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# Detection, fate and transport of estrogen family hormones in soil

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## HIGHLIGHTS

- The transport behavior of 17β-estradiol in soils was examined.
- Two metabolites, estrone and estrone-sulfate, were identified in non-autoclaved soil.
- Mass recovery decreased with increasing input concentration in non-autoclaved soil.
- Estrogens are significantly retarded compared to a conservative tracer.

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## ABSTRACT

Estrone (E1), 17β-estradiol (E2), and estrone-sulfate (E1-3S) are released into the environment in significant amounts. They are known to adversely affect the endocrine systems of aquatic organisms. Although previous studies clearly demonstrate that free hormones sorb strongly to soil and degrade quickly, significant amounts of free and the more persistent conjugated estrogens can be still detected in various environmental media. To date, E1-3S has been considered as a metabolite that forms either during the animal hormone cycle or as a degradation product of precursor hormones like E2-3S. We performed small-scale laboratory column tests to investigate two major features: transport and degradation of E2, and formation of E1-3S and E1. To evaluate the influence of soil microbial activity, one portion of soil was autoclaved and the background solution treated with sodium azide. The results demonstrate that (i) E2 is degraded to E1 and E1-3S in non-autoclaved soil, and to E1 in autoclaved soil, (ii) the formation of E1-3S is biologically driven, and (iii) the transformation of E2 to E1 does not require biological interaction. An inverse modeling approach was used to quantify the transport parameters and degradation rate coefficients.



## 1. Introduction

Human and animal waste-borne steroidal hormones are found in the environment at low nanogram per liter concentrations (Hanselman et al., 2003; Khanal et al., 2006); the detection of such concentrations requires advanced analytical techniques (Xu et al., 2006; Hsu et al., 2007; Xu et al., 2008). The estrogens 17β-estradiol (E2) and estrone (E1) are considered highly potent hormone-active compounds; they interfere with the normal operation of the endocrine system and physiologically affect fish and other aquatic vertebrate species at lower concentrations than other endocrine-disrupting compounds. For instance, environmentally-relevant concentrations of hormones were found sufficient to stimulate production of a female protein in male fish (Routledge et al., 1998), with threshold levels for E2 of 1–10 ng $L^{-1}$.

Animal manure applied to agricultural fields is considered to be a major source of estrogens and estrogen-sulfates in rural environments (Hanselman et al., 2003; Scherr et al., 2008). Estrogen-sulfates in animal waste were shown to adversely affect the endocrine system via deconjugation (Scherr et al., 2009a). The estrogenicity of estrogen-sulfates originates from the deconjugation and transformation to free estrogens; several batch studies deal with the estrogenicity of E1 and E2 (Roig, 2010). Where estrone-sulfate (E1-3S) estrogenicity has been observed, this effect is hypothesized to result from deconjugation of E1-3S to E1 (Isobe and Shimada, 2003).

In urban areas, wastewater effluents from treatment plants are the major source of hormones (Schlüsener and Bester, 2008) in surface waters. Current treatment technologies do not fully eliminate hormones and their conjugates; elimination rates differ significantly from one treatment plant (and technology or weather condition) to another, and metabolism even results in an apparent increase in hormones, e.g., of up to 360% for E1-3S, while the total hormone load between inlet and outlet remains equal (Schlüsener and Bester, 2008). The overall low, but effective, concentration of estrogen-family hormones, their complexity, the variety of

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chemical structures, and the transformation of active and conjugated forms makes it difficult to fully eliminate them or predict their behavior (Caliman and Gavrilescu, 2009).

Most studies focus on sorption and degradation of conjugated estrogens in batch tests (Hanselman et al., 2003). For instance, Scherr et al. (2009a) discussed the formation of E1-3S and E2 (which subsequently form E1) as the major metabolites of estradiol-sulfate (E2-3S) in incubation studies; formation of E1-3S is interpreted as a result of biological activity, dependent on temperature and soil type. However, these studies did not address the transport characteristics of hormones, which are required for comprehensive environmental assessment. Moreover, discussion continues as to whether the cause of the oft-observed transformation of E2 to E1 (and vice versa) is a result of microbial activity or redox conditions, because of experimental limitations such as incomplete sterilization or thermally stable enzymes (Hanselman et al., 2003; Khanal et al., 2006). Also, the effect of input concentration on hormone degradation is unclear; there is evidence that degradation rates decrease with time or with lower concentrations (Casey et al., 2005).

Small-scale laboratory column tests under well-controlled conditions allow identification of relevant transport processes and parameters by simplifying the naturally-occurring complex interaction among soil, water and hormones. However, only very few studies consider the fate and transport of estrogen-family hormones in column tests at environmentally-relevant concentrations, including metabolite formation. Different limiting factors of the column studies are reported. An elevated flow velocity and column size needed to obtain required sample volume causes preferential flow, a screening for metabolites is missing, and efforts to model the physical/chemical non-equilibrium resulted in low coefficients of determination (Sangsupan et al., 2006). Another study based on radio-labeled estradiol highlights also the lack of information about the transformation process, metabolic products and the large number of fitting parameters which results in non-uniqueness of the applied model (Casey et al., 2003). Most studies postulate that hormone transport is characterized by low mobility and low persistence (Casey et al., 2005) which cannot explain the low levels of estrogen-family hormones in the environment.

Although there is significant research on the fate and transport of 17β-estradiol and estrone in the environment (Khanal et al., 2006), there is still a need to understand the fate, transport and removal of estrogenic compounds including metabolites in soils and water, in particular in the presence or absence of soil microorganisms considering varying hormone concentrations that might stimulate degradation or transformation processes.

To address these issues, we conducted a series of experiments with three different input concentrations of E2 in small-scale laboratory columns. Natural autoclaved and non-autoclaved, hormone-free Bet Dagan soil (BDS) was used to study the fate and transport of E2 and its metabolites under saturated conditions. The experiments employed pulse tests simulating the input of a small volume of hormone-enriched water into the soil–water environment. Retardation coefficients for the hormones and a conservative tracer were calculated to demonstrate the extent of hormone adsorption/desorption, and to validate the functioning of the column and injection system. To quantify the influence of biological activity, transport parameters and recovery of each compound in autoclaved soil were compared to non-autoclaved soil.

## 2. Material and methods

### 2.1. Chemicals

Estrone (E1), estrone-3-sulfate (E1-3S), 17β-estradiol (E2), and estriol (E3) were purchased from Sigma–Aldrich, Steinheim, Germany. Isotopically-labeled estrone-$^{13}C$ (estrone 3,4-$^{13}C_2$) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Molecular weights are given in Table 1. Humic acid sodium salt, technical grade [68131-04-4] and sodium fluorescein (uranine) [518-47-8] were obtained from Sigma–Aldrich, Steinheim, Germany. Uranine was used as a conservative tracer. Water was purified and deionized on a Milli-Q purification system (Millipore Corp., USA). Details of other compounds used for this study are given in Supplementary material (S1).

### 2.2. Preparation of stock solutions and background solution

Stock solutions for calibration of each hormone and injection of E2 were prepared in acetonitrile and diluted with water. Final concentrations of 1–30000 ng $L^{-1}$ were used for calibration of each hormone. Injection solutions of 2.8, 14 and 28 mg $L^{-1}$ E2 were prepared by dilution with water from stock solutions containing acetonitrile. For the pulse injection, 1 mL of the solution was used, so that the input masses were 2.8, 14 and 28 μg E2, respectively; E2 solubility was ensured after EN ISO 7579 (2010).

Five milliliter aliquots of water samples and calibration solutions were spiked with 10 μL (300 ng $L^{-1}$) of internal standard. The estrogen stock solutions were stored at 4 °C in the dark. The limits of detection and quantification were calculated according to ISO 11843. The standards were linear ($R^2 \geqslant 0.99$, $n = 10$) and the detection limits according to ISO 11843 were 1.55 ng $L^{-1}$ (E1), 2.8 ng $L^{-1}$ (E2) and 1.03 ng $L^{-1}$ (E1-3S). The composition of local tap water (Chefetz et al., 2008) was chosen as background solution for the transport tests and prepared by diluting stock solutions. Further details are given in Supplementary material. Sodium azide (0.02%) was used to prevent microbial activity while running tests with autoclaved soil only.

### 2.3. Soil

A sandy clay soil (Solel et al., 1979; Yaron et al., 1989) with a clay content of 25.3% (mainly montmorillonite), 14% silt, 48.6% sand, 12.1% coarse sand, 0.5% organic matter and specific surface area 68 $m^2$ $g^{-1}$ was obtained from Bet Dagan, Israel. Additional details on the soil chemistry are given in Supplementary material (Table S1). Non-autoclaved soil was sieved (1 mm mesh) and air-dried at room temperature (22 °C). A portion of this soil was autoclaved using a published method (Carter et al., 2007). Briefly, small portions of soil (100 g) were sealed in autoclave bags and autoclaved at 121 °C (103 kPa) for 60 min on two consecutive days.

### 2.4. LC–MS/MS analysis

An online solid-phase extraction liquid-chromatography tandem mass spectrometry system (Waters, Aqua Analysis system) equipped with triple quadrupole mass spectrometer (Quattro micro API), electrospray ionization (ESI), two SPE columns Oasis® HLB (25 μm, 2.1 × 20 mm; Waters) and analytical $C_{18}$ column (Waters XBridge™ 3.5 μm, 2.1 × 50 mm) was used. The detailed analysis procedure is provided in Supplementary material (S2). The retention times were 5.42 min (E1 and E2) and 5.23 min (E1-3S). The estrogens were analyzed using the parameters and gradient system given in Tables 1 and S2 (Supplementary material). The total chromatographic run time for one sample including online SPE LC-MS/MS was 15 min. All analyzes were run in MRM mode (Multiple reaction monitoring), which allowed a separation of each compound on the basis of the fragmentation and retention times.