



## **Detection, transport and degradation of free and conjugated estrogens in soils**

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The behavior of Estradiol (E2), Estrone (E1) and Estrone-Sulfate (E1-3S) in a natural soil is analyzed in terms of transport and metabolic degradation properties. These compounds are released into the environment in significant amounts and have been shown to potentially contribute to endocrine disruption in humans and wildlife. Although literature data demonstrate that hormones sorb strongly to soil and also degrade quickly, significant amounts, especially of conjugated estrogens, can be still detected in various environmental media. Moreover, several recent studies note that hormones bind to colloidal organic carbon, which can facilitate hormone transport and decrease the degree of hormone degradation. These findings thus suggest a moderate mobility and persistence of estrogen compounds and their metabolites. However, many uncertainties regarding the transport and fate of estrogen compounds remain, which are complicated by difficulties in sampling and detection. Most research is performed at the catchment scale where large volumes of water are available for analysis. In addition, laboratory batch tests usually involve estrogen concentrations that are large and unrealistic for environmental conditions, or require use of labeled compounds for which it is unclear whether the substance measured is parent or metabolite. There is a serious lack of transport studies under controlled conditions, using small water volumes and low detection limits, which are crucial to understand the basic transport processes of estrogen transport and degradation. Here, we present a protocol for hormone measurement using an online-SPE LC-MS/MS for detection. We use the method to quantify free estrogens and metabolites at low concentrations in small sample volumes (~mL) of water and soil extracts. The study includes batch (sorption) and transport tests in flow-through columns, using natural clay-rich soil from Bet Dagan, Israel. Consideration of sorption and transport experiments using both autoclaved and non-autoclaved soil enables determination of the effect of enzyme activity on estrogen metabolic degradation. Three main hypotheses are verified: a) hormone degradation via metabolism yields more persistent conjugated estrogens, b) enzyme activity can seriously affect estrogen fate in soils and c) sorption of hormones to colloids affects transport and persistence.