The removal of pathogens in surface-flow constructed wetlands and its implications for water reuse

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Abstract Microbiological quality represents the biggest concern to the reuse of treated wastewater. This paper reports and discusses the results of an international survey on the removal of indicators of microbiological contamination in surface-flow constructed wetlands. Constructed wetlands consistently provide a reduction of 90–99% (1–2 log-removal) in the concentration of indicators such as coliform bacteria and faecal streptococci. This removal is found in wetlands treating water from different types of pretreatment (primary sedimentation, activated sludge, trickling filter, maturation ponds). On the other hand, when the influent is of high microbiological quality, wetlands act as sources of pathogenic contamination. The final water quality, however, is still compatible with medium to no-contact recreational activities and other final water uses. High variability in the effluent quality and seasonality might limit the opportunities for reuse. The role of constructed wetlands in different treatment schemes and the remaining open questions concerning removal mechanisms and reference pathogens are discussed.

Keywords FWS constructed wetlands; pathogens; wastewater reclamation; water reuse

Introduction

The quest for more sustainable water management practices has led in recent decades to an increased interest in water reuse practices worldwide. As a source of water that is relatively constant throughout the year and due to the opportunities of nutrient recycling, water reuse can contribute to increasing the reliability of water supply and to close the nutrient cycle.

Surface-flow constructed wetlands have been implemented in several wastewater reuse schemes worldwide as polishing steps of conventional wastewater treatment (Ghermandi *et al.*, 2007). Constructed wetlands require low maintenance costs and energy usage and produce an effluent that is devoid of unwanted harmful chemical by-products. Constructed wetlands provide further benefits including increased biodiversity, provision of wildlife habitat and creation of areas suitable for recreational activities (Knight, 1997). As such, they are regarded as promising components of sustainable wastewater treatment systems.

Microbiological quality represents the biggest threat to municipal wastewater reuse due to the large concentration of potentially infectious species that are routinely present in the effluent of conventional treatment plants. In spite of the increasing number of studies concerning the removal of pathogens in surface-flow constructed wetlands, the lack of a comprehensive research synthesis has so far hindered the correct framing of opportunities and limitations of these systems in wastewater reuse schemes. With the aim of filling this knowledge gap, a comprehensive international survey collected information from electronic journal databases, libraries and through contacting authors and relevant agencies. The survey led to the creation of a large database concerning the removal of

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pathogen indicators in surface-flow constructed wetlands worldwide. This paper summarises the main findings.

Microbiological quality: indicators and guidelines

Since it is practically unfeasible to monitor for all pathogenic microorganisms of concern, the use of indicators is a widely accepted practice. A reliable indicator should be easy to detect in water samples, show high resistance to treatment and environmental stress and have a direct pathogenic effect on humans. The most commonly used indicators belong to the group of coliform bacteria (total coliforms (TC), faecal coliforms (FC) and *Escherichia coli* (*E. coli*)). FC and *E. coli* identify faecal contamination more specifically than TC. Unlike FC, *E. coli* has a pathogenic effect on humans. Bacteria from the genus *Streptococci* are sometimes preferred to coliforms due to their higher resistance to environmental stress. More specific indicators include organisms with a direct pathogenic effect on humans (bacteria like *Clostridium perfringens, Listeria monocytogenes, Salmonella, Enterococcus faecalis* and protozoans like *Giardia lamblia* and *Cryptosporidium parvum*) or with a direct correlation to the presence of viruses and human parasites (coliphages, eggs of helminths or nematodes). Monitoring of such specific indicators has so far been limited due to technical and economic constraints. Table 1 summarises advantages and disadvantages of the most common microbiological indicators.

Indicator organisms are used as proxy for the microbiological quality of reclaimed water and for the associated human risk in virtually all water reuse guidelines. Table 2 illustrates the main categories of reuse and a selection of the microbiological water quality criteria for reuse established in the frame of the EU-RTD project Aquarec.

Results

The database contains results on pathogens removal from 70 surface-flow constructed wetlands, most of which are located in North America (47), Europe (13) and Australia (6). All the wetlands in the database treat wastewater from either domestic (54) or agricultural (14) sources. Only two wetlands treat stormwater. The database is focused on full-scale data (50). Results from relevant pilot-scale studies (20) are also included. The vegetation in most of the systems is dominated by emerging macrophytes (*Typha spp.*, *Phragmites spp.* and *Scirpus spp.*) (37), by free-floating macrophytes (*Lemna spp.* or *Eichornia crassipes*) (10) or by a combination of both (12).

Table 3 provides an overview of the behaviour of the wetlands in the database with respect to the main indicators of pathogenic contamination. The indicators are grouped into three categories: bacteria, viruses (including the nested subcategories of F-specific, F-RNA specific and MS-2 phages), helminth eggs and protozoa. Results are given

Table 1 Frequently used indicators of microbiological contamination in wastewater

Indicator	Analytical costs	Remarks
Total coliforms, faecal coliforms *Escherichia coli*	Low	• Always present in wastewater • Low correlation with pathogenic organisms
Streptococcus faecalis	Low	• More resistant than coliforms
Clostridium perfringens	Low	• Direct pathogenic effect on humans • More resistant than coliforms
Eggs of helminths and nematodes	Medium	• Related to presence of human parasites
Coliphages	Medium	• Related to presence of enteroviruses
Giardia lamblia, *Cryptosporidium parvum*	High	• Human parasites • Not always detected

Table 2 Selection of the microbiological criteria established by the Aquarec project for different reuse categories (adapted from Salgot *et al.*, 2006)

Category	Final use	Faecal coliforms	*Clostridium perfringens*	*Enterococci*	Coliphages [pfu/100 mL]	*Cryptosp.* and *Giardia* [cysts/50 mL]	Nematode eggs [eggs/L]
1	Residential uses; direct aquifer recharge	abs	abs–0	abs	<1	<1	<1–10
2	Bathing water	<20– < 1,000	abs–10	<1,000	<1	<1	<1
3	Urban uses; irrigation of raw-consumed crops, sprinkler irrigation; unrestricted irrigation	abs– < 1,000	<1	<20	<1,000	<10	<1
4	Irrigation of pasture for milking; of industrial and not raw-consumed crops; of fruit-trees (except with sprinklers); recreational impoundments (unrestricted, no bathing)	abs–10,000	<10	<1,000	–	–	<1
5	Restricted and landscape irrigation; aquaculture; aquifer recharge by percolation	abs– < 10,000	<100	<10,000	–	–	<1
6	Surface water quality; recreational impoundments and streams (no contact)	<200– < 10,000	<1	<20	<1,000	<10	<1
7	Industrial cooling (except food industry)	abs–10,000	<10	<1,000	–	–	<1

Where not otherwise specified, all values are expressed in (cfu/100 mL); abs = absent

Table 3 Average influent and effluent concentrations of pathogen indicators in surface-flow constructed wetlands

Indicator	**N**	**Domestic wastewater**				**Agricultural wastewater**	
		Secondary CW		**Tertiary CW**			
		Infl*	**Effl****	**Infl***	**Effl****	**Infl***	**Effl****
Bacteria [cfu/100 mL]							
Faecal coliforms	76	$1.9 \times 10^6 \pm 3.9 \times 10^6$	1.7×10^4 (7.6×10^4)	$8.0 \times 10^4 \pm 9.6 \times 10^4$	$4.5 \times 10^3 \pm (2.1 \times 10^5)$	$4.2 \times 10^5 \pm 7.3 \times 10^5$	$4.6 \times 10^4(8.4 \times 10^5)$
Total coliforms	21	$5.0 \times 10^7 \pm$ NA	4.3×10^6 (NA)	$1.1 \times 10^6 \pm 8.6 \times 10^5$	$4.6 \times 10^4(2.7 \times 10^5)$	$2.3 \times 10^6 \pm 2.3 \times 10^6$	$3.2 \times 10^5(1.4 \times 10^6)$
Escherichia coli	15	$9.4 \times 10^6 \pm$ NA	1.6×10^6 (NA)	$1.9 \times 10^4 \pm 3.1 \times 10^4$	$5.7 \times 10^2(2.5 \times 10^3)$	$7.6 \times 10^5 \pm$ NA	$8.3 \times 10^4(2.8 \times 10^5)$
Streptococcus faecalis	13	$3.9 \times 10^5 \pm$ NA	$5.2 \times 10^3(3.0 \times 10^4)$	$6.4 \times 10^3 \pm 3.0 \times 10^3$	$1.1 \times 10^3(8.0 \times 10^4)$	–	–
Enterococcus faecalis	4	622 ± NA	94 (NA)	3 ± NA	75 (NA)	–	–
Clostridium perfring.	4	434 ± 612	40 (864)	–	–	–	–
Listeria monocytog.	1	–	–	–	–	$1.06 \times 10^5 \pm$ NA	980 (NA)
Viruses [pfu/100 mL]							
Total coliphages	2	–	–	1,233 ± NA	742–NA	–	–
F-specific	1	$3.1 \times 10^5 \pm$ NA	3.3×10^3(NA)	–	–	–	–
F-RNA specific	2	$8.0 \times 10^4 \pm 1.1 \times 10^5$	$7.5 \times 10^2(1.0 \times 10^4)$	–	–	–	–
MS-2 phage	2	–	–	6.7 ± NA	2.3–NA	–	–
Protozoa and helminth eggs [cysts/100 mL, oocysts/100 mL or number of eggs/L]							
Giardia lamblia	4	27 ± NA	12 (40)	35 ± 28	8 (64)	–	–
Cryptosporidium p.	4	11 ± NA	6 (15)	7 ± 3	3 (12)	–	–
Helminth eggs	1	0.95 ± NA	1.05 (NA)	–	–	–	–

N = total number of measuring campaigns. Separate measurements from cells operating in parallel are treated as independent observations; *[avg ± stdev] ** [avg (max)] according to the source of the wastewater and – for domestic wastewater – according to the level of pretreatment.

Table 3 shows that constructed wetlands reduce the concentration of all considered indicators, with the exception of helminth eggs. In the only available study (Molleda *et al.*, 2005), the concentration of eggs in the wastewater is reduced during the summer months but increases during the autumn, leading on average to a slight increase of concentration in the wetland.

Figure 1 illustrates the average removal efficiency for the most commonly used indicator bacteria according to the source of the water, the level and the type of pretreatment. As it is standard practice for disinfection processes, the removal in Figure 1 is calculated in terms of units of log-removal. One unit of log-removal corresponds to a 90% reduction of concentration; two units correspond to a 99% reduction, etc.

Figure 1 shows that constructed wetlands achieve 1–2 units of log-removal for all considered indicators, with the exception of *E. coli* that is less efficiently removed in secondary wetlands and in wetlands receiving the effluent of maturation ponds. These values are consistent with those observed by Vymazal (2005). The removal is higher for wetlands receiving primary treated sewage. This is not surprising since the removal of pathogen indicators appears to be related to the influent concentration (Figure 2 (right)). The lowest removal is achieved in wetlands treating the effluent of maturation ponds. These systems are often designed to achieve high removal of pathogenic contamination. Wetlands treating previously disinfected water do not further contribute to the disinfection. On the contrary, they often act as sources of pathogens.

The microbiological quality of the effluent of constructed wetlands shows high fluctuations. The coefficient of variation (= standard deviation/expected value) of the concentration of FC is on average 128%, but in some cases it is higher than 200%. In some of the studied wetlands there is unambiguous evidence of seasonal trends in the effluent concentration of pathogen indicators. This is typically higher during the hot season when the need for reclaimed water is also higher. These aspects are well illustrated in Figure 2 (left). The results refer to the wetlands in Cooroy, Australia. This system receives chlorinated tertiary wastewater (source: D. Heerey, Noosa Council, personal communication).

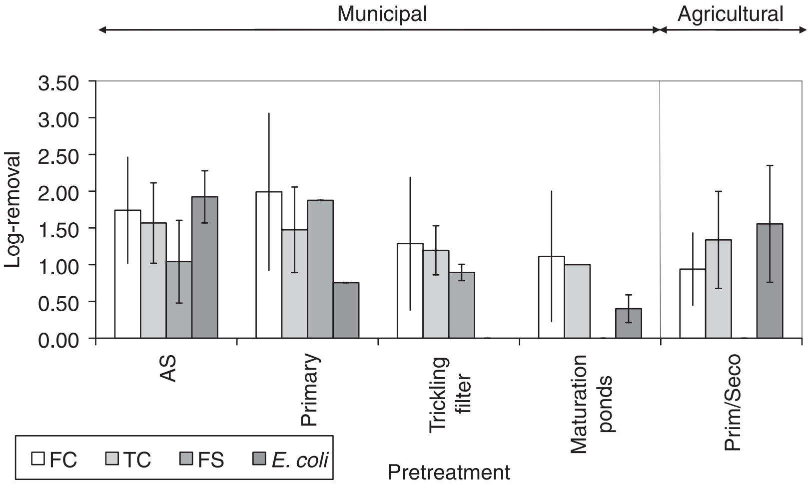

Figure 1 Mean removal efficiency (± SD) of FC according to the type of pretreatment and source of wastewater

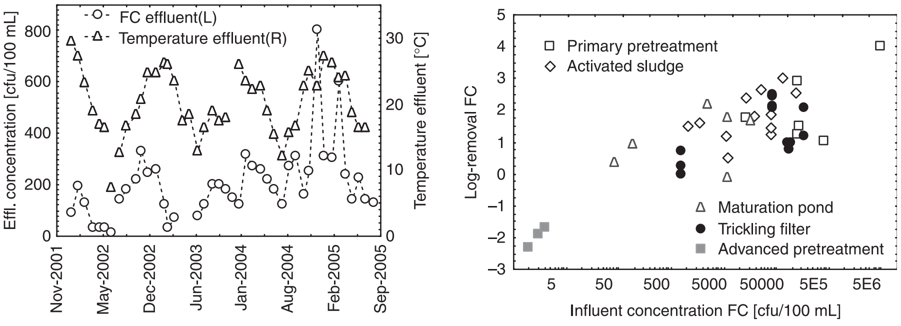

Figure 2 (left): Seasonal trend of the effluent concentration of FC in the constructed wetlands of Cooroy, Aus (source: D. Heerey, Noosa Council, personal communication); (right) average removal of FC in relation to the average influent concentration

The identification of correlations between pathogen removal and key parameters such as hydraulic retention time and vegetation cover was hindered by an incomplete reporting of the relevant aspects in many of the studies. However, clear evidence of a correlation between removal efficiency and hydraulic retention time emerges from the case studies investigating the behaviour of wetlands under different hydraulic conditions. The removal of indicator bacteria appears to be well correlated with the removal of total suspended solids (TSS). The correlation is highly significant statistically for wetlands receiving primary wastewater, which typically contains high TSS concentrations ($R^2 = 0.42$, $p = 0.0001$, $N = 37$). Data from tertiary treatment wetlands are more scattered. The correlation with TSS removal is, however, statistically significant for systems receiving influent concentrations of 15 mg/L of TSS or higher ($R^2 = 0.30$, $p = 0.01$, $N = 33$) (Figure 3 (left)). The correlation between the removal of indicator bacteria and of TSS is further illustrated in Figure 3 (right) by the results of a measuring campaign on FC in the constructed wetlands of Benton (KY) (cattail), USA.

The available studies provide conflicting indications about the existence of correlations in the removal of pathogen indicators. Several studies identify correlations between the removal of faecal streptococci (FS) and FC (Perkins and Hunter, 2000), *E. coli* and MS-2 phages (Schreijer *et al.*, 2003), *Giardia* and *Cryptosporidium* (Falabi *et al.*, 2002). In the frame of this study, a correlation was found between FC and FS in the treatment wetland in Columbia, Missouri, USA ($R^2 = 0.43$, $p = 0.0004$, $n = 25$, original data from C. Cuvellier, Columbia Regional Wastewater Treatment Plant, personal

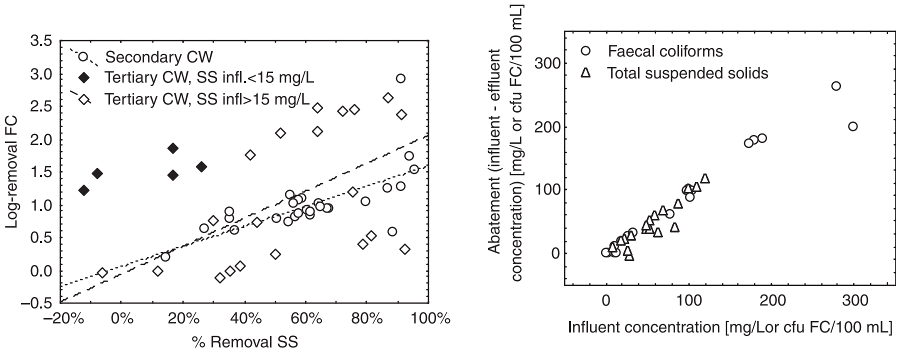

Figure 3 (left): correlation between removal of FC and TSS according to the level of pretreatment; (right) correlation between removal of FC and TSS during a measuring campaign in the wetland in Benton (cattail), USA (source of the data TWDB, 2000)

communication) as well as between FS and *E. coli* in Mågle, Sweden ($R^2 = 0.63$, $p < 0.01$, $n = 10$; original data from VA-Forsk, 2004). The analysis of other systems, however, did not reveal any correlation between indicators. The removal of pathogens in free-floating macrophytes systems (Fujioka *et al.*, 1999; Falabi *et al.*, 2002) and the removal due to interaction with biofilms (Stott and Tanner, 2005) appear to be related to the size of the microorganisms. The available comparative studies report lower removal efficiency for coliphages (average size 0.025–0.065 μm) with respect to bacteria (1–4 μm) and parasites (three studies). *Giardia* (8–12 μm) is removed more efficiently than *Cryptosporidium* (2–6 μm) in three of the four available comparative studies.

Discussion

Water reuse guidelines aim to achieve an adequate level of health protection by setting performance targets and water quality standards. Where the concept of tolerable health risk is applied, as in the revised WHO guidelines for wastewater use in agriculture (WHO, 2006), the quality standards do not depend only on the presence of pathogens in the water, but also on the results of dose-response studies and on disease burdens. In such a perspective, different water quality standards might apply to populations with different characteristics or in different climatic conditions. The performance of the treatment system may be combined with other health protection measures (such as human exposure control, irrigation techniques and crop restrictions) until the required quality standard is reached.

Based on the results of this survey, one can observe that constructed wetlands can contribute to the overall reliability of the water reuse scheme. Figure 4 illustrates the possible contribution to five different water reclamation schemes, which include surface-flow constructed wetlands as polishing step of conventional treatment with primary sedimentation, activated sludge (AS) systems, trickling filters and maturation ponds. The category “advanced treatment” includes all systems that achieve a very high microbiological quality, usually by means of membrane filtration or chemical disinfection. In Figure 4, the removal efficiencies of bacterial indicators such as FC typically achieved in conventional treatment (WHO, 2006) are combined with the removal efficiencies of constructed

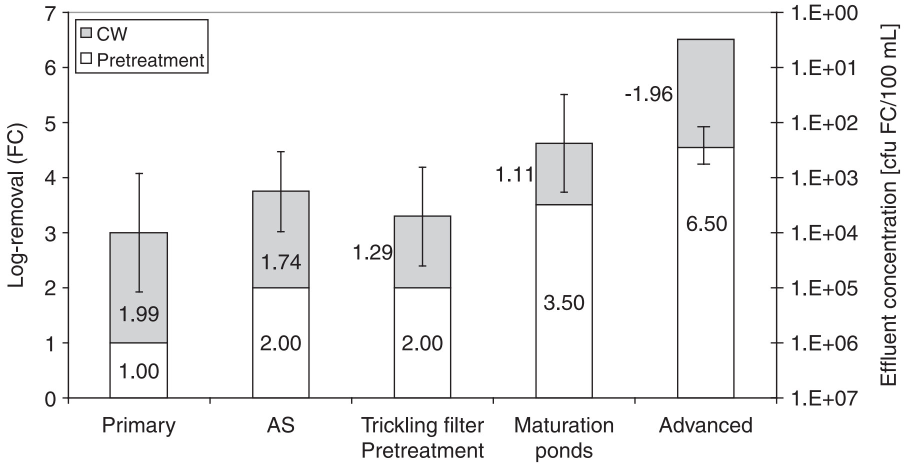

Figure 4 Average removal of pathogen indicators in wastewater treatment systems that include constructed wetlands. Numbers represent the removal magnitude by each component. The whiskers account for the standard deviation of removal in the constructed wetlands only

wetlands resulting from the present study. The concentration of FC in raw wastewater is assumed to be 10^7 cfu/100 mL.

Wetlands polishing the effluent of advanced treatment systems do not contribute to the removal of pathogens. The concentration of the indicator is, on the contrary, often increased to the level of the natural background concentration. This level is governed by factors like plant types, vegetation coverage, climate and wildlife species (US EPA, 1999). The effluent concentration of FC in these systems is about 100 cfu/100 mL. This quality is in the range observed in many natural wetlands (IWA, 2000) and is compatible with reuse in applications with medium to no-contact with the public, including recreational impoundments with restricted access (see Table 2).

Wetlands polishing the effluent of activated sludge systems, trickling filters or primary sedimentation achieve a lower microbiological quality. Their final concentration is in the range between 1,000 and 10,000 cfu/100 mL. According to Table 2, such an effluent might require further treatment (disinfection) or combination with health protection measures before reuse in agriculture and other applications. In reality, there is no clear international agreement on the quality standards. Some water guidelines, e.g. the Californian Title 22 Reuse Standards (State of California, 2000), set very stringent limits (concentration of TC $<$ 2.2 cfu/100 mL for non-processed food crops). Others, e.g. WHO, 1989 Water Reuse Guidelines (WHO, 1989), are less restrictive (concentration of FC $<$ 1,000 cfu/100 mL for non-processed food crops).

The high variability of the microbiological quality might be the major impediment for the direct use of the secondary effluent polished in surface-flow constructed wetlands (Perkins and Hunter, 2000). Similarly, a seasonal trend in effluent concentration of pathogens might represent a limitation of the opportunities of reusing the water for agricultural uses, since the concentration of pathogens is typically higher during the hot season when the need for reclaimed water is higher.

The evidence of the important role played by physical separation processes in the removal of pathogens highlighted in this study has important consequences in a water reuse perspective. Physical separation from the water column does not necessarily imply that the pathogens trapped in the solid matrix are no longer viable or potentially infectious. Karim *et al.* (2004) provide evidence of a longer survival of viruses and bacteria in the sediments of constructed wetlands. Bacteria mortality in the sediments appears to be correlated with sediment size, being lower in sediments containing large amounts of clay-sized particles (Burton *et al.*, 1987). Pathogens trapped in the sediments can be remobilised and resuspended into the water column as infectious agents. Several studies found a strong negative correlation between flow rate and removal efficiency of pathogen indicators in surface-flow constructed wetlands (Perkins and Hunter, 2000; Thurston-Enriquez *et al.*, 2004).

Physical separation processes seem to be less significant in wetlands with low influent concentration of suspended solids. Other mechanisms of removal prevail in such systems. Microorganisms that are not attached to solid particles are in fact less subject to physical separation processes, but are more exposed to chemical (oxidation, UV radiation, exposure to biocides) and biological (predation, competition with other bacteria and viruses) removal mechanisms (Roper and Marshall, 1974; Gerba and McLeod, 1976; IWA, 2000).

The removal of specific indicators of microbiological contamination in surface-flow constructed wetlands cannot be characterised with sufficient confidence on the basis of the knowledge that is currently available in the literature. The available studies provide conflicting indications about removals and correlations between indicators. Investigation of the correlation between removal and size of the microorganisms seems to be a direction of research for free-floating macrophytes systems and for wetlands in which removal by physical separation processes (Falabi *et al.*, 2002) and by interactions with biofilms prevail (Stott and Tanner, 2005). A better understanding of removal mechanisms and the identification of correlations between different indicators can significantly contribute to better frame the opportunities offered by constructed wetlands in water reuse schemes.

Conclusions

The literature survey about the removal of pathogens in surface-flow constructed wetlands prompted the following main conclusions:

- Secondary and tertiary surface-flow constructed wetlands consistently achieve a removal of 90–99% (1–2 log-removal) of the most frequently used indicators of pathogenic contamination.
- Wetlands treating a previously disinfected influent often act as sources of pathogenic contamination due to processes of internal generation.
- The microbial quality achieved by polishing wetlands allows reuse of the treated wastewater in medium to no-contact applications. Higher water quality can be achieved in combination with maturation ponds or advanced treatment.
- Physical separation and remobilisation processes play an important role in determining the microbiological quality of the effluent of wetlands with high influent concentrations of TSS.
- There is limited information in the literature about the removal of specific pathogen indicators in constructed wetlands.
- There is a lack of homogeneity in the reporting of monitoring results. Many studies fail to report about key aspects like the hydraulic retention time and vegetation cover. This calls for more standardised report procedures.

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