

# ATRAZINE BOUND RESIDUES FORMATION AND DISSIPATION IN SUBTROPICAL SOIL UNDER SWINE WASTEWATER APPLICATION

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**ABSTRACT:** The effects of swine wastewater on atrazine dissipation and formation of bound residues in subtropical clay soil were investigated in this study. The experiment was carried out in laboratory, under room conditions, where samples of Rhodic Hapludox soil received 168.61 mg kg<sup>-1</sup> of atrazine and were incubated for 60 days in the following treatments: T1 (sterilized soil + swine wastewater), T2 (sterilized soil + distilled water), T3 (Non sterilized soil + swine wastewater) and T4 (Non sterilized soil + distilled water). The extractable residues and bound residues of atrazine were extracted and analyzed by high performance liquid chromatography. The results showed no effect of swine wastewater on atrazine dissipation. However, the addition of swine wastewater favored the increase of bound residues, which can increase the persistence of atrazine in the environment and reduce its bioavailability.

**KEYWORDS:** herbicide, degradation, sorption, organic matter.

## DISSIPACÃO E FORMAÇÃO DE RESÍDUOS LIGADOS DE ATRAZINA EM SOLO SUBTROPICAL SOB APLICAÇÃO DE ÁGUA RESIDUÁRIA DA SUINOCULTURA

**RESUMO:** Os efeitos da aplicação de água residuária da suinocultura (ARS) na dissipação de atrazina e formação de resíduos ligados em solo subtropical argiloso foram investigados neste trabalho. O experimento foi conduzido em laboratório, sob condições controladas, onde amostras de Latossolo Vermelho distroférrico receberam a aplicação de 168,61 mg kg<sup>-1</sup> de atrazina e foram incubadas durante 60 dias, de acordo com os seguintes tratamentos: T1 (Solo esterilizado + ARS); T2 (Solo esterilizado + água destilada); T3 (Solo não esterilizado + ARS), e T4 (Solo não esterilizado + água destilada). Os resíduos extraíveis de atrazina e resíduos ligados foram extraídos e analisados por cromatografia líquida de alta eficiência. Os resultados mostraram que não houve efeito da aplicação de ARS na dissipação de atrazina. Entretanto, a adição de ARS ao solo favoreceu o aumento da formação de resíduos ligados, o que pode aumentar a persistência da atrazina no ambiente e diminuir sua biodisponibilidade.

**PALAVRAS-CHAVE:** herbicida, degradação, sorção, matéria orgânica.

## INTRODUCTION

Atrazine is a triazine herbicide used for the control of long-leaved weeds in corn crops. Its chemical structure is represented by a triazine ring replaced by chlorine, ethylamine and isopropylamine, which makes it become recalcitrant for biological degradation in the environment (COLLA et al., 2008; LIMA et al., 2009). It is the herbicide that is most detected in superficial and underground waters, as a result from lixiviation, surface flow and draining of agricultural areas (FAVA et al., 2007; MUDHOO & GARG, 2011).

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In a swine farm, the daily amount of wastewater depends, among other factors, on the number and age of animals, on the amount of water wasted in the fountain and bay sanitation, body and feed wastes, fur, dust and other materials from the process. These factors, when associated, determine the concentration of solids, organic charge, heavy metals, nutrients and microorganisms in the wastewater, that define which treatment is needed in order to prevent wastewater from causing environmental pollution.

Recent studies have evaluated the potential and effects of swine wastewater in the plant (PELISSARI et al., 2009; DAL BOSCO et al., 2008; BAUMGARTNER et al. 2007), soil (SAMPAIO et al., 2010a; CAOVIOLA, et al., 2010; PRIOR, et al. 2009; SUSZEK, et al. 2007), groundwater (MAGGI et al., 2011; DOBLINSKI et al. 2010; SAMPAIO et al. 2010b; SMANHOTTO et al. 2010; ANAMI et al. 2008; DAL BOSCO et al. 2008; ANAMI et al. 2007) and hydraulic systems (SAMPAIO et al, 2010c; SAMPAIO et al. 2007a; SAMPAIO et al. 2007b; FRIGO et al. 2006).

When adequately applied, swine wastewater promotes improvements in physicochemical and biological properties of the soil. However, organic wastes in soil can modify the pesticides fate in the environment, due to the high content and quality of organic material and diversity of microorganisms (MÜLLER et al., 2007; KADIAN et al., 2008; WANG et al., 2009; AGUILERA et al., 2009; OSMAN et al., 2009). According to the International Union of Pure and Applied Chemistry, 'bound residue' is the name given to the chemical species originated from pesticides, which can only be extracted by methods that change the nature of the molecule and/or the matrix, including soil, plants and animals (BARRACLOUGH et al., 2005).

Studies about the effects of organic material use on soil, in atrazine degradation, have been reported by some researchers (HOUOT et al., 1998; BIGWANEZA et al., 2003; TSUI & ROY, 2007; KADIAN et al., 2008; AGUILERA et al., 2009). In a general sense, atrazine degradation is improved by adding microorganisms and nutrients. However, when any kind of organic material is added into a soil, to which herbicides will be sprayed later, it can influence in the increase of sorption due to the formation of residues, linked to the additional organic material and so decreasing the bioavailability and retarding biological degradation (WANG et al., 2009). Besides, wastewaters application in soil can also suppress the action of atrazine to degrade microorganisms, either by the incorporation of inorganic nitrogen or by the competition among the species of bacteria and fungi (SHAPIR et al., 2000; GHOSH & PHILIP, 2006).

In sub-tropical Brazilian ecosystems, the researches regarding the effects of organic residues over the factors that interfere on the dynamics of soil pesticides, such as bound residues, are still incipient. Thus, this essay aimed at evaluating dissipation of atrazine and formation of non-extractable residues (bound residues), in Rhodic Hapludox soil when moistened with water from the swine farm wastes.

## MATERIAL AND METHODS

The soil used in this experiment, clay-textured and classified as Rhodic Hapludox (EMBRAPA, 2006), was collected in Ubitatã city, State of Paraná, in an area with no records of atrazine application, but with a vegetable coverage composed by grass, and samples collected from 0-20 cm underground. Roots that remained in the soil were manually removed, and soil dried in open air, lumps were broken, and sieved in a fabric with 2-mm openings. Swine wastewater and chemical and physical characteristics of soil were determined (Tables 1 and 2).

TABLE 1. Characterization of soil used in the experiment.

pH (CaCl <sub>2</sub> )	Sand	Silt	Clay	CEC	C	OM
		(g kg <sup>-1</sup> )		(cmol <sub>c</sub> kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	
5.20	150.00	250.00	600.00	11.77	17.81	30.63

Cation exchange capacity (CEC); Carbon (C); Organic Material (OM).

TABLE 2. Characterization of swine wastewater.

pH	BDO	CDO	TOC	TKN
	(mg L <sup>-1</sup> )			
7.22	627.70	1.965.00	377.00	710.00

Biochemical demand of oxygen (BDO); Chemical Demand of Oxygen (CDO); Total Organic Carbon (TOC); Total Kjeldahl Nitrogen (TKN).

Swine wastewater was collected in a rural property in Toledo city (Paraná - Brazil), treated in an integrated bio-system constituted by a bio-digester, a sedimentation tank, two stabilization ponds, and separate tanks for algae and fish. The sample was collected in a sterile vial in the way out of the second stabilization pond.

### Treatments and measured parameters

Previous studies in this field enabled the definition of the following treatments, in order to reach the objectives: sterile soil + swine wastewater (T1); sterile soil + distilled water (T2); non-sterile soil + swine wastewater (T3); non-sterile soil + distilled water (T4).

The parameters evaluated in each of the treatments were the extractable and bound residues, and dissipated atrazine.

### Conduction of the experiment

Amounts of 100g of soil were transferred to 250-ml Erlenmeyer vials. The samples were moistened with an adequate volume of sterile distilled water or SW, according to the treatment, until humidity was raised up to 60% field capacity. The field capacity of the soil was determined by the technique of cob separation by wetness front. The incubation was conducted in the dark, at 23° C, and at constant agitation of 100 rpm, during 60 days.

The soil was sterilized in autoclave for treatments T1 and T2, and for all the treatments, 168.61 mg kg<sup>-1</sup> of commercial atrazine were applied. During incubation, soil humidity was preserved in 60% of field capacity, by adding sterile distilled water, according to the vials weighing.

The removal of extractable residues of atrazine was carried after 60 days of incubation. A few 25-g samples were dried in open air, sieved in a fabric with 2-mm openings and put into 250-ml Erlenmeyer vials with 25ml of methanol. After five minutes in ultrasound bath, the samples were stirred for 1 hour at 220 rpm and centrifuged for 30 minutes at 2000 rpm. The supernatant was reserved and the procedure was repeated twice. The supernatants were grouped and 10 mL at 40°C were evaporated in a rotary evaporator until the final volume was 1 mL in order to determine atrazine by high-performance liquid chromatography (HPLC). The soil was reserved for further extraction of bound residues.

The same described extraction procedure was performed, in triplicate, to obtain a fortified soil sample with 50 mg L<sup>-1</sup> of atrazine to determine the percentage of the method recovery, which was 97.26%.

The soil samples, after going through the removal of extractable residues, were autoclaved at 120°C and 9.8x10<sup>4</sup> Pa pressure, for 30 minutes, for three days in a row to release bound residues. Later, the samples went through the same process of extraction with methanol, as previously described.

Atrazine concentration in extracts was determined by high-performance liquid chromatography (HPLC) techniques. The samples were filtered in a filter unit with a membrane of 0.45 µm pores and then injected into chromatograph, according to the following conditions: column C-18 (150 x 4.6 mm), mobile stage methanol: water (50:50, v/v), UV detector – 230 nm, continuous flow of 1 mL min<sup>-1</sup>, furnace temperature of 35°C, 15 minutes run and injection volume of 20 µL.

The amount of dissipated atrazine was calculated according to the difference between the initial concentration of applied atrazine and the sum of extractable and bound residues concentrations after the incubation period.

### Data analyses

The treatments were established in a 2 x 2 factorial scheme in a complete randomized lineation, with 5 replications, adding up to 20 experimental plots. The obtained data were subjected to variance analysis and to the multiple mean comparison test, by using Scott-Knott Test at 5% of significance.

## RESULTS AND DISCUSSION

The obtained data presented normality according to Anderson-Darling test at 5% of significance and there was no interaction among the studied factors in none of the analyzed parameters, so that only the medium concentration of bound residues showed significant difference among the treatments (Table 3).

TABLE 3. P-value values for extractable residue, bound residue and atrazine dissipated in soil.

Variation source	Extractable residue	Bound residue	Dissipated atrazine
Water (A) <sup>1</sup>	0.87 <sup>ns</sup>	0.00 <sup>*</sup>	0.53 <sup>ns</sup>
Sterilization (E) <sup>2</sup>	0.32 <sup>ns</sup>	0.10 <sup>ns</sup>	0.44 <sup>ns</sup>
Interaction (A x E)	0.22 <sup>ns</sup>	0.89 <sup>ns</sup>	0.20 <sup>ns</sup>
C.V. (%)	22.55	22.67	23.58
General mean	75.99	17.22	75.39

\* Significant at 5% probability; "ns" not significant. <sup>1</sup>Two levels of water added: distilled water or SW; <sup>2</sup>Two levels of sterilization: sterile or non-sterile soil.

The results for the extractable residues corresponded, in treatments T1, T2, T3 and T4, at 43.3%, 54.3%, 45.4% and 37.1%, to atrazine initially applied to soil, respectively (Table 4). The amount of remaining extractable residues after the incubation period is an indicative of atrazine biodegradation and formation of bound residues. Treatment T4, in which the soil did not go through the process of sterilization, presented the lowest mean value for extractable residues, indicating that a biological degradation of atrazine occurred. This result corroborates with MUNIER-LAMY et al. (2002), who also observed that the presence of the natural soil microbiota led to a decrease in the amount of extractable residues of atrazine after 120 days of incubation.

TABLE 4. Average concentration of extractable residue, bound residue and atrazine dissipated in soil after 60 days of incubation.

Treatment	Extractable residue (mg kg <sup>-1</sup> )	Bound residue (mg kg <sup>-1</sup> )	Dissipated atrazine (mg kg <sup>-1</sup> )
T1	73.24 <sup>a</sup>	20.60 <sup>b</sup>	74.78 <sup>a</sup>
T2	91.51 <sup>a</sup>	10.77 <sup>a</sup>	66.33 <sup>a</sup>
T3	76.59 <sup>a</sup>	23.92 <sup>b</sup>	68.10 <sup>a</sup>
T4	62.62 <sup>a</sup>	13.60 <sup>a</sup>	92.39 <sup>a</sup>

The means followed by the same letter in the column do not differ among themselves at 5% significance by the Scott-Knott Test.

The application of swine wastewater in soil influenced the formation of bound residues (Table 4). The organic material on soil along with organic material from the added swine wastewater promoted an increase in the formation of atrazine bound residues. The presence of organic matter in swine wastewater was expressed from the values of BOD, COD and TOC (Table 2) and approached the values found by DAL BOSCO et al. (2008) and SAMPAIO et al. (2010a). According to KHAN (1991), the organic material is the main site of formation of atrazine bound residues. The herbicide and its products of degradation are strongly retained by humid fractions, probably by a process that

involves sorption of the external surfaces and penetration of the internal voids of links among molecules with a sieve-like structure. In this context, WANG & KELLER (2009), studying sorption and desorption of atrazine and diuron in soil fractions, have mentioned that, due to their higher soil organic carbon content, clay fractions were much more effective sorbents for pesticides than silt and sand fractions.

Studies have shown increased formation of bound residues of herbicides in soils receiving organic materials. HOUOT et al. (1998) reported that the addition of compounds of solid urban residues and composted straw residues to the soil increased the formation of non-extractable atrazine residues. DAMIN (2005) observed higher formation of bound diuron residues in samples of Rhodic Hapludox soil with clay-like texture, added with sewage sludge, corroborating with the results shown on Table 4.

There was no significant difference among the treatments for dissipated atrazine, which shows that there was no effect of swine wastewater application. BRICEÑO et al. (2010) also found that cow slurry applications had no effect on atrazine dissipation. It can be observed that soil with natural microbiota and without addition of swine wastewater (Treatment T4) showed a higher mean value of atrazine dissipation, besides, the lowest mean value of extractable residue, evidenced biological degradation during the incubation period. In an experiment performed by NAKAGAWA & ANDRÉA (2000), biomineralization of atrazine was detected only in soil with natural microbiota, when compared to the sterilized soil and contaminated with pure culture of *Pseudomonas putida*.

In soils that received SW, atrazine dissipation was lower when compared to the natural soil (treatment T4). Such circumstance possibly occurred due to the fact that the addition of organic materials to the soil may influence on the atrazine degradation rate, either by the providence of nutrients that stimulate or suppress the degrading microorganisms, or by the reduction in atrazine bioavailability, due to the increase in the sorption of atrazine residues in the additional organic material (HOUOT et al., 1998; BIGWANEZA et al., 2003; AGUILERA et al., 2009; BRICEÑO et al., 2008). Treatment T2 showed the lowest value of atrazine dissipated, possibly due to the sterilization process, which corroborates the results found by KE-BIN et al. (2008).

It was observed that the formation of bound residues was larger in the treatments that received swine wastewater (T1 and T3), decreasing atrazine bioavailability in soil and complicating mineralization. The formation of bound residues has a great importance in studies about pesticides fate in the environment, because when these residues are formed, bioavailability decreases and, as consequence, its dissipation is also biologically reduced (MUDHOO & GARG, 2011). BIGWANEZA et al. (2003) reported that swine wastewater application and treated sewage to soil did not show significant effects on atrazine dissipation, in which adsorption was positively correlated to the amount of organic material in fertigated soils.

Considering soils which received application of SW, the sterile soil (T1) showed a higher value of dissipated atrazine in relation to the non-sterile soil (T3), with a possible occurrence of competition among the species of microorganisms involved. Atrazine degradation may be reinforced by the synergism among the species in the swine wastewater and soil microbiota, but on the other hand, the degradation activity may be negatively influenced by antagonistic soil microorganisms. The antagonism is not linked to a unique mechanism of action, but to associations of mechanisms such as antibiosis, competition for nutrients, direct interaction with the species, predation or resistance induction (MENDEZ & MONDINO, 1999). SHAPIR et al. (2000) observed that the addition of sewage sludge to the soil along with fertigation with treated sewage, aiming to achieve atrazine mineralization, increased the competition among native populations of the soil and the added bacteria. Thus, soil microorganisms that are better adapted to the environment may repress the activity and maybe even the capacity of microorganism's survival, added via SW.

According to the obtained results, it was observed that there was atrazine dissipation also in the sterile soil with only the addition of distilled water. Abiotic activities, as chemical oxidation

reactions, reduction and hydrolysis, can also contribute to the transformation and dissipation of atrazine in soil, besides microbiological degradation. The main mechanism of chemical reaction in the pesticide transformation on soil is hydrolysis, which is influenced by the pesticide pH value, temperature and sorption. In this case, in treatment T2, the atrazine transformation may have occurred by chemical processes (HOUOT et al., 1998; NAKAGAWA & ANDRÉA, 2000; DOLAPTSOGLU et al., 2007).

## CONCLUSIONS

From the obtained results, it is possible to conclude that:

- The application of swine wastewater showed no effects on atrazine dissipation in Rhodic Hapludox soil;

- The addition of swine wastewater to the soil favored an increase in the formation of bound residues, which may raise atrazine persistence in the environment and reduce its availability to plants and to biological degradation.

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