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# A comparison of fluorescein and deuterated water as tracers for determination of constructed wetland retention time

**Research Article**

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**Abstract:** Retention time of treated water in a horizontal subsurface flow constructed wetland was determined in the non-vegetative period using fluorescein and deuterium oxide. Fluorescein served as one of the most frequent tracers detectable at extremely low concentrations by fluorimetry; however, deuterated water (concentrations of deuterium measured by IRMS and expressed as δ (‰) against VSMOW) was used to precisely simulate the treated water flow movement. Tracer retention time (TRT) of fluorescein was 194 h while deuterated water TRT was 192 h. TRT and nominal hydraulic retention time (nHRT, 190 h) were nearly exactly equal. The tracer behavior of deuterated water was almost ideal. On the other hand, the fluorescein movement through the system was slightly influenced by the interaction with the vegetation bed (sorption causing the tailing of tracer-response curves). Nevertheless, both tracers can be successfully used and provide similar results. Retention time is a very important characteristic of a constructed wetland. It is closely connected with the efficiency of the contaminant removal from treated water. It has to be determined correctly when wetland operation parameters are optimized. The choice of the suitable and reliable tracer is always necessary. Fluorescein takes preference with respect to its simple and inexpensive determination.

**Keywords:** *Environmental analytical chemistry • Wastewater treatment • Reed bed • Water tracing • Isotope-ratio mass spectrometry*



## 1. Introduction

Wetlands are transitional environments between terrestrial and aquatic ecosystems where the water table is usually at or near the surface or where shallow water covers the land [1]. Constructed wetlands (CW) for wastewater treatment are engineered systems that have been designed to utilize the natural processes involving wetland vegetation, soils, and their associated microbial assemblages to assist in treating wastewaters. Wastewater treatment using constructed wetlands is simple, and requires less power and financial means than traditional technologies (*e.g.* mechanical-biological wastewater-treatment plants). The function of constructed wetlands as solar radiation dissipaters is also very important from the ecological point of view [2].

Retention time of treated water in the constructed wetland is an important characteristic of the system. Longer retention time means a longer time for microbial activities. Thus, it increases the purification efficiency potential of the wetland (*e.g.* removal of organics and

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nitrogen). Longer retention time also increases the removal of suspended solids and the sedimentation of insoluble particles [3]. Therefore, suitable methods should be available to determine retention time. Water retention inside the system can be mathematically expressed by a nominal hydraulic retention time (nHRT, $\tau$). Rash and Liehr [4] likened water behavior in CWs to chemical reactor theory with the simplest way of calculating $\tau$ (h) using wetland volume $V$ ($m^3$) and average flow rate $Q$ ($m^3\ h^{-1}$). Garcia *et al*. [5] described in more detail the nominal wetland volume, relating it to porosity $\varepsilon$ (dimensionless) of the substrate, water depth of the vegetative bed $h$ (m), and area of the wetland $S$ ($m^2$): $\tau = V / Q$ where $V = \varepsilon \cdot h \cdot S$. The mathematical tool for analyzing non-ideal flows in real systems is contained in a residence time distribution function (RTD) [6-8]. Developing the RTD requires injecting a conservative tracer into the system and measuring its concentration in the effluent time. If two (or more) tracers are injected simultaneously (at the same time, under the same conditions) their behavior in the wetland can be compared on the basis of their response (breakthrough) curves and times when their highest concentrations are detected at the outflow from the system. Various tracers, particularly, fluorescent dyes (*e.g.* fluorescein, rhodamine WT) or metal ions (*e.g.* lithium) can be used [9].

The aim of this study is to compare fluorescein and deuterated water capabilities to serve as tracers to determine the retention time of water in a CW with a horizontal subsurface flow (vegetation wastewater-treatment plant). Fluorescent dyes have already been used during several similar studies, while the use of deuterium oxide represents a novel approach to this research. Stable isotope studies have only been conducted in order to investigate the wetland hydrology [10].

Fluorescein belongs to the widely used conservative tracers in surface and subsurface water studies [11]. It is water-soluble, strongly fluorescent, and therefore detectable at extremely low concentrations [12]. When excited, fluorescein emits a green fluorescence radiation with maximum intensity at 510-520 nm. It is inexpensive, non-toxic, and non-mutagenic. However, fluorescein can sorb with sediments inside the studied system (CW). The sorption can markedly retard its flow and thus negatively affect its use as a tracer. The sorption is most pronounced at low pH [13].

Deuterated water is expected to be an excellent tracer due to its similarity to water, chemical stability, non-reactivity, and ease of handling and sampling [14]. Isotopic exchange causing a retardation of this tracer when it interacts with materials holding a large amount of water near their surface is only expected [15]. However, the determination of deuterium (hydrogen isotope ratio) in water samples is rather expensive and requires the use of special instrumentation - an isotope ratio mass spectrometer (IRMS).

The present study was conducted during the non-vegetative (winter) period. Thus, the impact of the wetland vegetation (*Phragmites australis*) was minimized. It is well known that a significant amount of water can be depleted by evapotranspiration during the vegetative period [16]. The outflow water rate can be even negligible in the case of the CW in summer. This would cause a significant loss of a tracer (deuterated water) and disable the experiment. The interaction of tracers with the vegetation bed (gravel, sediments, roots, and rhizomes) and a layer of clay separating the bed from the subsoil was the subject of this study.

# 2. Experimental procedure

## 2.1. Study site

The studied system was a CW with a horizontal subsurface flow located in Slavošovice, 15 km east of České Budějovice (South Bohemia, Czech Republic). This wastewater treatment plant started operations in August 2001. The system consists of a storm overflow, a pretreatment (screens, horizontal sand trap, and sedimentation basin), and two vegetated beds planted with common reed (*Phragmites australis* (CAV.), TRIN. ex. STEUDEL). The size of each reed bed is 17 m (length), 22 m (width), and 0.9 m (water depth). The constructed wetland substrate consists of gravel (1.0-2.0 cm). The porosity of the wetland bed is 36-50%. The wetland was designed for 150 person equivalents (PEs). The area for one PE is *ca*. 5 $m^2$. The CW treatment beds are separated from the subsoil by a natural layer of clay, which minimizes loss of water by leaching. The inflows and outflows of the CW are exactly determined and measurable. The main hydrological parameters and characteristics of the studied system are summarized in Table 1.

## 2.2. Tracer injection

A mixed solution of both tracers was prepared. Fluorescein (60.0 g, free acid, Fluka-Chemie, Buchs, Switzerland) was dissolved in ethanol (6.5 L, 97%, V/V) and 200 mL of $D_2O$ (Merck, Darmstadt, Germany) were added. This solution was injected to the influx after pretreatment (IN, see Fig. 1) in the non-vegetative period.

**Table 1.** Hydrological parameters and characteristics of the Slavošovice CW.

| Parameter | Value |
|---|---|
| Air temperature (°C) | 1.3 ± 7.6 |
| Water temperature at 0.2 m (°C) | 2.7 ± 3.6 |
| Water temperature at 0.5 m (°C) | 3.6 ± 2.5 |
| Inflow rate (L $s^{-1}$) | 0.219 ± 0.047 |
| Outflow rate (L $s^{-1}$) | 0.184 ± 0.043 |
| Daily sunshine (h) | 2.0 ± 2.9 |
| Precipitation (mm) [a] | 2.2 |

*Average values and standard deviations for the experimental period.*
*[a]Total amount during the experimental period.*

## 2.3. Water sampling

Water samples were taken at the outflow (OUT) and along a central transect running the length of a wetland bed from the inflow to the outflow (at a 60-cm depth) on days following the tracer injection (daily for two weeks). Eight sampling sites were designated at 1, 2, 3, 4, 5, 7, 10, and 13 m (S1-S13) from the inflow (Fig. 1). All samples were taken into plastic bottles, which were immediately wrapped in aluminum foil to protect them from direct sunlight and transported to the laboratory. There, the samples were filtered using glass-microfiber filters (0.40 µm, Macherey-Nagel, Düren, Germany) and placed in cold storage until analysis.

## 2.4. Sample analyses

A Shimadzu RF-1501 fluorimeter was used to determine fluorescein concentrations. It was operated at 490-nm (excitation) and 514-nm (emission) wavelengths. Relative fluorescence intensities of individual samples were measured using a 10-mm long quartz cell. Samples were diluted with deionized distilled water when fluorescein concentrations exceeded a linear dynamic range (LDR). Limit of detection of $2.4\times10^{-3}$ µg $L^{-1}$, limit of quantification of $9.1\times10^{-3}$ µg $L^{-1}$, LDR of $9.1\times10^{-3}$ - 20.0 µg $L^{-1}$, sensitivity of 34.66 L $\mu g^{-1}$, and repeatability of 3.33% were assessed for the fluorescein determination.

A Thermo Finnigan DELTA$^{plus}$ XL isotope ratio mass spectrometer was used to determine deuterium concentrations in individual samples. The deuterium concentrations in the analyzed water samples were expressed as δ(‰) parameter that is defined as: $\delta(‰) = (R_{sa}/R_{st} - 1)\cdot1000$ where $R_{sa}$ and $R_{st}$ are $^2H/^1H$ abundance ratios in a sample and a suitable standard. Hydrogen δ(‰) values are related to the VSMOW (Vienna Standard Mean Ocean Water) standard. This reference material is the basis of an international scale accepted to express a relative deuterium abundance

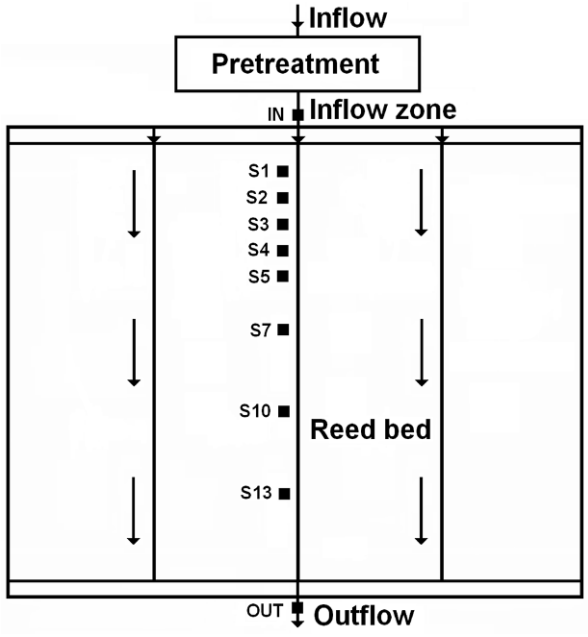


**Figure 1.** Scheme of the constructed wetland reed bed with the pretreatment section.

in samples, and its δ = 0‰ [17]. A $^2H/^1H$ ratio ($\times10^6$) is 155.75 for VSMOW. Samples enriched by deuterium are characterized by δ > 0‰; however, δ is less than 0‰ for samples deprived of deuterium.

## 2.5. In-situ monitoring

Continual measurements of inflow and outflow water intensities were taken using ultrasonic probes US-10 (Fiedler, Electronics for Ecology, Czech Republic), with the data recorded by dataloggers (M4216, Fiedler, Electronics for Ecology, Czech Republic).

# 3. Results and discussion

## 3.1. Flow movement of tracers

Both tracers were injected to the inflow water (Fig. 1) in the non-vegetative period. Water samples were taken along a central transect running the length of the wetland bed from the inflow to the outflow and at the outflow on following days (daily for two weeks), and concentrations of tracers were determined. The flow movement of fluorescein (selected fluorescein-response curves for 2, 4, 7, 10, and 13 m from the inflow zone) is shown in Fig. 2. Similarly, deuterium-response curves are shown in Fig. 3. The average water flow rate through the wetland bed was rather fast in the non-vegetative period being approximately 2 m per day (the longitudinal and transversal heterogeneity of the flow rate must be taken in the account). The maximum concentration of

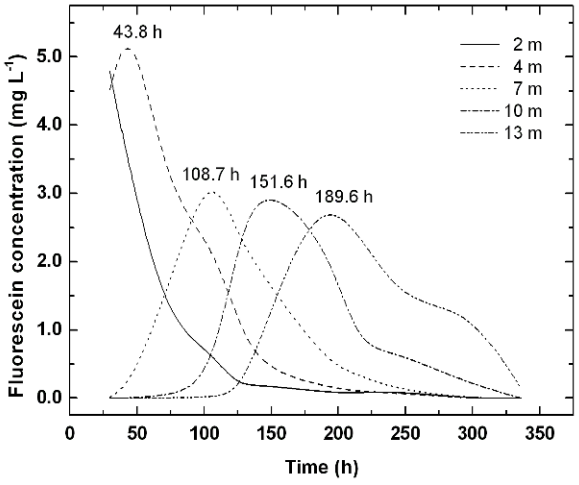


**Figure 2.** Fluorescein flow movement in the constructed wetland bed.

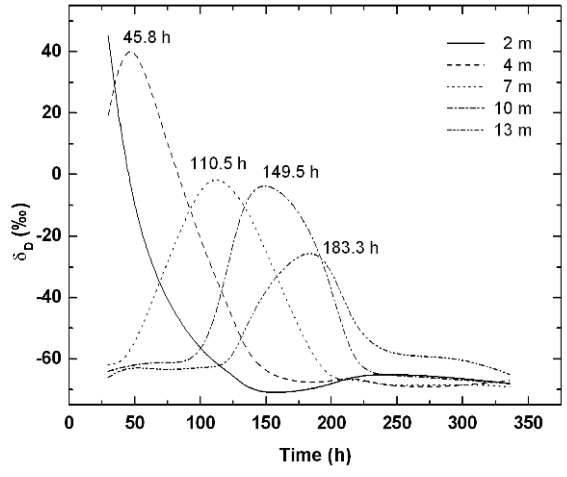


**Figure 3.** Deuterated water flow movement in the constructed wetland bed.

fluorescein at 10 m from the inflow zone (2.9 mg $L^{-1}$) was detected 151.6 h after the tracer injection. The maximum concentration of deuterium (δ = –3.6‰) at the same site was detected almost at the same time (149.5 h). Water without $D_2O$ addition is characterized by δ value of *ca*. –65‰. The movement rates of the tracer maximum concentration zones are very similar for both tracers; however, the shapes of tracer-response curves at individual distances from the inflow zone vary to some extent for fluorescein and deuterium. The fluorescein-response curves are wider with a more intensive tailing effect in comparison to the deuterium curves. It documents the slight ability of this compound to sorb in the vegetation bed (*e.g.* to sediments or gravel). The tracer properties of deuterated water were almost ideal in the wetland bed. With respect to the similar behavior of the tracers, both compounds can be used to determine water retention time in the CW. However, since fluorescein can be applied and determined at minimum expense, is readily available, non-toxic, and degradable in direct sunlight, it is preferred under winter time condition.

### 3.2. Tracer retention times and nominal hydraulic retention time

Tracer retention time (TRT) of fluorescein was determined 194 hours, while TRT obtained for deuterated water was 192 hours. These values are almost equal to nHRT which was 190 hours. The retention characteristics calculated on the bases of the treated water flow rate and the wetland bed volume can be successfully used as retention times in the non-vegetative period. This is due to the practically ideal flow of treated water, which gradually flows through the whole volume of the wetland bed. The effect of evapotranspiration can be neglected in winter.

## 4. Conclusions

Both fluorescein and deuterium oxide can be successfully used as conservative tracers in order to determine retention time in a CW with a horizontal subsurface flow in the non-vegetative period. The results are very similar for both tracers; however, the fluorescein-response curve was observed to tail out. It is due to the fluorescein sorption in the CW bed (*e.g.* to sediments). On the other hand, the interaction of deuterated water with the wetland bed was less pronounced. Deuterium oxide is an excellent tracer. However, the tailing of the fluorescein-response curve was relatively slight with a negligible effect on the obtained data. The much lower financial requirements on fluorescein determination in comparison to IRMS determination of deuterium, in conjunction with the very high sensitivity of the fluorimetric determination, fluorescein non-toxicity, and its ability to rapidly decompose in direct sunlight after the outflow from the wetland system, means that fluorescein can serve as a suitable tracer to determine retention time in a constructed wetland.

Treatment efficiency for parameters such as biochemical oxygen demand ($BOD_5$), chemical oxygen demand (COD), suspended solids, total nitrogen and phosphorus corresponds to a longer retention time in a constructed wetland [2]. The longer water remains in the wetland the greater chance of sedimentation, adsorption, biotic processing, microbial activities, chemical reactions, volatilization, or retention of contaminants (*e.g.* nutrients). Therefore, retention time is a very important characteristic of a constructed wetland that

should always be correctly (and of course with adequate financial expenses) determined and controlled. The possibility to prolong the retention time depends mainly on construction parameters of the wetland, porosity of the vegetated bed, flow rate of treated water, and the influence of the vegetation. The correct choice of the suitable and reliable tracer is the prerequisite to ensure correct data when constructed wetlands are designed and built and whenever their operation parameters and functions are tested and optimized.

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