

Occurrence and mobility of antimicrobials and hormones in Oxisol with application of swine slurry

Ocorrência de antimicrobianos e hormônios em Latossolo Vermelho com aplicação de dejetos de suínos

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ABSTRACT

The use of swine slurry as fertilizer in agriculture is a common practice in areas where the material is available. However, the presence of chemicals known as emerging organic pollutants in these wastes can contaminate water bodies through runoff or leaching. In this work, we evaluate the occurrence of antimicrobials and hormones in soil profile in an experimental agricultural area by applying different doses of swine slurry. The work was performed in the twelfth year of an experiment involving two annual applications of swine slurry in an oxisol at doses of 0, 50, 100, and 200 m³ ha⁻¹ and a control, to restore the amounts of P and K exported by grain crops. Soil solution samples were collected using suction lysimeters installed in soil layers at average depths of 20, 40, and 80 cm to determine the concentrations of antibiotics and hormones over a period of 60 days. The concentrations were measured by high-efficiency liquid chromatography. The results showed the occurrence of sulfadimidine antimicrobials, chlortetracycline, oxytetracycline, doxycycline, and the hormones estrone, 17 β -estradiol, and 17 α -ethinylestradiol at depths of up to 80 cm during the collection period. The concentrations of antimicrobials and hormones in the soil solutions varied over the sampling period and were not affected by the doses of swine slurry applied. An analysis of the concentrations observed in different layers showed that the application of swine slurry in an oxisol can potentially cause groundwater contamination.

Keywords: Agricultural waste. Emerging organic pollutants. Pollutant transport

RESUMO

A utilização de dejetos líquidos de suínos como fertilizantes na agricultura é uma prática comum nas regiões onde há disponibilidade desse material. Contudo, a presença de substâncias químicas, conhecidas como poluentes orgânicos emergentes, nesses dejetos, pode causar a contaminação de corpos de água por meio do escoamento superficial e, ou, lixiviação. Este trabalho teve por objetivo avaliar a ocorrência de antimicrobianos e hormônios no perfil do solo em uma área agrícola experimental com aplicação de diferentes doses de dejetos líquidos de suínos. O trabalho foi realizado no décimo segundo ano de condução de um experimento com duas aplicações anuais de dejetos líquidos de suínos em Latossolo Vermelho distrófico, nas doses de 0, 50, 100 e 200 m³ ha⁻¹ e uma testemunha, com reposição das quantidades de P e K exportados pelos grãos das culturas. Amostras de solução do solo foram coletadas com o uso de lisímetros de sucção instalados nas camadas de 20, 40 e 80 cm de profundidade, sendo determinadas as concentrações de antimicrobianos e hormônios durante um período de 60 dias. As concentrações foram determinadas em cromatografia líquida de alta eficiência. Os resultados demonstraram a ocorrência dos antimicrobianos sulfadimidina, clortetraciclina, oxitetraciclina e doxiciclina e dos hormônios estrona, 17 β -estradiol e 17 α -etinilestradiol até 80 cm de profundidade, durante o período de coleta. As concentrações de antimicrobianos e hormônios na solução do solo variam ao longo do tempo de amostragem e não foram afetadas pelas doses de dejetos líquidos de suíno aplicadas. As concentrações observadas nas diferentes camadas analisadas mostram que a aplicação de dejetos líquidos de suínos em Latossolo Vermelho distrófico apresenta potencial de contaminação das águas subterrâneas.

Palavras Chave: Resíduos agropecuários. Poluentes orgânicos emergentes. Transporte de poluentes

INTRODUCTION

Waste from agricultural activities can potentially cause environmental degradation, especially with regard to water resources (BURKHOLDER et al., 2007). In this scenario, the use of swineslurry stands out as a potentially polluting activity given that its inception in a confinement system led to increased availability of manure on swine farms (BOXALL et al., 2003).

Contaminants found in animal waste, such as drugs used for therapeutic purposes and prevention (WATANABE et al., 2010; DUTTA et al., 2012), a common practice to ensure the productivity and competitiveness of the swine sector, can enter the environment. Of the drugs used, antimicrobial agents and hormones are among the most widely prescribed classes (THIELE-BRUHN, 2003). Veterinary antimicrobials are used in swine to prevent and control diseases, and promote animal growth (GUARDABASSI et al., 2010). Hormones responsible for endocrine regulation in the animal body may be produced naturally by the individual (17β -estradiol and estrone) or be fed externally (17α -ethinylestradiol) (LINTELMANN et al., 2003).

Many of the applied pharmaceutical compounds are not fully metabolized in the animal body and are excreted in urine and faeces either in the original form or as partially metabolized forms (SARMAH et al., 2006; CASEY et al., 2003). The application of animal manure to soil for nutrient supply to aculture is one of the major pathways for the dispersal of these compounds in the environment (JACOBSEN et al., 2004). Once in the environment, antimicrobial residues may accumulate in the soil, be leached, or even be transported to water bodies through runoff (BURKHOLDER et al., 2007).

The fate and environmental behaviour of the aforementioned compounds are influenced by many factors (KEMPER, 2008) including the physicochemical properties of the molecule, its rate of decomposition, photo-stability, surface adsorption in minerals, ion exchange, and sorption by organic material (DIAZ-CRUZ et al., 2003), as well as prevailing environmental and management conditions (KEMPER, 2008). When these compounds are released into the soil, sorption processes, degradation, and transport (leaching or runoff) primarily govern their fate in the environment (SARMAH et al., 2006).

According to Casey et al. (2003), it is essential to understand the processes of transformation and transport of drugs in the environment and assess their effects on soil, surface water, and groundwater. The antimicrobials present in the environment can negatively affect aquatic and terrestrial organisms (KEMPER, 2008), bio-accumulate in plants (BOXALL et al., 2002a) and animals (KINNEY et al., 2008), and possibly influence (in-

crease) the resistance of microorganisms to antimicrobial agents (CHANDER et al., 2005). The importance of hormones lies in their potential to adversely affect the reproductive systems of aquatic organisms (BILA; DEZOTTI, 2007). Furthermore, the presence of residual drugs in water can damage the endocrine organs, altering their function and metabolism, as observed by Seki et al. (2002).

A few researchers have analyzed the transport of veterinary medicinal products in the environment (LUO et al., 2011; KAY et al., 2005; BLACKWELL et al., 2007). However, in Brazil, this information is still scarce. Therefore, it is difficult to assess the risk that these substances pose when released in terrestrial and aquatic environments (KAY et al., 2005), as well as when applied to clay soils through swine slurry (BOXALL et al., 2002b); preferential flow has been identified as an extremely important mechanism of water pollution in these soils (KAY et al., 2004). Soils to which manure is applied are characterized by high levels of ammonia, which increases soil pH and alters the speciation of veterinary medicinal products, thus affecting their adsorption (BOXALL et al., 2002a).

In this sense, in the present study, we aimed to evaluate the occurrence of antimicrobial molecules and veterinary hormones in the profile of an Oxisol subjected to application of swine slurry in different doses.

MATERIALS AND METHODS

Study area and treatments

The study was developed in experimental plots established 11 years ago in an agricultural area in Campos Novos, a municipality in the midwest of the state of Santa Catarina, located at latitude $27^{\circ} 22'59''S$, longitude $51^{\circ} 15' 33''W$, and 896 meters above sea level. The soil in the plots is of the Hapludox class, clayey, deep (greater than 2.0 m deep), low slope and high water storage capacity, and developed from the saprolite of the eruptive rocks in the Serra Geral formation (riodacite) group, São Bento, with primarily subtropical savanna vegetation (EMBRAPA, 2004). The climate in the region is humid subtropical with mild summers or of the Cfb type according to the Köppen classification (PANDOLFO et al., 2002). The physical and chemical attributes of the soil samples collected from different layers in these plots are summarized in Table 1. The soil was characterized physically using the methodologies described by Veiga (2011). For chemical characterization, we employed the methodologies described by Tedesco et al. (1995).

Table 1 - Physical and chemical attributes of oxisol after 12 years of application of liquid swine slurry.

| Layer cm | Physical attributes | | | | | | Layer cm | Chemical attributes | | |
|-------------|---------------------|---------------------|---------------------|--------------------|--------------------------------|--------------------------------|-------------|-----------------------|-----|-----|
| | Sand | Silt | Clay | SHC | TP | MA | | pH | CEC | OM |
| | kg kg ⁻¹ | kg kg ⁻¹ | kg kg ⁻¹ | cm h ⁻¹ | m ³ m ⁻³ | m ³ m ⁻³ | | cmol dm ⁻³ | % | |
| 0-5 | 28 | 350 | 622 | 96.3 | 0.58 | 0.18 | 20 | 4.5 | 8.0 | 5.0 |
| 5-10 | 19 | 316 | 666 | 26.7 | 0.58 | 0.16 | 40 | 4.6 | 4.5 | 4.0 |
| 12-17 | 20 | 333 | 648 | 10.6 | 0.61 | 0.12 | 80 | 4.7 | 4.0 | 3.5 |

CHS – saturated soil hydraulic conductivity; PT - total porosity; MA: macropores; CEC - cation exchange capacity; MO: organic matter

The treatments consisted of application of semi doses (half in the fall and half in the spring) of 50, 100, and 200 m³ha⁻¹yr⁻¹ of swine slurry (PS50, PS100, and PS200, respectively) and of a witness, in which the amounts of P and K exported through the grain harvested in each plot were applied, using as sources, respectively, triple superphosphate and potassium chloride, the doses of which were varied according to the type of crop cultivated. The experiments were of the randomized complete block type with three replications in plots of 5 × 6 m in a three-year crop rotation system in the following sequence: 1) black oat (*Avena strigosa* L.) + vetch (*Vicia sativa* L.) in autumn/winter and maize (*Zea mays* L.) in the spring/summer; 2) oats in the fall/winter and soybean (*Glycine max*) in the spring/summer; and 3) oats in the autumn/winter and beans (*Phaseolus vulgaris* L.) in the spring/summer.

In the year of soil sampling for this study, the PS used was taken from an anaerobic lagoon in a swine finishing farm in the municipality of Campos Novos and applied on 06 December 2011 with the help of watering cans; the distribution mechanism was adapted to ensure homogeneous distribution of the material in each plot. P and K were reset in one step by using

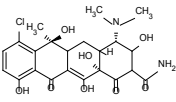
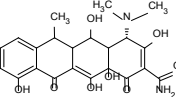
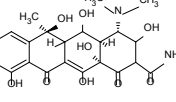
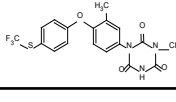
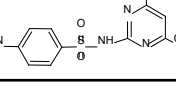
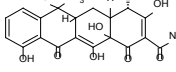
simple superphosphate and potassium chloride, respectively, as sources before sowing the subsequent summer crop. Common bean was sown throughout the area on stubble of black oat crop, 10 days after the application of PS.

Soil solution sampling

Soil solutions were sampled using suction lysimeters (HANNA, 2011), which allow the extraction solution to be retained in the porous soil matrix. In each plot, we installed three lysimeters with porous capsules positioned in layers at average depths of 20, 40, and 80 cm. The lysimeters were installed six months before the start of collection, about 100 cm from the end of the lower topographic height, with a distance of 10 cm between them.

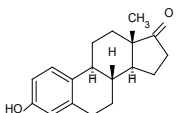
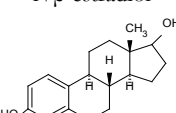
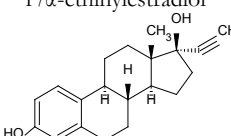
Soil solutions were sampled between November 2011 and February 2012, with the first sample being taken 15 days before the application of PS to evaluate the occurrence of residues from previous application (long term). The other samples were

Table 2 - Physicochemical properties of studied antimicrobials

| Substances | W _s | Log Kd | pKa | t _{1/2} | References |
|--|------------------------------|----------|-----------|------------------|--|
| | (mg L ⁻¹ a 20 °C) | | | | |
| Chlortetracycline  | 600 | 5706 | 3.3 – 9.3 | 79 | Anderson et al. (2005); Jacobsen; Halling-Sorensen (2006). |
| Doxycycline  | n.e | n.e | 3.0 – 9.5 | 533 | Anderson et al. (2005); Jacobsen; Halling-Sorensen (2006); Walters et al. (2010). |
| Oxytetracycline  | 1000 | 417-1026 | 3.3 – 8.9 | 578 | Anderson et al. (2005); Jacobsen; Halling-Sorensen (2006); Pan et al. (2009). |
| Toltrazuril  | Insoluble | n.f. | 6.47 | n.f. | Bilandzick et al., 2012. |
| Sulfadimidine  | 1500 | 2.4-4.2 | 7.4 | 0.3 a 47.6 | Pan et al. (2009); Aust et al. (2010); Baran et al. (2011). |
| Tetracycline  | 1700 | 1140 | 3.3 – 9.6 | 578 | Anderson et al. (2005); Jacobsen; Halling-Sorensen (2006); Pan et al. (2009); Walters et al. (2010). |

W_s: water solubility; Log Kd: Partition coefficient of soil/water; p Ka: acid ionization; t_{1/2}: half-life; n.f.: not found.

Table 3 - Physicochemical properties of studied hormones

| Substances | W_s (mg L ⁻¹ a 20°C) | Log Kd | pKa | $t_{1/2}$ (d) | References |
|--|--------------------------------------|-----------|-----------|------------------|--|
| Estrone  | 12.4 | 2.44-2.72 | 10.3-10.8 | 2 a 3 | Ying et al. (2002); Chen et al. (2009); Combalbert; Raquet Hernandez(2010). |
| 17β-estradiol  | 13.3 | 2.68-2.83 | 10,5-10.7 | 0.2 a 9.0 | Ying et al. (2002); Pan et al. (2009); Chen et al. (2009); Combalbert; Raquet Hernandez(2010). |
| 17α-ethinylestradiol  | 4.8 | 2.65-2.86 | 10.4-10.7 | 4 a 6 | Ying et al. (2002); Pan et al. (2009); Combalbert;RaquetHernandez, 2010. |

W_s : water solubility; Log Kd: Partition coefficient of soil/water; p Ka: acid ionization; $t_{1/2}$: half-life time.

collected 1, 3, 7, 15, 30, and 60 days after the application of PS for evaluating the occurrence of antimicrobial residues and hormones in the short and medium terms. The samples were stored in 300 mL polyethylene bottles, packed in boxes with thermal insulation, kept in refrigerator at 3° C, and transported to a laboratory, where they were analyzed immediately.

Antimicrobial and hormone analysis

We determined the concentrations of the hormones estrone, 17β-estradiol, and 17α-ethinylestradiol, and those of the antibiotics chlortetracycline, doxycycline, oxytetracycline, tetracycline, sulfadimidine, and toltrazuril, all of which are most widely used in the swine farm from where the PS was collected. The main physicochemical properties of the studied antimicrobials and hormones are summarized in Tables 2 and 3, respectively. The samples were analyzed by the direct method and quantified using high-performance liquid chromatography (Dionex®, model Ultimate 3000), coupled with Diode Array detector and Acclaim 120Å C18 column (5µm, 4.6 × 150 mm); Chromaleon® software version 6.80 was used for interpreting the obtained chromatograms. The limit of quantification was determined by the standard deviation of the calibration curve multiplied by 10, and the limit of detection was calculated as the slope of the calibration curve multiplied by 3.14, as recommended by Kowalski et al. (2003).

Reagents

For chromatographic analysis of the samples, we use

dacetonitrile (UV-IR-HPLC-isocratic, Panreac) and phosphoric acid (H₃PO₄) (P.A, Dynamic). Patterns of 17-α-etinil17β-estradiol (98%), chlortetracycline (87%), doxycycline (98%), estrone (99%), 17β-estradiol (98%), oxytetracycline (99%), sulfadimidine (99%), tetracycline (88%), toltrazuril (99.7%) supplied by Sigma-Aldrich® were used. All solutions and standardsolutions were prepared with ultrapure water (Direct-Q® 3 UV, Millipore) characterized by 18.2 MΩ of resistivitycm at 25° C.

Chromatographic method for hormones and toltrazuril

The hormones and toltrazuril were analyzed using a method adapted from that of Verbinnen et al. (2010). The method involves the elution of four molecules using acetonitrile and water in a 54:46ratio at a flow rate of 1 ml min⁻¹, analysis time of 20 min, and wavelengths of 280 nm for the hormones and 244 nm for toltrazuril.

Chromatographic method for chlortetracycline, doxycycline, oxytetracycline, and tetracycline

The analyses were performed using the “Tetracyclines in Pork” method (DIONEX, 2010). The reagents used were acetonitrile and phosphoric acid (0.15%, pH 2.15). Elution was started with 10% acetonitrile,90% phosphoric acid, and flow rate of 1 ml min⁻¹ for 3 min, and these values were increased subsequently to 45% acetonitrile,55% phosphoric acid, and 1.2 mL flow min⁻¹ for 14 min. The wavelength for all molecules was 370 nm.

Chromatographic method for sulfadimidine

The method presented by Santos et al. (2007) was employed for the analysis of antibiotics. We tested for sulfadimidine using acetonitrile and water in a ratio of 30:70 and flow rate of 0.250 ml min⁻¹. The analysis time was 18 min and the wavelength employed was 270 nm.

Statistical analysis

The results were analyzed statistically using analysis of variance, complemented by the application of the mean comparison test (Tukey, P<0.05) when the F test showed significance at 95%.

RESULTS AND DISCUSSION

Total rainfall during the sampling period (75 days) was 447 mm, and it was distributed relatively well temporally. This amount was below the climatological average for the region, which was close to 538 mm in the study period, according to the National Institute of Meteorology (INMET, 2012). The rainfall, air temperature, dates of PS application, and collection of solution samples in the lysimeters during the study period are shown in Figure 1. Three rainfall events occurred in the study period, and the total precipitation was around 50 mm. The first event occurred when we first performed sampling, the second before the application of PS, and the third during sampling after the application of PS. The precipitation in other rainfall events was considerably lower. Average daily temperatures ranged between 12.7 and 23.7°C over the study period.

The chromatograms obtained from the analysis of hormones and antibiotics in the samples show an orderly progression from baseline. This is reflected in the coefficients of determination (R²) of the calibration curves obtained using the respective standards. The linear regression coefficients were

greater than 0.9843, the value obtained for toltrazuril. The calibration curve for 17- α -etinylestradiol showed the highest value, with R² = 0.9999.

Table 4 summarizes the amounts of samples analyzed, number of samples with values above the quantification and detection limits, no detection, indication features, as well as the maximum and minimum concentrations determined in the solutions sampled from the suction lysimeters. The trace values indicate that was found chromatographic signal, with the possibility of quantifying the sample using a more sensitive analytical process. With the exception of toltrazuril, all substances were detected in at least one of the samples collected in the sampling period (Table 4). The non-detection of toltrazuril can be attributed to its high stability in soil and low solubility in water (BILANDZICK et al., 2012).

We found concentrations above the limit of quantification in 39 samples as follows: estrone (1), 17 β -estradiol (6), 17 α -ethinylestradiol (1), and sulfadimidine (31). The concentrations in another 19 samples were between the detection and quantification limits as follows: 17 β -estradiol (4), 17 α -etinylestradiol (3), sulfadimidine (9), chlortetracycline (1), oxytetracycline (1), and tetracycline (1).

The concentrations of 17 α -ethinylestradiol in four samples were detected and quantified in one of them. The highest concentration achieved was 68.5 ng L⁻¹. Lai et al. (2000) suggested that owing to its hydrophobic properties, this hormone is susceptible to adsorption on organic matter and particles of swine slurry. Andersen et al. (2005) studied the adsorption of estrogen during removal processes and estimated that 76% of the amount of 17 α -ethinylestradiol was adsorbed onto the solid phase. This may explain its low quantification in the soil solutions used in this study, which contained large amounts of organic matter such as clay (Table 1).

Molecules of chlortetracycline, doxycycline, oxytetracycline, tetracycline, estrone, and 17 β -estradiol were found in measurable concentrations. Chlortetracycline (2 samples), oxytetracycline (1), and tetracycline (1) were found in concen-

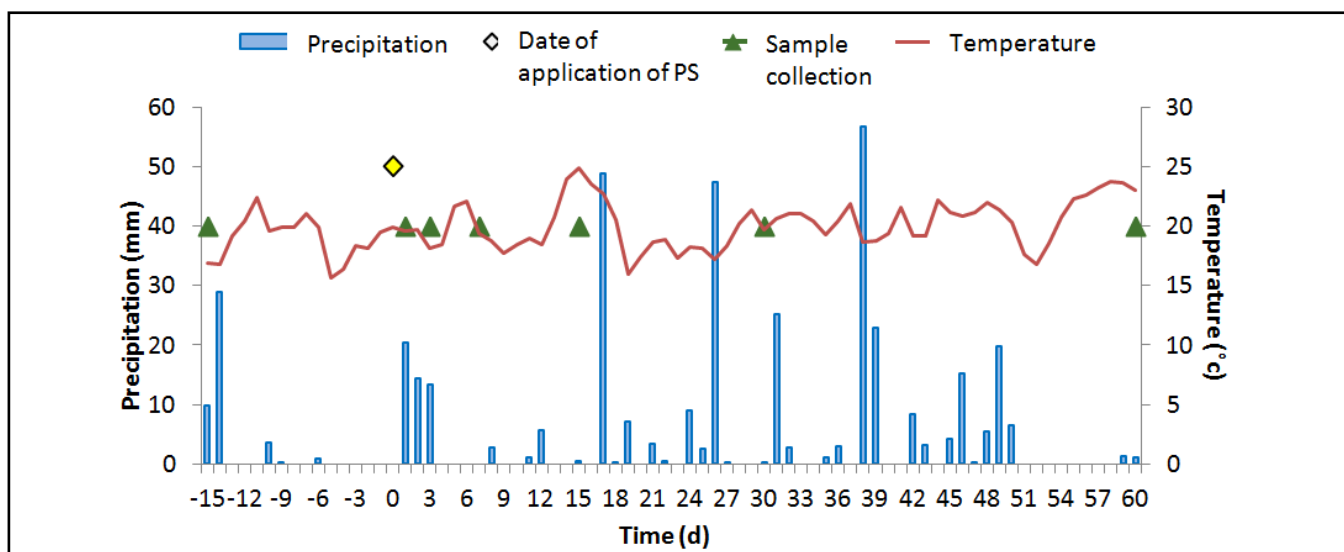


Figure 1 - Precipitation, temperature, date of application of PS, and sample collection during sampling period.

Table 4 - Antimicrobial and hormones in solution samples collected in suction lysimeters

| Substances | LD ----ng L ⁻¹ ---- | LQ | Samples n° | ALQ n° | ALD n° | ND | Cmax -----ng L ⁻¹ ---- | Cmin |
|----------------------|-----------------------------------|-------|---------------|-----------|-----------|-----|--------------------------------------|--------|
| Estrone | 36.5 | 121.7 | 200 | 1 | 0 | 198 | 137.3 | 137.3* |
| 17β-estradiol | 28.4 | 94.8 | 200 | 6 | 4 | 184 | 429.5 | 117.7 |
| 17α-ethinylestradiol | 18.4 | 61.2 | 199 | 1 | 4 | 193 | 68.5 | 59.9 |
| Toltrazuril | 8.7 | 28.9 | 201 | 0 | 0 | 201 | | |
| Sulfadimidine | 9.7 | 32.2 | 199 | 31 | 10 | 153 | 813.4 | 41.6 |
| Chlortetracycline | 3.3 | 11.1 | 200 | 0 | 1 | 200 | | |
| Doxicycline | 5.9 | 19.9 | 200 | 0 | 0 | 200 | | |
| Oxitetracline | 1.8 | 6.1 | 198 | 0 | 1 | 190 | | |
| Tetracycline | 1.8 | 6.1 | 200 | 0 | 2 | 366 | | |

*: drug found in only a determination; LD - limit of detection; LQ - limit of quantification; ALQ - number of samples above limit of quantification;ALD - number of samples above detection limit; ND - not detected;Cmax - maximum concentration;Cmin - minimum concentration

trations above the limit of detection. Doxicycline was highly adsorbed onto the soils with high organic matter content (BO-XALL et al., 2002a), which was proven by the high value of the coefficient k_d of the linear isotherm (THIELE-BRUHN, 2003). This explains why it was detected in a large number of samples (200). Low concentrations of chlortetracycline were also found by Hamscher et al. (2002) and Li et al. (2011), who studied the occurrence of this molecule in agricultural areas with application of PS. The low concentrations can be explained by the fact that chlortetracycline interacts strongly with clay minerals (GU et al., 2007) and soil organic matter (KULSHRESTHA et al., 2004). Low concentrations of oxytetracycline, too, were found by Hamscher et al. (2002) and Wu et al. (2011).

A likely explanation for the low concentrations found in the Hapludox might be the degradation of oxytetracycline in anaerobic lagoons because during the PS maturation period, the degradation of oxytetracycline can reach 94%, causing the waste, when applied in the field as fertilizer, to not show more

of this substance in quantifiable levels. This degradation occurs due to microorganisms (WU et al., 2011) and abiotic factors such as pH, temperature, and solar radiation (HALLING-SORENSEN et al., 2002). Tetracyclines are strongly connected to the organic matter present in soil (HAMSCHER et al., 2002), which is based on their ability to form complexes with cations such as calcium, which are found in the soil at high concentrations (CHRISTIAN et al. 2003). This explains the low occurrence of tetracyclines in the current study and in other similar studies of soils fertilized with swine slurry (WATANABE et al., 2010).

Estrone is highly absorbed in soil with high organic matter content (LAI et al., 2000). According to Casey et al. (2003), the probability of estrone adsorption in soil is high, especially when the organic matter content in the soil is high, such as in this Hapludox (Table 1), as well as the iron oxide content, which can result in the formation of bound residues (LAI et al., 2000), justifying the large number of samples (200) in which estrone was detected.

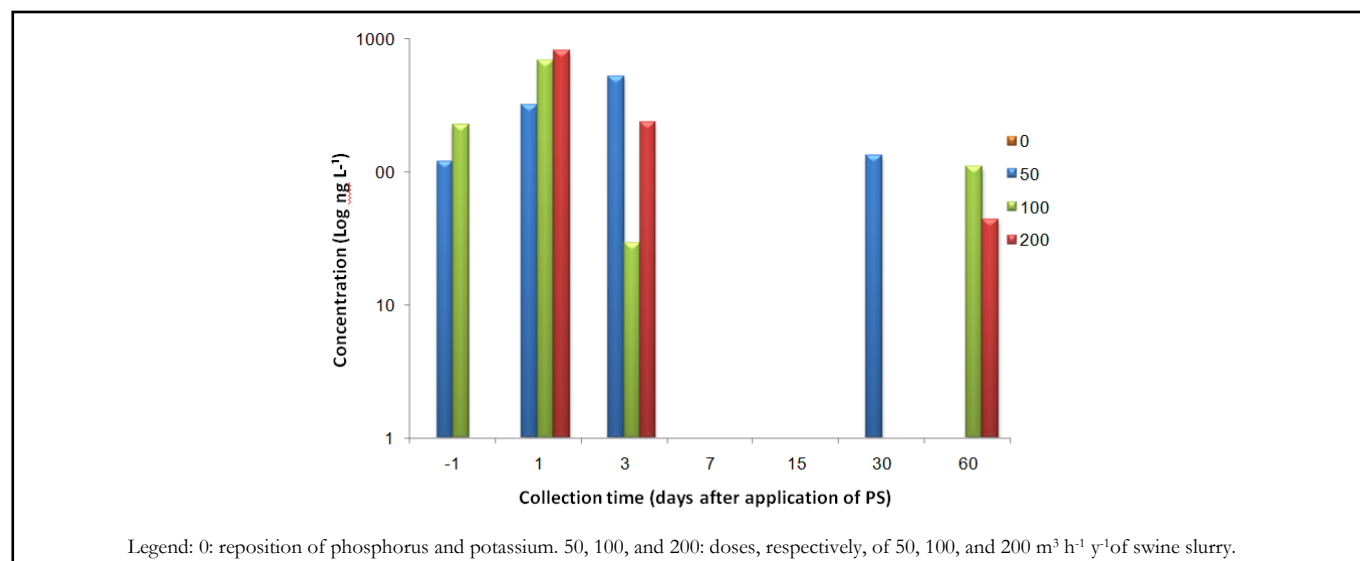


Figure 2 - Average sulfadimidine concentrations (ng L⁻¹, logarithmic scale) with values above detection limit, determined in solution collected in suction lysimeters

Table 5 - Maximum concentrations of antibiotics and hormones at different soil depths after application of swine slurry

| Maximum depth | Substances | | | | | | | | | |
|---------------|-------------------------------|-------|------|-----|-------|-----|------|-----|-------|--|
| | E1 | E2 | EE2 | TTL | SMD | CTC | Doxi | Oxi | Tetra | |
| | -----ng L ⁻¹ ----- | | | | | | | | | |
| 20 cm | 0.0 | 429.5 | 0.0 | 0.0 | 689.4 | 0.0 | 0.0 | 0.0 | 0.0 | |
| 40 cm | 0.0 | 127.6 | 48.1 | 0.0 | 550.7 | 0.0 | 0.0 | 0.0 | 0.0 | |
| 80 cm | 137.3 | 133.5 | 68.5 | 0.0 | 813.4 | 0.0 | 0.0 | 0.0 | 0.0 | |

E1: estrone, E2: 17 β -estradiol, EE2: 17 α -ethinylestradiol, TTL: toltrazuril, SMD: sulfadimidine, CTC: chlortetracycline, Doxi:doxycycline, Oxi: oxtetracycline, Tetra:tetracycline

A likely explanation for the low concentrations of 17 β -estradiol found in this Haplouox is the association of 17 β -estradiol with soil colloids (ARNON et al., 2008) and organic matter from the soil and from the PS itself (LAI et al., 2000), probably favoured by the change in pH, which is more acidic in the soil than in the PS.

Sulfadimidine concentrations were found to be 41.6–813.4 ng L⁻¹ (Figure 2). Before applying PS, the mean concentration was 229.6 ng L⁻¹, which shows the persistence of this molecule in the environment, as observed by Christian et al. (2003), who studied the occurrence of antibiotics in soils amended with PS in Germany. They found a concentration of 15 mg kg⁻¹ at seven months after the application of fertilizers, indicating high retention and stability of the substances in soil, having the same half-life of 0.3–47.6 days (BARAN et al., 2011).

An increase in sulfadimidine concentration occurred immediately after the application of PS to soil surface (Table 4) because the maximum concentration (813.4 ng L⁻¹) was observed one day after the application, even without rainfall. Sulfonamides are often found in high concentrations in the environment (HU et al., 2008; CHRISTIAN et al., 2003; LI et al., 2011) owing to their high water solubility and consequent mobility in soil solution (HALLER et al., 2002). After application, they are quickly transported in the soil profile (BOXALL et al., 2002a), as can be seen three days after the application, when the concentration reached 519.8 ng L⁻¹. Boxall et al. (2002a) stated that in acidic soils absorb higher amount of sulfadimidine. This can be observed at seven and 15 days after application, when the substance was not detected. At thirty days, the concentration rose again to 134.9 ng L⁻¹, possibly owing to high rainfall in the days prior to the collection, causing sulfadimidine leaching in the soil profile. This was also observed at 60 days after the application of PS, when the concentration was 256.4 ng L⁻¹ (Table 4).

Table 5 summarizes the average concentrations of antibiotics and hormones at average sampling depths of 20, 40, and 80 cm. The molecules of 17 β -estradiol, 17 α -ethinylestradiol, and sulfadimidine were found at measurable concentrations at various depths, including 80 cm. The remaining molecules were not quantified in the soil profile. 17 β -estradiol concentrations were also observed at 15 cm (ZITNICK et al., 2011), 60 cm (THOMPSON et al., 2009), and 100 cm (KJAER et al., 2007) in evaluations of the transport of 17 β -estradiol in soil fertilized with swine slurry. We observed that the maximum concentration of the substance in the soil solution in the first layer was observed in most samples. Herman and Mills (2003) attributed

such concentrations to lower degradation rates owing to milder temperatures, as observed in the study site (average of about 20° C), and the abundant energy provided to the microorganisms by the PS applied, which prevents total degradation and allows for a residual fraction in the environment.

We showed that the maximum concentration of 17 β -estradiol was observed in deeper layers. Ivashechkin et al. (2004) attributed this increase in the concentration of the substance to the process of desorption of 17 α -ethinylestradiol molecules from soil constituents. This desorption can be caused by an increase in negative charge and deprotonation, thus reducing adsorption onto colloids in the soil (IVASHECHKIN et al., 2004). This result was also observed by Karnjanapiboonwong et al. (2011) when studying the occurrence of hormones at a depth of 30 cm.

CONCLUSIONS

1. The hormones estrone, 17 β -estradiol, and 17 α -ethinylestradiol and the antimicrobial sulfadimidine were found in the Haplouox soil at concentrations higher than the limit of quantification. The antibiotics chlortetracycline, oxytetracycline, and tetracycline were found only at concentrations higher than the detection limit.

2. The frequencies of occurrence at concentrations higher than the detection and quantification limits of hormones and antibiotics were low, with the exception of sulfadimidine, for which the frequency was higher than 10%.

3. Sulfadimidine and 17 α -ethinylestradiol were detected in the Haplouox solution up to 60 days after the application of swine slurry.

4. The antimicrobial sulfadimidine and the hormone 17 α -ethinylestradiol were detected in the soil profile up to depths of 80 cm.

5. The high concentrations of sulfadimidine and 17 α -ethinylestradiol found in the soil solutions show that the application of swine slurry to agricultural land can potentially contaminate groundwater, especially when the water table is near the surface and heavy rainfall occurs a few days after PS application.

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REFERENCES

- ANDERSEN, H. R.; HANSEN, M.; KJØLHOLT, J.; STUER-LAURIDSEN, F.; TERNES, T.; HALLING-SØRENSEN, B. Assessment of the importance of sorption for steroid estrogens removal during activated sludge treatment. *Chemosphere*, v.61, p.139-146, 2005.
- ANDERSON, C. R.; RUPP, H.S.; WU, W. Complexities in tetracycline analysis-chemistry, matrix extraction, cleanup, and liquid chromatography. *Journal of Chromatography*, v.1075, 23–32, 2005.
- ARNON, S.; DAHAN, O.; ELHANANY, S.; COHEN, K.; PANKRATOV, I.; GROSS, A.; RONEN, Z.; BARAM, S.; SHORE, L. Transport of testosterone and estrogen from dairy-farm waste lagoons to groundwater. *Environmental Science & Technology*, v.42, p.5521-5526, 2008.
- AUST, M. O.; BRUNH-THIELE, S.; SEEGER, J.; GODLINSKI, F.; MEISSNER, R.; LEINWEBER, P. Sulfonamides leach from sandy loam soils under common agricultural practice. *Water Air Soil Pollution*, v.211, p.143-156, 2010.
- BARAN, W.; ADAMEK, E.; ZIEMIANSKI, A. J.; SOBCZA, K. A. Effects of the presence of sulfonamides in the environment and their influence on human health. *Journal of Hazardous Materials*, v.196, p.1-15, 2011.
- BILA, M. D.; DEZOTTI, M. Desreguladores endócrinos no meio ambiente: Efeitos e consequências. *Química Nova*, v.30, p.651-666, 2007.
- BLACKWELL, P. A.; KAY, P.; BOXALL, A. B. A. The dissipation and transport of veterinary antibiotics in a sandy loam soil. *Chemosphere*, v.67, p.292-299, 2007.
- BILANDZICK, N.; VARENINA, I.; BOJIE, D.; KOLANOVIE, S. B. Toltrazuril primjena i farmakokinetika. *Veterinarska Stanica*, v.43, p.135-144, 2012.
- BOXALL, A. B. A.; BLACKWELL, A.; CAVALLO, R.; KAY, P.; TOLLS, J. The sorption and transport of a sulphonamide antibiotic in soil systems. *Toxicology Letters*, v.131, p.19-28, 2002a.
- BOXALL, A. B. A.; FOGG, L.; BLACKWELL, P.; KAY, P.; PEMBERTON, E. *Review of veterinary medicines in the environment*. Bristol, Environment Agency, R&D Technical Report P6-012/8/TR, 2002b.
- BOXALL, A.; KOLPIN, D.; HOLLING-SØRENSEN, B.; TOLLS, J. Are veterinary medicines causing environmental risks? *Environmental Science & Technology*, v.37, p.285-294, 2003.
- BRADFORD, S. A.; SIMUNEK, J.; BETTAHAR, M.; GENUCHTEN, M. TH.; YATES, S. Modeling colloid attachment, straining, and exclusion in saturated porous media. *Environmental Science & Technology*, v.37, p.2242-2250, 2003.
- BURKHOLDER, J.; LIBRA, L.; WEYER, P.; HEATHCOTE, S.; KOLPIN, D.; THORNE, S. P.; WICHMAN, M. Impacts of waste from concentrated animal feeding operations on water quality. *Environmental Health Perspectives*, v.115, p.308-312, 2007.
- CASEY, F. M.; LARZEN, G.; HAKK, H.; SIMUNEK, J. Fate and transport of 17 α -17 β -Estradiol in soil-water systems. *Environmental Science & Technology*, v.37, p.2400-2490, 2003.
- CHANDER, Y.; KUMAR, K.; GOYAL, S. M.; GUPTA, S. C. Antibacterial activity of soil bound antibiotics. *Journal of Environmental Quality*, v.34, p.1952-1957, 2005.
- CHEN, M.; QI, RONG; NA, W.; ZHANG, H.; WEI, Y.; ZHOU, Y. New concept of contaminant removal from swine wastewater by a biological treatment process. *Frontiers of Biology*, v.4, p.402–413, 2009.
- CHRISTIAN, A. T.; SCHNEIDER, A. R. J.; FARBERB, H. A.; SKUTLAREK, B. D.; MEYER, C. M. T.; GOLDBACH, A. H. E. Determination of antibiotic residues in manure, soil, and surface waters. *Acta Hydrochimica et Hydrobiologica*, v.31, p.36-44, 2003.
- COMBALBERT, S.; RAQUET-HERNANDEZ, G. Occurrence, fate, and biodegradation of estrogens in sewage and Manure. *Applied Microbiologic Biotechnologic*, v.86, p.1671–1692, 2010.
- DIAZ-CRUZ, M. S.; LOPEZ DE ALDA, M.; BARCELÓ, D. Environmental behavior and analysis of veterinary and human drugs in soils, sediments and sludge. *Trends in Analytical Chemistry*, v.6, p.340-351, 2003.
- DIONEX, *Tetracycline in pork*. Dionex Application #76. Chromeleon, D-Library. 2010, 141 p.
- DUTTA, S. K.; INAMDAR, P. S.; TSO, J.; AGA, S. D. Concentrations of free and conjugated estrogens at different landscape positions in an agricultural watershed receiving poultry litter. *Water, Air, & Soil Pollution*, v.223, p.2821-2836, 2012.
- EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária - Centro Nacional de Pesquisa em Solos. Solos do Estado de Santa Catarina. Rio de Janeiro: Embrapa CNPS, 2004. 1CD Rom; mapa color. Embrapa solos. *Boletim de Pesquisa e Desenvolvimento*, n.46.
- GU, C.; KARTHIKEYAN, K. G.; SIBLEY, S. D.; PEDERSEN, J. A. Complexation of the antibiotic tetracycline with humic acid. *Chemosphere*, v.66, p.1494-1501, 2007.
- GUARDABASSI, L.; JENSEN, L. B.; KRUSE, H. *Guia de antimicrobianos em veterinária*. Porto Alegre: Artmed, 2010, 268 p.

- HALLER, M. Y.; MULLER, S. R.; MCADELL, C. S.; ALDER, A. C.; SUTER, M. J. F. Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. *Journal of Chromatography*, v.952, p.111-120, 2002.
- HALLING-SØRENSEN, B.; SENDELØV, G.; TJØRNELUND, J. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. *Archives of Environmental Contamination and Toxicology*, v.42, p.263-271, 2002.
- HAMSCHER, G.; SCZESNY, S.; PER, H.; NAU, H. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. *Analytical Chemistry*, v.74, p.1509-1518, 2002.
- HANNA INSTRUMENTS, HI83900 - Suction Lysimeter - Instruction manual, 12 p. <http://hannaindia.com/manuals/manHI_83900.pdf>. 6 Ago. 2011.
- HERMAN, J. S.; MILLS, A. L. Biological and hydrogeological interactions affect the persistence of 17- β -17 β -Estradiol in an agricultural watershed. *Geobiology*, v.1, p.141-151, 2003.
- HU, X. G.; YI, L.; ZHOU, Q. X.; XU, L. Determination of thirteen antibiotics residues in manure by solid phase extraction and high performance liquid chromatography. *Chinese Journal of Analytical Chemistry*, v.36, p.1162-1166, 2008.
- INMET - Instituto Nacional de Meteorologia - <<http://www.inmet.gov.br/html/clima/mapas/?mapa=prec>>. 15 out. 2012.
- IVASHECHKIN, P.; CORVINI, P.F.; DOHMANN M. Behaviour of endocrine disrupting chemicals during the treatment of municipal sewage sludge, *Water Science and Technology*, v.50, p.133-40, 2004.
- JACOBSEN, A. M.; HALLING-SØRENSEN, B. Multi-component analysis of tetracyclines, sulfonamides and tylosin in swine manure by liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, v.384, p.1164-1174, 2006.
- JACOBSEN, A. M.; HALLING-SØRENSEN, B.; INGERSLEV, F.; HANSEN, S. H. Simultaneous extraction of tetracycline, macrolide and sulfonamide antibiotics from agricultural soils using pressurised liquid extraction, followed by solid-phase extraction and liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, v.1038, p.157-170, 2004.
- KAY, P.; BLACKWELL, P. A.; BOXALL, A. B. A. Fate of veterinary antibiotics in a macroporous tile drained clay soil. *Environmental Toxicology and Chemistry*, v.23, p.1136-1144, 2004.
- KAY, P.; BLACKWELL, P. A.; BOXALL, A. B. A. A lysimeter experiment to investigate the leaching of veterinary antibiotics through a clay soil and comparison with field data. *Environ. Pollut.*, n.134, p.333-341, 2005.
- KARNJANAPIBOONWONG, A.; SUSKI, J. G.; SHAH, A. A.; CAI, Q.; MORSE, A. N.; ANDERSON, T. A. Occurrence of PPCPs at a wastewater treatment plant and in soil and groundwater at a land application site. *Water, Air, & Soil Pollution*, v.216, p.257-273, 2011.
- KEMPER, N. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecol. Indicat.*, n.8, p.1-13, 2008.
- KINNEY, C.; FURLONG, E.; KOLPIN, D.; BURKHARDT, M.; ZAUGG, S. Bioaccumulation of pharmaceuticals and other anthropogenic waste indicators in earthworms from agricultural soil amended with biosolid or swine manure. *Environmental Science & Technology*, v.42, p.1861-1870, 2008.
- KJAER, J.; OLSEN, P.; BACH, K.; BARLEBO, H.C.; INGERSLEV, F.; HANSEN, M.; HALLING-SØRENSEN, B. Leaching of estrogenic hormones from manure-treated structured soils. *Environmental Science & Technology*, v.41, p.3911-3917, 2007.
- KOWALSKI, W.J.; NOWACKA-KRUKOWSKA, H.; DROZDZEWSKA, K.; SIŁOWIECKI, A.; SZARPAK, A. Application of high performance liquid chromatography (HPLC) for determination of 1,3-dichloropropane-2-ol in food matrices. *Journal of Food and Nutrition Sciences*, v.12, p.51-57, 2003.
- KULSHRESTHA, P.; ROSMANN, F. G.; AGA, D. Investigating the molecular interactions of oxytetracycline in clay and organic matter: insights on factors affecting its mobility in soil. *Environmental Science & Technology*, v.38, p.4097-4105, 2004.
- LAI, K. M.; JOHNSON, L. K.; SCRIMSHAW, M. D.; LESTER, N. J. Binding of waterborne steroid estrogens to solid phases in river and estuarine systems. *Environmental Science & Technology*, v.34, p.3890-3894, 2000.
- LI, Y. W.; WU, X. L.; MO, C. H.; TAI, Y. P.; HUANG, X. P.; XIANG, L. Investigation of Sulfonamide, Tetracycline, and Quinolone Antibiotics in Vegetable Farmland Soil in the Pearl River Delta Area, Southern China. *Journal of Agricultural and Food Chemistry*, v.59, p.7268-7276, 2011.
- LINTELMANN, J.; KATAYAMA, A.; KURIHARA, N.; SHORE, L.; WENZEL, A. Endocrine disruptors in the environment. *Pure Applied Chemistry*, v.75, p.631-681, 2003.
- LUO, Y.; XU, L.; RYZ, M.; WANG, Y.; ZHANG, H.; ALVAREZ, P. J. J. Occurrence and transport of tetracycline, sulfonamide, quinolone and macrolide antibiotics in the Haihe River Basin, China. *Environmental Science & Technology*, v.45, p.1827-1833, 2011.
- PAN, B.; NING, P.; XING, B. Sorption of pharmaceuticals and personal care products. Part V. *Environmental Science and Pollution Research*, v.16, p.106-116, 2009.

PANDOLFO, C.; BRAGA, H.J.; SILVA JÚNIOR, V.P.; MASSIGNAM, A. M.; PEREIRA, E. S.; THOMÉ, V. M. R.; VALCI, F.V. *Atlas climatológico digital do Estado de Santa Catarina*. Florianópolis: EPAGRI, 2002. CD Rom.

SANTOS, J. H. Z. ; PIZZOLATO, T. M.; CUNHA, A. C. B. Desenvolvimento de Metodologia Analítica de Fármacos em meio Aquático por Extração em fase Sólida e HPLC. *Revista de Ciências Ambientais*, v.1, p.19–34, 2007.

SARMAH, A. K.; MEYER, M. T.; BOXALL, A. B. A. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere*, v.65, p.725-759, 2006.

SEKI, M.; YOKOTA, H.; MATSUBARA, H.; TSURUDA, Y.; MAEDA, M.; TADOKORO, H.; KOBAYASHI, K. Effect of ethinyl17 β -Estradiol on the reproduction and induction of vitellogenin and testis-ova in medaka (*oryzias latipes*). *Environmental Toxicology and Chemistry*, v.21, p.1692-1698, 2002.

TEDESCO, M. J.; GIANELLO, C.; BISSANI, C. A.; BOHNEN, H.; VOLKWEISS, S. J. *Análise de solo, plantas e outros materiais*. Porto Alegre: Departamento de Solos, UFRGS, 1995. 174p.

THIELE-BRUHN, S. Pharmaceutical antibiotic compounds in soils - a review. *Journal of Plant Nutrition and Soil Science*, n.166, p.145-167, 2003.

THOMPSON, M.L.; CASEY, F. X. M.; KHAN, E.; HAKK, H.; LARSEND, G. L.; DESUTTER, T. Occurrence and pathways of manure-borne 17 β -Estradiol in vadose zone water. *Chemosphere*, v.76, p.472-479, 2009.

VEIGA, M. *Metodologia para coleta de amostras e análises físicas do solo*. Florianópolis: Epagri, 2011. 52p. (Epagri. Boletim Técnico, 156).

VERBINNEN, R. T.; NUNES, G. S.; VIEIRA, E. M. Determinação de hormônios estrógenos em água potável usando CLAE - DAD. *Química Nova*, v.33, p.1837-1842, 2010.

WALTERS, E.; MCCLELLAN, K.; HALDEN, R. U. Occurrence and loss over three years of 72 pharmaceuticals and personal care from biosolids – soil mixtures in outdoor mesocosms. *Water Research*, v.44, p.6011–6020, 2010.

WATANABE, N.; BERGAMASCHI, B.; LOFTIN, K.; MEYER, M.; HARTER, T. Use and environmental occurrence of antibiotics in freestall dairy farms with manured forage fields. *Environmental Science & Technology*, v.44, p.6591-6600, 2010.

WU, X.; WEI, Y.; ZHENG, Z.; ZHAO, X.; ZHONG, W. The behavior of tetracyclines and their degradation products during swine manure composting. *Bioresource Technology*, v.102, p.5924–5931, 2011.

YING, G. G.; KOOKANA, R. S.; RU, Y. J. Occurrence and fate

of hormone steroids in the environment. *Environment International*, v.28, p.545–551, 2002.

ZITNICK, K. K.; SHAPPELL, N. W.; HAKK, H.; DESUTTER, T. M.; KHAN, E.; CASEY, F. X. M. Effects of liquid swine manure on dissipation of 17 β -Estradiol in soil. *Journal of Hazardous Materials*, v.186, p.1111-1117, 2011.

Contribuição dos autores

Daniela Aparecida de Oliveira: este trabalho fez parte de sua dissertação de mestrado.

Adilson Pinheiro: foi o orientador da dissertação de mestrado.

Milton da Veiga: foi o co-orientador e responsável pelo dispositivo experimental.

Thiago Caique Alves: realizou os procedimentos analíticos.