

Viral indicators for fecal contamination - A one year viral metagenomic study of treatment efficiency in Danish waste water treatment plants

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Introduction

Viral pathogens in irrigation water are a major threat to public health due to their possibility to cause human illness via e.g. contaminated crops. When using reclaimed water for irrigation it is thus important to assure it is free from pathogens.

Objective

- To investigate with viral metagenomic sequencing, if the treatment processes can make the water sufficiently clean to be used for irrigation
- To evaluate the applicability of Human Adenovirus (HAdV) or JC Polyomavirus (JCPyV) as indicator for the presence of human fecal contamination.

Methods

Two waste water treatment plants (WWTP) in Copenhagen, an urban WWTP and a highly sophisticated hospital WWTP, was sampled monthly during a one year period at three steps during the treatment process, see figure 1.

Microorganisms were concentrated from the water using positively charged Monolithic Affinity Filters.

- 53 samples were tested quantitatively using qPCR for HAdV, JCPyV, norovirus GI and GII, *Salmonella* and *Campylobacter*
 - 29 samples were sequenced using Illumina MiSeq
- The sequenced data was analyzed for the viral metagenome using the MGmapper pipeline developed at DTU.

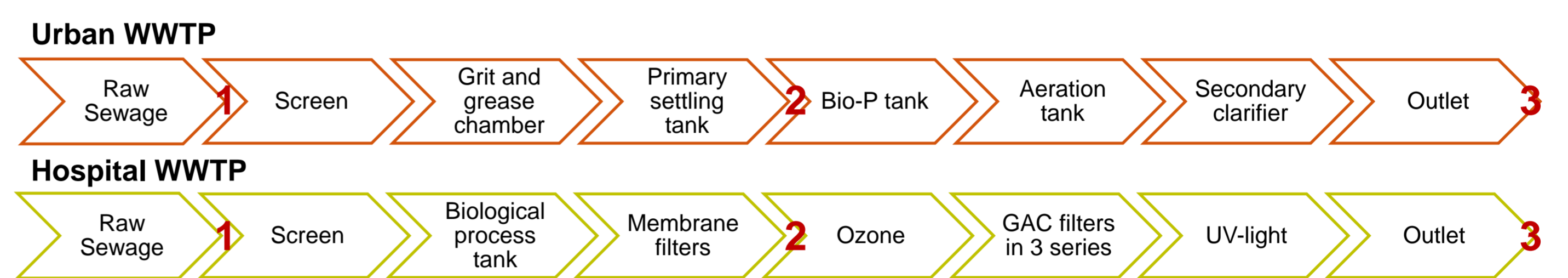


Fig 1. Treatment processes at the two tested WWTPs
Each treatment process is shown as a flow chart, Urban WWTP (top) and Hospital WWTP (bottom). The three sampling points at each WWTP is marked 1-3.

Results

We found that the hospital WWTP more efficiently eliminated the selected microbes tested with qPCR, fig 2. In addition:

- After membrane filtration (step 2) at the hospital most microbe genomes were eliminated, except in two samples containing either norovirus GI or *Salmonella*
- No microbial genomes was detected in hospital outlet water (step 3)
- A 2-3 log reduction in microbe concentration (gc/L) was obtained at the urban WWTP
- The presence of indicator viruses, HAdV and JCPyV, correlated with the presence of norovirus, although in lower concentrations

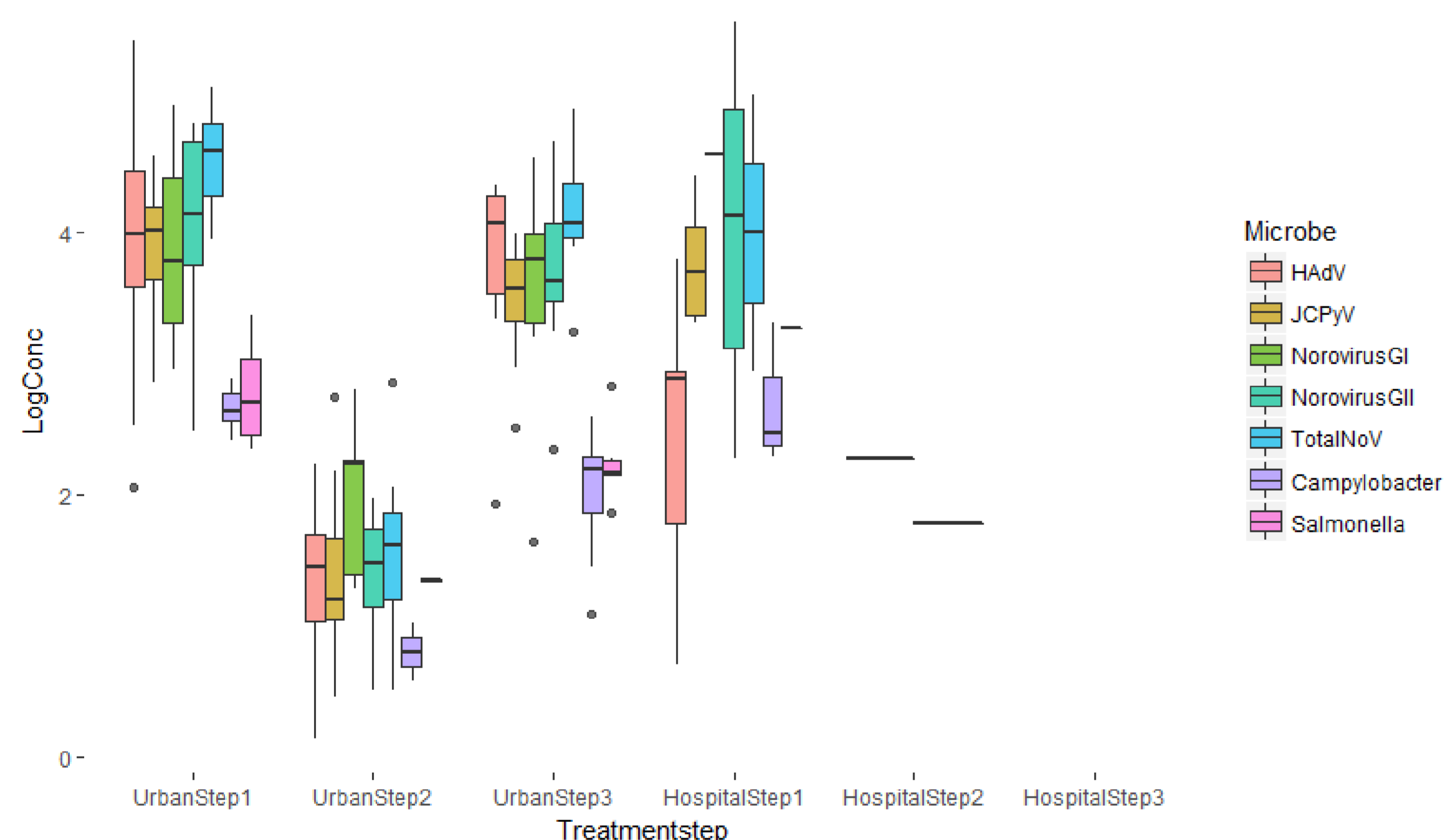


Fig 2. Microbial concentration in different water samples
Each box represent the measured log concentration per L for the tested microbe. Each microbe is represented by an individual color (see figure legend). The samples are grouped together dependent on each sampling point, urban WWTP step 1-3 and hospital WWTP step 1-3.

Sequencing results

- The relative abundance of seven families containing suspected human pathogens presented in fig 3
- A decrease in abundance compared to the incoming raw sewage (step 1) could be seen in samples of urban outlet (step 3) and after post membrane filtration at the hospital (step 2-3), fig 3

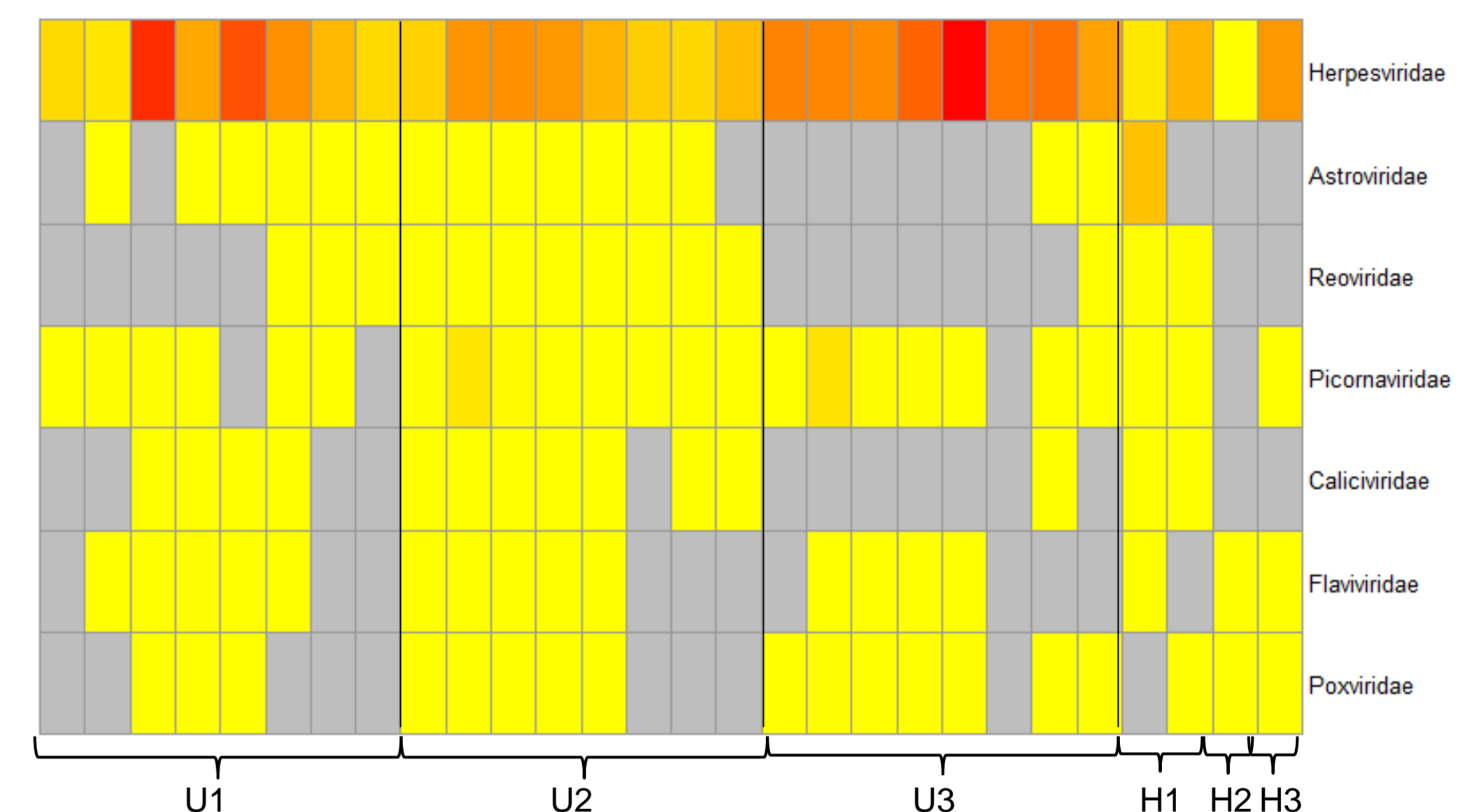


Fig 3. Detection of pathogenic viral families
Heatmap over the relative abundance of 7 pathogenic viral families detected in the 28 samples. The colour ranges from grey=no detection to red=high abundance. U1-U3=Urban WWTP step 1-3, H1-H3= Hospital WWTP step 1-3.

The PCoA plot, fig 4, illustrates the resemblance in viral composition between the samples. The following observations can be made from the plot:

- Most urban samples (step 1-3) clustered with hospital inlet samples (step 1)
- Hospital WWTP step 2 clustered with the negative control
- Hospital outlet sample (step 3) clustered separately

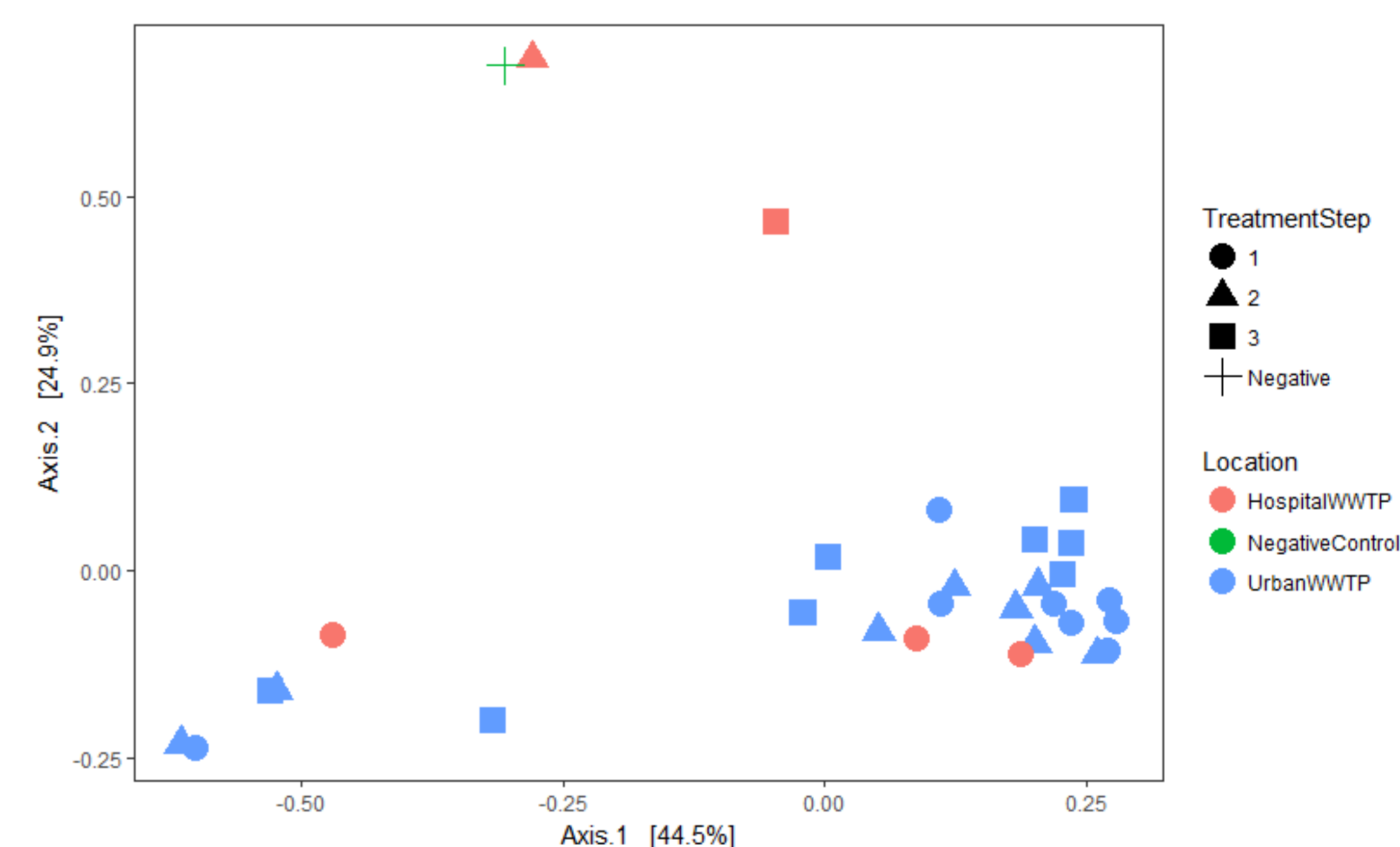


Fig 4. Detection of pathogenic viral families
PCoA plot made using the relative abundance of identified viruses with an relative abundance >1e-5. Each sample is separated in colour and shape depending on the sample location.

Conclusion and future perspectives

Our results indicate that the outlet water from the hospital WWTP may be used to irrigate crops for human consumption, as samples from this step did not

- test positive by qPCR for any of the analyzed virus and bacteria
- cluster with the other tested samples, but in contrast has a viral composition more similar to the negative control.

To further support this conclusion more samples from the hospital WWTP needs to be sequenced to see if the pattern is consistent.

HAdV and JCPyV could likely be used as indicators for human fecal contamination. However, our data shows that they might be found in lower concentrations than the pathogenic norovirus.

In conclusion the study emphasize the need of highly advanced treatments to ensure the quality of reclaimed sewage water.

Acknowledgements

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