



## **Quantification of fluorescent staining tracer in soil profiles: calibration and influence of adsorption**

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Staining techniques are widely used to visualize solute movement or leaching of chemicals, and to investigate the influence of preferential flow paths on water movement in soils. For that purpose different staining substances are usually applied to the soils by sprinkling. Different staining substances (including both fluorescent and common dyes) have been used for flow visualization. However, for calibration of spatially explicit models of solute transport, actual dye concentrations are needed rather than merely staining indicators. Compared to common dyes (e.g., the most popular staining agent Brilliant Blue FCF), fluorescent substances have some remarkable advantages: (1) they are visible in soils of different colors, including very dark soils; (2) two or more fluorescent dyes with separated excitation and emission wavelengths can be used simultaneously; and (3) they permit examination of the soil profile for any wavelength different from excitation range of the staining dye employed.

Here we develop a novel method for simple, but accurate quantification of fluorescent staining tracer Uranine in soil profiles. To make this procedure economic and easily applicable, only widely available components were utilized. For fluorescence excitation common black light luminescent lamps with a peak wavelength of 350 nm were chosen, and a setup with four lamps embedded into two reflection panels was used. To detect staining patterns, an Olympus camera (Olympus E-330, lenses Olympus Zuiko Digital 14-15 mm 1:3.5-5.6) was used. A glass-soot panel was developed for soil reflection estimation, and pink light lamps were used for assessing heterogeneous optical properties of soil profiles. Calibration procedures are known to be complicated as they denote mixing of disturbed soil with known amounts of dye solution. To simplify this procedure, undisturbed soil was used, and a time-saving in situ calibration method was developed, where calibration was performed simultaneously with a staining experiment in a slice adjacent to the excavated soil.

Both experimental and theoretical investigations of fluorescence in soils showed that the molar absorption coefficient of the dye and excitation wavelength play a crucial role in utility of a particular tracer. It was also found, that adsorption of dye in soil plays an important role and causes fluorescence quenching (at least for Uranine). We speculate that it is much more efficient to consider fluorescence to be excited by incident light and relate its intensity to concentration in solution.

Finally, an easily applicable and inexpensive visualization and quantification technique for a fluorescent tracer in natural soil profiles was developed and its applicability was demonstrated at a field site. A new in situ calibration is proposed, which simplifies this procedure and makes it more realistic. This method can be used not only to quantify fluorescent dyes, but also for common dyes like Brilliant Blue FCF. It was shown that reflectance, fluorescent layer depth, and scattering and absorption properties of soils can be assessed not only by complicated diffuse reflectance spectroscopy, but also with simple equipment used in this study. Adsorption and its kinetics are important factors to consider during calibration and quantification and should not be ignored; however, there are still some uncertainties that should be studied in more detail. Different image filtering and correction procedures were examined and their importance for particular equipment was established. The cost of equipment and expenditures (except for the camera) was less than \$700US. There is an evidence, that fluorescence quenching by adsorption occurs mostly in organic substances and its mechanisms are different from those for mineral soil grains; currently we are working to solve these uncertainties.