

Improving the Efficacy of Wastewater-Polishing Reed Beds

In wetlands, human-pathogenic microorganisms are physically removed and biodegraded by sedimentation, filtration, and evapotranspiration-driven attachment to plant roots, natural die-off, UV radiation, straining and sorption by biofilm, and protozoan predation. Horizontal wetlands usually discharge to surface waters that are frequently used for recreation or drinking water production. It is commonly assumed that human pathogens identified in wetlands effluents originate from wastewater. However this was never proven because studies on human pathogens in wetlands did not utilize molecular epidemiology techniques that allow for simultaneous qualitative and quantitative assessment of pathogens in wetland influents and final effluents, and characterization of their viability.

Because *Cryptosporidium*, *Giardia*, and microsporidia can infect a variety of non-human hosts, identification of human-pathogenic species represents a challenge. Another challenge is determination of viability of these pathogens. Both challenges are addressed by the fluorescence *in situ* hybridization (FISH) technique used in this project. FISH employs fluorescently labeled oligonucleotide probes targeted to species-specific sequences of 18S rRNA, and therefore identification of pathogens is species-specific. As rRNA has a short half-life and is only present in numerous copies in viable organisms, FISH allows for differentiation between potentially viable and non-viable pathogens. *Cryptosporidium parvum*, *Giardia duodenalis*, and human-virulent microsporidia, (i.e., *Encephalitozoon intestinalis*, *E. hellem*, *E. cuniculi*, and *Enterocytozoon bieneusi*) are waterborne enteropathogens that have a broad zoonotic reservoir. Their transmissive stages, i.e., oocysts, cysts, and spores, respectively, are resistant to environmental stressors and are therefore long-lasting and relatively ubiquitous in the environment and can cause human health issues.

Research Scope and Findings

Wastewater samples from four constructed wetlands were tested qualitatively and quantitatively for *Cryptosporidium parvum*, *Giardia duodenalis*, and human-pathogenic microsporidia, with assessment of their viability. Overall, four species of human enteropathogens were detected in wetland influents, vegetated areas, and effluents: *C. parvum*, *G. duodenalis*, *Encephalitozoon hellem*, and *Enterocytozoon bieneusi*. The results of this project demonstrated that:

- Composition of human enteropathogens in wastewater entering and leaving constructed wetlands was highly complex and dynamic.
- Constructed wetlands may not always provide sufficient remediation for human enteropathogens.
- Wildlife can contribute a substantial load of human zoonotic pathogens to constructed wetlands.
- Most of the pathogens discharged by wetlands were viable.



Constructed wetlands are an attractive alternative to conventional tertiary treatment processes for municipal wastewater, on-site domestic wastewater, and concentrated animal feeding operations.

BENEFITS

- Helps wastewater treatment plant and water reclamation facility operators, epidemiologists, and engineers concerned with wastewater treatment processes assess the quality of surface water.
- Demonstrates how constructed wetlands can be excellent for absorbing, removal, and storage of nitrogen and phosphorus from wastewater.

RELATED PRODUCTS

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- Large volumes of wetland effluents can contribute to contamination of surface waters used for recreation and drinking water abstraction and therefore represent a serious public health threat.

The presence of pathogens at higher concentrations in wetland-polished wastewater, vegetated areas, and outfalls can be explained by the facts that these pathogens were propagated in the wetlands by residing wildlife; contributed to the wetland water by visiting wildlife; or originated from other sources, e.g., surface runoff from wetland banks utilized by rodents as habitats or visiting areas.

Aquatic birds and mammalian wildlife that frequently inhabit wetlands can disseminate human-virulent species of *Cryptosporidium*, *Giardia*, and microsporidia, i.e., *E. hellem* and *E. bienersi*. Wildlife that inhabits or visit constructed wetlands has previously been demonstrated to significantly contribute fecal coliforms (e.g., *Escherichia coli* and *Klebsiella pneumoniae*) to wetlands. It has been suggested that wildlife plays an important role in the elevation of total and fecal coliform levels in wetland effluents due to their fecal deposition, and the spontaneous multiplication of wildlife-derived coliforms in wetlands during summer months. Because the wastewater resides in wetlands, these areas can act as endemic sites supporting both propagation and transmission of human zoonotic pathogens. Sizing reed-bed systems for a residence time of five days has become a standard practice, leaving plenty of time for propagation and spreading of wastewater-derived pathogens in wetland habitats via a wide variety of wildlife. Any temporal or permanent malfunctioning caused by clogged inlet pipes can additionally increase wastewater retention time in wetlands. The research team concludes that, in addition to fecal coliform bacteria, wildlife can also substantially contribute human-pathogenic protozoan and fungal microorganisms to wetland systems.

There are also alternative explanations why the levels of *Cryptosporidium* oocysts, *Giardia* cysts and microsporidian spores in the wetland outfalls were considerably higher than in the influents:

- All wetlands operated without implemented means to prevent animal access.
- Robust vegetation (i.e., *P. australis*) and tall trees around the wetlands reduced exposure to sunlight, and prevented heating and full exposure of pathogens to UV light (preventing their desiccation/destruction).
- In all wetlands, precipitation caused a) inflow of runoff water to the wetland from wetland banks inhabited by rodents, and b) surface runoff from other sources. Potential malfunctioning caused by clogged inlet pipe(s) could cause temporal hydraulic short circuits that bypass part of the wetland filtration area consequently resulting in reduction or collapse of removal performances.

The minimal levels of non-viable pathogens in this study indicate that the pathogen walls become permeable to compounds and microorganisms present in large quantities in wastewater and they undergo fast biodegradation. Such a phenomenon was observed previously for human-pathogenic microorganisms in wastewater matrices. Loss of pathogen viability in constructed wetlands was attributed to the lytic action of bacteria and bacteriophages, oxidation reactions, adsorption, and exposure to plant and microbial toxins.

Recommendations for Constructed Wetlands

The researchers concluded that in order to increase performance of constructed wetlands in removal of zoonotic pathogens from the wastewater, the transmission and propagation of these pathogens within constructed wetlands should be eliminated. They recommend that constructed horizontal wetlands used for polishing of secondary treated wastewater must prevent animal access to the wetland operations. The access of wildlife to these areas should be limited by using protective barriers such as fencing. Also, surface runoff resulting from precipitation and rainfall should be diverted out of wetland areas as it may contribute to load of human pathogens into the wetlands.

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