydrodynamic dispersion, especially in periods of higher precipitations. Although the artificial channels are considered as lotic systems it must be taken into account that they have very low water velocity and receive pesticide inputs from upstream and surrounding farming lands, existing then minor dilution possibilities.

The harvested rainwater presented an average ATZ concentration of 0.73 µg/L during the humid period. Similar values were found by Alonso *et al*. (2018) who studied the presence of

ATZ in rainwater in the agro-productive region of the Pampean region in Argentina finding average concentrations of 0.93 µg/L. The maximum ATZ value measured by these researchers in rainwater (26.9 µg / L) was higher than those reported in the USA (19 µg/L) and in other countries, possibly as a consequence of the higher agronomic doses used in Argentina (Vogel *et al*., 2008).

With regard to the monitoring carried out in the dry season, atrazine residues were detected in 80% of the water samples, including surface water samples and the rainwater sample (n=5). The mean detected concentration ranged between 0.06 (A6) and 0.23 µg/L (L4 and P59) (Table 1). The channel, which only transports water in the summer, could not be sampled during this period. Also, the sample from the monitoring point A7b could not be collected for further analysis because it corresponds to an ephemeral stream.

Table 1. Location of monitoring sites and average atrazine concentrations in surface water, soil extracts and rainwater samples

ReferenceLatitude Longitude			**Mean concentration of ATZ (µg/L)**	
Surface water			**Humid season**	**Dry Season**
(Ca2)	32°33'36.30"S	63°41'51.90"O	15.6	-
(L4)	32°30'37.60"S	63°38'22.10"O	0.52	0.23
(L3)	32°37'9.35"S	63°32'30.24"O	0.24	0.22
(A6)	32°34'51.20"S	63°14'47.80"O	0.22	0.06
(A2)	32°23'16.50"S	63°29'52.20"O	0.05	<0.04
(A7b)	32°29'53.40"S	64°12'11.50"O	0.62	-
Soil extracts			**Mean concentration of ATZ (µg/Kg)**	
			Humid season	**Dry season**
(SDalmacio)	32°34'47.68"S	64°12'11.50"O	ND	4.7
(STancacha)	32°12'40.90"S	64° 0'18.60"O	0.3	4.65
(S43)	32°28'50.77"S	63°51'37.65"O	1.2	4.7
Rainwater			**Mean concentration of ATZ (µg/L)**	
			Humid season	**Dry season**

(P59)	32°19'30.76"S	64° 6'59.05"O	0.73	0.23

Ref. (-): Not sampled; ND: not detected. (LD: 0.04 µg / L)

If the results obtained between the humid and the dry season are compared, the atrazine values were higher in the humid period at each monitored site. This is also related to the application period of the pesticides. It is noteworthy that the ATZ is applied to the soil during low coverage, coinciding generally with periods of high rainfall. This situation could have favoured the herbicide transport by runoff, explaining the frequent detection of ATZ in water bodies in average concentrations that oscillated between 0.22 and 15.6 µg / L. On the other hand, although the detected ATZ values in the dry season are lower than in the wet period (0.06 to 0.23 µg/ L), it would demonstrate the persistence of this compound in the studied aquatic environments.

The analysis of the AZT measured concentrations was then compared with the regulatory framework in the national scope Law 24,051 related to hazardous wastes (Regulatory Decree 831/93, 1993) and the Regulations of the Water Resources Office of the Province of Córdoba (SSRH), which has been implemented in 2016 and establishes the Provincial Standards for Water Quality and Control for Drinking Water. The obtained results were also compared to the international guidelines for the quality of drinking water (WHO, 2011) and the regulations of the United States Environmental Protection Agency (US EPA, 2009), Directive of the European Community (directive 98/83/EC, 1998) and the Canadian Environmental Quality Guidelines (CCME, 2006). This last regulation also contemplates the impact of toxic compounds on aquatic organisms (Table 2).

With the exception of the average concentration detected in the channel, the average concentrations of ATZ measured in the rest of the water samples did not exceed the values established by the regulations of the SSRH at the provincial level, Law 24.051 at the national level and the WHO, the USEPA and the CCME at the international level. However, it should be noted that the average concentrations recorded in all the samples exceeded the maximum value established for ATZ by the EC (0.1 µg/L) and, additionally, samples Ca2, L3, A7b and P59 exceeded the value established by the CE for ATZ plus its metabolites (0.5 µg / L) (Table 2).

Table 2. Reference values for atrazine in drinking water established by national and international regulations

Reference values (µg/L)					
WHO (2011)	US EPA (2009)	CE (1998)	CCME (2006)	SsRH (2016)	Law 24,051 (1993)
100 (ATZ, DEA, DIA) 200 (HA)	3	0.1 (ATZ) 0.5 (ATZ + their metabolites)	1.8	3	3

In some studies of ATZ detection in surface water in the province of Córdoba the concentrations exceed the established limit (0.1µg/L) by the European Economic Community (Bachetti *et al*., 2013; 2015, Becher Quinodóz *et al*., 2013; Lutri *et al*., 2015). In another study carried out in different freshwater courses (river, channels and streams) in the Ctalamochita River basin (Córdoba) ATZ values were determined in average concentrations ranging from 0.06 to 2 µg/L (Urseler *et al*, 2014).

Soil extracts

Regarding to soil extracts, in the humid season 67% of positive samples were obtained (n=3). The highest concentration of the pesticide was obtained in soil S43 and corresponded to 1.2 µg/kg (Table 1). In the dry season, the concentrations of atrazine far exceeded the values found during the humid season. The degradation of atrazine in the soil is conducted by both biological and chemical reactions. Biological degradation occurs through the activity of microorganisms and is considered as the main process by which this herbicide is transformed. Weather is a main factor related to the degradation herbicide rate, mainly controlled by soil humidity and temperature. Thus, degradation accelerates in warm and humid regions; and it slows down in dry and cold conditions (Montoya *et al*., 2011).

In the study area, the finding of higher atrazine values during the dry season, compared to the humid season, may be due to the pesticide application in winter to prepare the soil for summer. During winter the microbial activity is lower therefore the ATZ degradation diminishes. In studies carried out in agricultural fields in Georgia, California and Minnesota (USA), atrazine expectance of 13, 58 and 261 days were obtained, respectively. The differences between these expectances are attributed to the temperature variation between the studies, concluding that atrazine is more persistent in cold weather, given the lower microbial activity in those climates (USEPA, 2009). In addition to having less water during the dry season the leaching process is diminished, then the pesticide is more concentrated in the soil. The mobility of atrazine in the soil depends on the texture, the content of organic matter, the soil drainage characteristics and the amount of water applied either by rain or irrigation. Clay soils with a high content of organic matter tend to retain atrazine on the soil surface which implies a limited presence of ATZ in the drained water (Hang *et al.*, 2010). In sandy soils, atrazine tends to infiltrate and transport with the drained water (Montoya et al., 2006).

The obtained results allow to determine that in the soils with a sandy texture (Tancacha soil), the rainwater is able to remove the atrazine that is poorly adsorbed to the clasts. The amount of clay in this soil is only 8-13%, being one of the parameters that mediates to a greater extent its capacity for the herbicide retention. In Dalmacio's soil, however, atrazine was not detected in the soil solution. It has a clay percentage of approximately 19%, which generates a greater possibility of herbicide retention diminishing its mobilization and transport by rainwater. In soil S43, the atrazine concentration was the highest (1.2 µg/kg), which may be due to its higher content of organic matter, given that up to 38 cm it has more than 1% of OM, characteristic that promotes the herbicide retention and its future degradation.

ELISA test

In relation to the detection method for ATZ, it is important to highlight the great usefulness of the ELISA tests for the detection and quantification of pesticides such as atrazine. During 2004, an atrazine monitoring program was carried out on water samples from water treatment plants in the USA using the Abraxis ELISA test kit, highlighting the authors the accuracy of the used methodology (Graziano *et al.*, 2006). Sánchez Camazano *et al.* (2005) used enzymatic immunoassay tests for spatial and temporal evaluation of the atrazine in surface and groundwater, obtaining concentrations in the range of 0.04-25.3 µg/L and 0.04-3, 45 µg/L, respectively. These concentrations were generally linked to the rainy season. The authors also correlated the results obtained with enzymatic immunoassays by the ELISA test with those obtained by high performance liquid chromatography (HPLC), finding correlations of 0.96 (p <0.001), which supports the great usefulness of this tests. Other authors suggested that the values obtained from the determination of ATZ by ELISA can be used in addition to the analyses performed by GC-MS to improve the spatial and temporal resolution of water quality monitoring studies (Byer *et al*, 2011).

In contempt of their important advantages, immunochemical assays are not exempt from limitations. The response of the method to analogous compounds, metabolites or other components that may be present in the sample, is another problem to be taken into account. Therefore, selectivity (cross-reactivity) and the matrix effect are the two factors that provide the greatest uncertainty to immunochemical methods (Garcés García, 2008). The manufacturer of the ELISA kit lists several possible triazine herbicides that may confuse the accuracy of detection and measurement. This method possesses cross-reactivity with 2 of ATZ metabolites: DEA and HA and 4 other triazine herbicides. Byer *et al.* (2011) performed comparative studies between the ELISA test and GC-MS confirming the presence of these compounds, namely: simazine, DEA, cyanazine and ametryn. The main metabolite of atrazine, DEA, was added to the GC-MS comparison by changing the slope of the line without affecting the linear regression, which indicates an overestimation of the ELISA kit that can be explained by a cross-reaction with similar metabolites in the sample.

These data show a much closer agreement between the techniques, possibly indicating that the ELISA kit detects both atrazine and its metabolites. These results would indicate a great advantage of immunoenzymatic analysis, because it has been reported that the metabolite DEA has a stronger effect than ATZ itself on aquatic life. Both substances are particularly recalcitrant to biodegradation and have strong leaching properties (Mauffret *et al.*, 2017). The advantage would lie in the ability of the ELISA technique to quantify both ATZ and its main metabolite, indicating the sum of the concentrations of both substances harmful to human and animal health.

CONCLUSIONS

Although the use of ATZ has been banned and restricted in several parts of the world, in Argentina this herbicide is still being used without any restriction and the results show that it affects water quality in one of the most important agricultural areas of the country. The data collected through the ELISA test determine the impacts of agricultural activities on surface waters in the fluvial- aeolian plain in the central part of the province of Cordoba.

From the obtained results it possible to affirm that the atrazine detection in surface water and rainwater is an undeniable indicator of the impact that agricultural activities generate on atmospheric water and surface water bodies. The sampling period also coincides with the period of herbicide application, so it is expected that these values decrease over time, as it moves away from the fumigation period. Taking into account the atrazine values obtained in surface water and rainwater samples, it is concluded that they were higher in the humid season compared to the dry season, for each monitoring site. This situation is related to the major water availability and consequently the higher possibility of herbicide transportation but is also strongly related to the herbicide application epoch. The lentic aquatic ecosystems showed a greater contamination degree than the lotic ecosystems. This is interpreted as the result of the greater water flow velocity in the streams and the consequent possibility of greater dilution by hydrodynamic dispersion, especially in the periods of higher precipitations. Considering that there is already a provincial regulatory framework, we can say that, with the exception of the channel, where there is a very low water flow velocity, the analysed samples comply with the local regulations

In reference to the studied soils, the obtained results make it possible to determine that in a soil with a sandy texture and lower clay percentage, rainwater is able to remove atrazine poorly adsorbed to the clasts which may favor the herbicide transportation to the water bodies. On the other hand, soil with higher percentage of clay or organic matter, show higher atrazine concentration, a situation that promotes the retention of pesticide and its probable future degradation.

In the present study it was possible to demonstrate the advantage of using the enzyme-linked immunosorbent assay (ELISA) for the spatial and temporal atrazine monitoring program in surface water, rainwater and soil extracts. A major advantage would lie in the ability of the ELISA technique to quantify both atrazine and its main metabolite, indicating the sum of the concentrations of both substances harmful to human and animal health. The ELISA analytical methodology was fast and easy used, relatively cost-effective and did not require, in general, any pre-treatment of the sample.

It is recommended in the future to carry out a major monitoring campaign to evaluate the temporal evolution of this herbicide in surface waters. It is also suggested to perform groundwater monitoring to determine if atrazine could reach the unconfined aquifer.

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