Fate and transport of compounds with estrogenic activity in reconstructed soil columns

Destino e transporte de compostos com atividade estrogênica em colunas de solo reconstruídas

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ABSTRACT

Endocrine disruptors (ED) are exogenous agents capable of deregulating the normal functioning of the endocrine system in humans and animals. The ED entry routes in the environment highlight the launch of domestic and agricultural wastewater in water bodies. This work aims to evaluate the occurrence, mobility and biosorption attenuation of hormones and antibiotics in soil columns in the application of agricultural and domestic waste and rain simulation. Soil columns, which were filled with soil from an agricultural area, were installed under the conditions of a swine wastewater application (ARS) at a dose of 50 m$^3$/ha with and without the biosorbent effect and human urine at a rate equivalent to nitrogen compared with ARS. The estrogen concentrations were observed by High-performance liquid chromatography coupled with diode array detector (HPLC-DAD), and the mass balance was developed to evaluate the estrogen removal in the soil profile. The occurrence of hormones in all analyses was approximately 20%. Pine bark adsorbent was found to be considerably efficient in removing hormones from liquid matrices. The mass balance of the soil columns with adsorbent exhibited an estrogen recovery in the matrix applied to less than a 1% rate. For comparison, the soil columns without adsorbent obtained a mass transit about 20% of the total applied.

Keywords: Endocrine disruptors; Lysimeter; Adsorption.

RESUMO

Os desreguladores endócrinos (DE) são agentes exógenos capazes de desregular o funcionamento normal do sistema endócrino em humanos e animais. Dentre as rotas de entrada de DE no meio ambiente, destacam-se o lançamento de águas residuais domésticas e agrícolas em corpos d'água. Este trabalho tem como objetivo avaliar a ocorrência, mobilidade e atenuação de biossorção de hormônios e antibióticos na coluna do solo sob aplicação de resíduos agrícolas e domésticos e simulação de chuvas. Colunas de solo, preenchidas com solo de uma área agrícola, foram instaladas nas condições de aplicação de águas residuais de suínos (ARS), na dose de 50 m$^3$/ha sob e sem efeito de biossorvente, e na urina humana a uma taxa equivalente em nitrogênio comparada para ARS. As concentrações de estrógeno foram observadas por HPLC-DAD, e foram desenvolvidos balanços de massa para avaliar a remoção de estrogênio no perfil do solo. As ocorrências de hormônios em todas as análises foram de aproximadamente 20%. Observou-se ainda uma eficácia considerável do adsorvente da casca de pinheiro na remoção de hormônios de matrizes líquidas. O balanço de massa das colunas do solo com adsorvente mostrou uma recuperação de estrógenos presente na matriz aplicada a uma taxa inferior a 1%. Para comparação, as colunas de solo sem adsorvente obteriam um transporte de massa de cerca de 20% do total aplicado.

Palavras-chave: Disruptores endócrinos; Lisímetro; Adsorção.
INTRODUCTION

The transport of organic contaminants in soil profiles is a physicochemical phenomenon dependent on variables, such as equilibrium constants, soil physicochemical characteristics and the compounds involved (Goepfert et al., 2014).

Among these variables, the characteristics of the soil–water system, such as the octanol–water partition coefficient (K_{ow}), soil–water partition coefficient (K_d), pH and chemical composition favouring adsorption (or retention), are considered the most critical ones (Rabølle & Spliid, 2000). Particularly, steroidal compounds (e.g. hormones) tend to move fast through the soil profile because of their aromatic centres charged by electrons and high solubility in water due to their high polarity (Pinheiro et al., 2013). However, only a few studies have been conducted on this topic. The speed of the movement of emergent organic compounds (EOCs) in the soil profiles is directly linked to their stability (Casey et al., 2004; Karnjanapiboonwong et al., 2010) and their possible degradation or microbiological conversion (Deng et al., 2016; Grossberger et al., 2014; Mashhare et al., 2013). Furthermore, these molecules can be interconverted because of the oxidation and reduction potential characteristics of the medium in which they occur (Nguyen et al., 2016).

The transport of contaminants such as EOCs may be affected even by small fluctuations in these characteristics because of their high occurrence in the environment at low magnitudes (µg L^{-1} and ng L^{-1}). Therefore, understanding this occurrence and which variables are preponderant in the transport of this class in soil profiles can be a crucial point in studies on contaminated area degradation or even in the promotion of the natural biodegradation process (Casey et al., 2004). The by-product compounds of EOCs from enzymatic pathways can reach water bodies. They are potential contaminants capable of disrupting the animal endocrine system and the balance of plant communities, and sometimes they are even more dangerous to the environment than the original compound (Zheng et al., 2012). For example, the 17α-oestradiol biotransformation occurs because of the metabolic pathway of anaerobic bacteria that transforms the α-isomer into β (Ying et al., 2002). Observing the occurrence, or even the effects, of these compounds in environmental matrices is difficult if their role is not elucidated.

Mashhare et al. (2013) observed oestrone and oestradiol biotransformation in 17-α and 17-β-oestradiol in compacted columns with sandy and clayey soils. The column with the higher organic matter content of clay soils had a degradation rate two times higher than that with sandy soils. Moreover, the CO₂ production in the columns was four times higher in the clayey columns, indicating a high activity of microorganisms and/or degradation.

Llorca et al. (2014) reported that dehydrogenase enzyme activity is an essential variable in the degradation of hormones in the soil and is positively correlated with the oxidation of 17-β-oestradiol.

In their modelling study on hormone transport in soil columns, Thiebault et al. (2015) found that adsorption depends not only on the organic matter present in the soil but also on the clay content of the soil. In analyses such as mestranol, which is transformed into EE2 in the presence of anaerobic microorganisms through the demethylation metabolic process, the larger the mass of mestranol entry is, and the larger the EE2 formation (Ying et al., 2002).

Thus, this research aimed to examine the EOC transport of the class of endocrine disruptors (EDs) in soil columns in a wastewater application and their relationship with the inorganic profile of percolates in the occurrence of steroidal compounds during a one-year experiment under rain simulation.

MATERIAL AND METHODS

Target compounds

Physicochemical properties are important to prevent quantification problems that may be related to secondary reactions with impurities or possible molecular degradation during the analytical method application (Comerton et al., 2009).

Compounds with low solubility in water and a high octanol–water partition coefficient (K_{ow}) are generally found in the fatty tissues of biota and cause bioaccumulation in the food chain. The K_{ow} can determine the effective sorption and affinity for organic matter of these substances. The contaminants must have high solubility in water (q) to be easily transported by water bodies (Raimundo, 2007).

These physicochemical properties can categorise endocrine interferents into three broad groups: lipophilic compounds, which have high K_{ow} values; basic or non-ionic compounds; and acidic compounds, which are hydrophilic and ionic. Natural and synthetic hormones are lipophilic in nature. The cyclin group and sulphadimidine are hydrophilic compounds and tend to dissociate easily in an aqueous medium. The factors that can significantly influence the solubility and fate of these substances in the environment are temperature, pH, salinity and existence of humic substances or particulate matter (Raimundo, 2007). Table 1 presents the physicochemical characteristics of the target molecules examined in this research.

Soil columns and rain simulation

The columns were made of polyvinyl chloride (PVC) pipes 110 cm in height and 20 cm in diameter. Their lower part was made of aluminium as a conical support 20 cm in height. Inside each column is a filter medium that consists of a geotextile blanket, which prevents fine soil material and gravel from passing through. The conical base was coupled with the PVC pipe, constituting the column lysimeter. The columns were gathered together in a metal support in groups of six so that they were suspended. The soil was compacted upwards. Then, three lateral outlets were fixed. Further information on the soil compaction is presented in Supplementary Material S1.

After the compaction, water volumes were applied to the upper part of the columns, and the hydrodynamic behaviour of the columns was evaluated. All the means of the flows were statistically compared by ANOVA tests.

Twelve soil columns were created. In the upper layer of each column, different residues were applied with potential hormone contamination in triplicate. These residues swine slurry
liquid (ARS), yellow water, upper layer without residue application (white) and ARS with 50% (m/m) of Pinus Elliottii spp. Bark. The residues were applied after the soil compaction. Three application processes were performed on the experimental days 8, 123 and 187. In the entire experiment, the precipitation simulation was conducted in the soil columns through the direct method using one year of high average precipitation. Collections in all four exit points were performed in each soil column with fortnightly periodicity and characterised by nutrients, hormones and organic and inorganic carbon. The daily rainfall simulation values and the flow volumes are presented in Supplementary Material S2.

**Hormone quantification**

Hormone quantification was conducted using a high-performance liquid chromatography (HPLC; Dionex, Model Ultimate 3000-DAD), with the methodology adapted from Almeida & Nogueira (2006). The mobile phase consisted of an aqueous solution of acetonitrile (10% V/V) and acetonitrile BP at a gradient of 0%-66% of acetonitrile PA for 40 min and a flow of 1 mL.min⁻¹. The column used was C18 (150 mm × 4 mm, 5 µm). The detector maintained the range of 200-400 nm for 17α-estradiol, 17β-estradiol, 17α-ethinylestradiol and mestranol and 240 nm for progesterone. The column temperature was 25 ºC, and the injection flow was 200 µL.min⁻¹.

The residues applied in the soil columns were characterised using the methodology of Matějíček et al. (2007). The mass used for extraction was 5 g. The microwave equipment was operated in a heating ramp system from 0 ºC to 150 ºC for 30 min, at a rate of 25 ºC every 5 min, at a power of 780 W. All the tests were performed in quintuplets. The results of the standard deviation were attained, and Student’s t-test was used to check for the homogeneity of the samples.

**Data treatment**

The mass balance was obtained from each sampling point per soil column based on the average of each triplicate. Four values were generated for each triplicate in accordance with the four outputs (three intermediate and one final). Equation I quantifies the total mass of each lysimeter obtained from the drained volume product and the observed concentrations.

\[ m = \sum_{i=1}^{n} c_i V_i - V_T \]

where \( c_i \) is the sample I concentration, \( V_i \) is the water volume collected in sample I and \( V_T \) is the total water volume drained from the lysimeter.

**Soil characterisation**

The soil used in the experiment was collected in a plot of approximately 1 m³ of an area used for agriculture located in the municipality of Lontras in Santa Catarina, Brazil. Two horizons were used for this collection. The collected soil was separated into horizon A (0-50 cm deep) and B (50-100 cm deep).

The samples were collected for physical soil characterisation with 140 cm³ volumetric rings. They were collected every 5 cm deep and every 20 cm at a 10 cm interval. A total of 12 samples were obtained at 0-100 cm deep. The soil samples were determined through the density, porosity and granulometry of the soil. The samples were collected in the same layers with 475 cm³ volumetric rings to determine the saturated hydraulic conductivity of the soil. The rings containing the samples were removed from the soil, covered with cloths to prevent soil loss and placed in appropriate containers for analysis.

Soil characterisation was performed in the soil physics laboratory of Campos Novos Agricultural Research Company–EPAGRI. After harvesting, the soil was dried in the sun without mixing the two horizons and then homogenised in its own nomenclature (A and B) in a concrete mixer. Afterward, the soil was sifted for later use.

**RESULTS**

**Soil columns**

The daily rainfall volume applied in each soil column was 31.5-3,687.9 mL, corresponding to the precipitation height of approximately 0.5-120 mm, respectively. The volume extracted corresponded to approximately 30% of the volume applied.
These values are not consistent with the data obtained by Cunha & Wendland (2015). In their research, the precipitation during that period was estimated at 788.40 mm, corresponding to 55.6% of the value 1,416.90 mm recorded in the year 2002.

In a study on atrazine and diuron transport in the profile of undisturbed soil, the algal Red–Yellow Argisol Tb A was moderated, and 31.95 L of water was applied in the lysimeters. This value represented 650.85 mm of rain for about six months. The mean total volume collected was 23.19 ± 1.60 L, representing 72.57 ± 8.40% of the total applied (Kaufmann et al., 2012).

The different collection values recorded in the mentioned studies can be related to the different types of soil, whether deformed or undeformed. Several factors affect the soil–water movement (Pott & De Maria, 2012), such as porosity, moisture, biological activity, vegetation cover and surface roughness (Santos et al., 2016). In this context, the managed soils can generate changes in their physical properties, causing an increase in density, a reduction of total porosity and an increase in resistance to penetration, thus reducing air and water fluxes and potentiating the surface flow (Schreiner et al., 2010). Regardless of the application, no statistical difference was found in the recoveries observed in the different treatments, as shown in Figure 1. Therefore, this is not an important variable to explain the flow between soil columns, as it is only a transport vector.

Soil characterisation

Soil characterisation (Table 2) presents the average values of hydraulic conductivity, density, composition and porosity for the two study horizons.

| Sample/Parameter | CHS cm.h⁻¹ | Density g.cm⁻³ | Sand g/Kg | Silte | Clay | Porous C (μm) (cm⁻³.cm⁻³) |
|------------------|------------|----------------|----------|-------|------|--------------------------|
| A                | 11.52      | 1.24           | 279.9    | 348.3 | 371.8 | 0.472                    |
| B                | 11.93      | 1.18           | 272.7    | 265.0 | 462.2 | 0.509                    |

CHS – Saturation Hydraulic Conductivity.

Figure 1. Flow and Rain Simulation. ARS: water swine residuary; AM: yellow water; ADS: Adsorbent Treatment.

Note that porosity is approximately 50%. That is, half of the volume of the lysimeter is space that can be filled with water and/or constituents of the applied residues.

The soil density values determined from the different horizons collected were within the range found in mineral soils (1.10-1.60 g.cm⁻³) and considered ideal for the good development of the plant root system (Kiehl, 1979). However, the density decreased as the depth of the collected sample increased.

The porosity of the post-cultivated soil was significantly reduced because of the increased compaction, which is evidenced by the increased soil density. As horizon A was more compacted, porosity was smaller than that of horizon B, which was heavier and less compacted. This porosity difference refers to the values observed in the soil macroporosity, that is, the decrease in the size of the larger aggregates, which consequently reduces the pore size (Trindade et al., 2009). According to Dias Junior (2000), the compaction process refers to the compression of unsaturated soil that promotes the soil’s increased density and reduced volume caused by the expulsion of air from the soil pores, thus reducing the total porosity, mainly the macroporosity.

Hydraulic conductivity represents the flow capacity of water in the porous medium. It is associated with granulometry, which makes up the soil. Campbell (2006) found that the textural class of sand presents a thicker texture, tending toward a more significant drainage and resulting in an increased hydraulic saturation conductivity as the amount of sand in the composition increases.

Analytical method

Given the low levels of concentration, the calibration exhibited satisfactory linearity. Some points were maintained, and the linearity of the curves was evaluated by determining the coefficient of line. A tendency of low concentrations was observed above the trend line of the curves.

The chromatographic area units (mAU) for the analytes mestranol and 17-α-oestradiol resulted in lower values than the other analytes, as observed from the detection and quantification limits. The chromatographic peak resolutions are presented in Figure 2. These resolutions showed an excellent peak separation and a proper resolution in the two wavelengths used in the methodology.

The analytical curves were observed in the standard deviation of the replicates, the determination coefficient and the slope. All pattern replicates reproduced well. Table 2 presents the values, standard deviation, determination coefficient, slope of the calibration curve, Limit of detection (LD) and limit of quantification (LQ). For each calibration curve was also evaluated the hypotheses that the replicates reproduced, or the inverse, being.
null and alternative hypotheses. For all curves, the null hypothesis was accepted, thus proving the homogeneity of the results of the replicate as evaluated by the P-value in the ANOVA tests at a significance level of 5%.

The detection and quantification limits were in the range of ng.L\(^{-1}\), which was very low but still reached the adequate levels of hormone quantification in the liquid matrices. To ensure the assay quality, the reading of the patterns was conducted on different days to evaluate the calibration curve maintenance and the method confidence. A discrepancy in the results at 4%-8% of the injected patterns was observed. The patterns were randomly injected between the samples and used in the calibration curves. As the HPLC was used exclusively for these analyses, these limits were accepted as real. Moreover, the patterns between the samples were analysed.

As the HPLC was used exclusively for these analyses, these limits were accepted as real. Moreover, the patterns between the samples were analysed.

The ultraviolet (UV) spectra of the calibration curve were correlated by the scores of 0-1,000, which were acceptable in the samples above 950. The retention times of the samples were stable, varying at a maximum of 0.5 min in the samples and patterns.

The patterns used were packed in the refrigerator at temperatures below 4 °C and checked for each chromatographic run. New patterns were made every six months from standard solution dilutions of 1,000 ng.L\(^{-1}\).

All the residues applied were submitted to hormone characterisation and the determination of antibiotics presence. A sample of each residue from a standard hormone solution, which was composed of 17-α-oestradiol, 17-β-oestradiol, 17-α-oestradiol, mestranol and progesterone, at a concentration of 2 mg.L\(^{-1}\) was used. The same step was performed for the standard cyclin solution, which consisted of tetracycline, chlortetracycline, doxycycline and oxytetracycline, in the same concentration as above.

Two grams of each residue was added to 2 mL of each standard solution to maintain the contamination concentration of 2,000 ng.g\(^{-1}\) residues. The results obtained from the contaminated samples and the standard deviation and recovery are presented in Table 3.

Recovery values of 89.26%-93.67% were obtained for the hormones, thus proving that the technique recovered acceptable concentrations and was validated for use. The same technique for the antibiotics extraction was used, and the recovering values obtained were 2.08%-3.07%. These values indicate an inefficiency in the extraction for this contaminant class.

These values were also observed through Student’s t-test to determine the homogeneity results (Table 4). Through this analysis, only the analytes 17-α-oestradiol and mestranol were found to be homogeneous, that is, belonging to the same group. As for the other analytes, a small discrepancy was found in the case of the other hormones observed, whereas a large discrepancy was found in the antibiotics. The latter was not considered homogenous by the replicates.

Whereas the extraction method was validated for the hormones and rejected for the antibiotics, it did not work for the analyte quantification in the ARS. The mass balance was decreased for the antibiotics because of this problem in the extraction methodology.

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**Table 3. Calibration curve data for hormone determination.**

| Compound       | R\(^2\)   | Slope   | LD (µg.L\(^{-1}\)) | LQ (µg.L\(^{-1}\)) |
|----------------|----------|---------|-------------------|-------------------|
| β-Estradiol    | 0.9946   | 1.15225 | 0.99              | 3.01              |
| α-Estradiol    | 0.9998   | 0.65516 | 1.56              | 4.73              |
| 17-α-Estradiol | 0.9667   | 1.05874 | 2.49              | 7.55              |
| Progesterone   | 0.9855   | 2.40718 | 1.63              | 4.94              |
| Mestranol      | 0.9967   | 0.03498 | 0.77              | 2.33              |

LD - detection limit; LQ – quantification limit.
### Waste quantification

From the microwave-assisted extraction, the results of the characterisation showed that the analytes were more available in the yellow water. This outcome can be explained by the acidic media and the absence of organic matter compared with the other matrices. Table 5 shows the average concentrations of hormones in the matrices applied with water swine residuary and yellow water.

A discrepancy was observed in the mestranol concentrations when evaluating applications 1 and 2. This discrepancy was due to the pig cycle or even the menstrual cycle of women that contributes to urine collection (Goeppert et al., 2015).

High concentrations of AE2 and ME (Figure 3) were obtained in the samples as both are natural analytes (Table 6). The metabolic process of mestranol demethylation transformed EE2 in the presence of anaerobic microorganisms in the same way that the AE2 could be converted to the $\beta$-E2 isomer (Ying et al., 2002).

Manure from animal breeding, such as pigs, cattle, sheep and birds, is considered a natural source of hormones (Casey et al., 2003).

### Occurrences of the target compounds

In total, 1,320 samples were collected and analysed. Among them, 9 analytes were observed, and 14,850 analyses were conducted. Quantifiable concentrations were obtained in 1,264 (8.51%) analyses. Some analytes were detected in 1,531 (10.31%) analyses, and no occurrence was found in 12,055 analyses (81.18%). Thus, in 18.82% of the analyses, some form of hormone occurrence was observed over experiment time (Figure 4).

This occurrence discrepancy, which is 2%-10% in previous literature (Pinheiro et al., 2013; Zhang et al., 2007; Casey et al., 2003), can be explained by the formation of the E1 sulphate conjugate (E1-3s) and E2-3s (Scher, 2009). However, some studies on estrogen transport in soil columns found a polar metabolite formation that had not yet been identified and that could be a sulphated conjugate (Casey et al., 2003). In most of the analyses, a strong chromatographic signal with a UV spectrum was found in the chromatograms, thus confirming the presence of estrogen in the first few minutes. As an apolar column was used, the compound was assumed to be a polar one.

The free E1-3S is formed from a biological component. However, the identification of this enzyme or microorganism in this conversion has not been studied (Goeppert et al., 2014).

No occurrences were observed in the water samples collected in the lysimeter application of textile sludge and in the lysimeter verification. The most significant amounts of occurrences were in the lysimeters with urine application (AM), accounting for 0%-35% of the quantification and 7.95%-14.77% of the detection.

In the soil columns submitted to the biosorbent, only 39 of the 990 analyses were detected, and none of them were quantified. That is, compared with the other treatments, a relative decrease occurred because of the biosorbent action.

Interestingly, the appearance of $17\beta$-oestradiol, which was not detected or quantified in the residues, should be emphasised. This substance formation occurs because of a metabolic pathway.
of anaerobic bacteria, transforming the α isomer into β (Ying et al., 2002). The kinetics of this compound formation was not examined. However, a significant occurrence was found in the observed concentrations.

In compacted columns with sandy and clayey soils, Mashtare et al. (2013) observed the estrone and oestradiol biotransformation in 17-α and 17-β-estradiol. The column with the highest organic matter content in clayey soils presented a degradation rate two times higher than that of the column with sandy soil. Moreover, CO₂ production in the columns was four times higher in the clayey columns, indicating a high microorganism activity and/or degradation. Chun et al. (2005) reported that the activity of the enzyme dehydrogenase is a critical variable in hormone degradation in the soil and is positively correlated with the oxidation of 17-β-estradiol.

In their study on modelling hormone transport in soil columns, Mohammed et al. (2013) observed that adsorption does not depend only on the organic matter content of the soil but also on its clay content.

Thus, when observing analytes such as mestranol, which is transformed into EE2 in the presence of anaerobic microorganisms through the metabolic process of demethylation, the higher the mestranol mass is, the greater the EE2 recovery. This analogy is corroborated by Ying et al. (2002), who obtained similar results.

According to Chen et al. (2007), the probability of estrogen being adsorbed on the soil is five times higher than it being desorbed. Most of the masses transported were adsorbed and/or degraded in the soil column itself. This fact explains why only a few masses was recovered.

### Mass balance

At least some removal, attenuation, or degradation occurred in the secondary metabolites of all the molecules observed. However, 17-β-oestradiol exhibited an atypical behaviour. It was not included in the composition of the waste matrices applied in the upper part of the soil columns, and the same occurrence was observed at the beginning of the study. This outcome could be related to the interconversion of the enzyme-linked molecular pathway. This phenomenon is not yet well established in the literature. However, E1 could be transformed to E2 through the preferential formation of the more potent 17β isomer up to ~30 41 mol%, suggesting that the isomer interconversion occurred through E1. Thus, the sediments could serve as both a sink and a source of the more estrogenic compound E2. Hormone transformation was markedly slower in autoclaved sediments than in non-autoclaved sediments. The results support the inclusion of an isomer-specific behaviour and the potential for a reversible transformation and interconversion.
Fate and transport of compounds with estrogenic activity in reconstructed soil columns in anaerobic sediments in modelling fate in stream networks and developing risk management strategies (Mashtare et al., 2013). This hypothesis is evidenced by the conversion from 4.38 to 51.10 nmol of BE2 in the lysimeter study. Whereas all the constituent masses of the residues were applied in the soil columns, only mestranol was transported steadily on the experimental days. The global recovery of mestranol for the ARS and AM matrices reached 20%, and the remaining 80% was adsorbed, degraded or converted to other steroids.

Observing a similarity between the recovery profiles in the lysimeters and in the wastewater application from swine and human urine was possible, with EE2, progesterone and mestranol having the highest recoveries. However, this pattern was not maintained in the adsorbent lysimeters, where progesterone had the lowest recovery of mestranol.

Figure 4. Profile of the analyte occurrence studied over time. ARS: water swine residuary; AM: yellow water; ADS: Adsorbent Treatment; EE2: 17-α-Estradiol; PG: Progesterone; Me: Mestranol, E2: Estradiol.
The masses recovered in the second application were considerably smaller. However, the masses applied from the residues were also smaller than those in the first application. Thus, the input masses were smaller. In terms of the recovery rates, the soil columns with urine maintained their behaviour, reaching a mass recovery rate of 1.4%–11.0%. The adsorbent material had an efficiency decrease in the succeeding mass balance because of a possible saturation at the sorption sites.

In the comparison profile, the columns with an adsorbent layer showed a distinct dynamics of the other treatments in the two types of transport, namely, runoff and superficial. By contrast, all analytes present in the yellow water were transported more easily because they were more available in the medium (Figure 5).

The relationship between the compound sorption in the soil and the competition of soil with organic matter is not yet understood in the literature. However, some studies pointed out that this competition could be a false positive because of the affinity between the EDs and the organic matter. Biologically, organic matter is degraded, and the pollutant returns to the environment and is transported later (Yu et al., 2008).

Emphasising the role of a soil column with an adsorbent material on the surface is important. This soil column is effective as it removes 99% of the observed contaminants. Adsorption is considered the best technique to be applied in the removal of this pollutant class (Altmann et al., 2016) because it does not produce degradation products and can be easily applied to existing effluent treatment plants.

**CONCLUSION**

The occurrence of hormones from residues applied as biofertilisers was observed in approximately 20% of the samples. Even after three months after the residue application in the soil columns, observing the occurrence of some analytes was still possible. This result demonstrates the retention capacity and the delays in the release of hormones into the soil. Hormones tend to flow rapidly at a depth of 10 cm or 100 cm, with the intermediate depths serving only as passing zones.

The matrix constituting the biofertilisers affected the mobility of the analytes. Progesterone presented a different mobility when the sources of the application were observed. This phenomenon can be explained by the amount of organic matter. The analytes could have been possibly retained in the ARS organic matter, whereas they were available in the yellow water and were displaced along with the profile with greater ease.

The use of a biosorbent was shown to be effective in attenuating the hormones in soil columns. About 95% of the hormone mass applied was retained by the adsorbent. However, adsorption studies should be conducted to determine whether desorption occurs and how soon it occurs. Therefore, advancing the adsorption phenomenon in the characterisation and performing adsorption tests outside the soil column (e.g., in a reactor) will be necessary to evaluate the effectiveness of the process.

The high mobility of disturbed soil hormones leads to the necessity of studies on removing or attenuating the analytes.

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**Figure 5.** Compound recoveries by depth. EE2: 17α-Estradiol; PG: Progesterone; Me: Mestranol; E2: Estradiol.
present in the biofertilisers before applying them to the soil. Note that most of the recovered masses occurred at a 100 cm depth and in greater magnitude soon after the application, thus proving the rapid movement of these molecules.

The biosorbent application showed a good ability to attenuate hormones by retaining these molecules in the upper part of the soil column and preventing them from moving along the soil profile.

Studies on the attenuation and removal of these analytes in both water bodies and sanitary effluents are necessary. Hormone occurrences are alarming because, even if they are close to the concentrations observed in the literature, they are detrimental to the human organism, specifically to the endocrine system.

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Thiago Caique Alves: Writing, data processing and analysis execution

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SUPPLEMENTARY MATERIAL

Supplementary material accompanies this paper.
Supplementary Material (S1).
Supplementary Material (S2).
This material is available as part of the online article from http://www.scielo.br/RBRH