

Concentration of Enteric Viruses from Wastewater Using NanoCeram Electropositive Filters

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Abstract

In this study, the NanoCeram and 1MDS electropositive filters were compared for the detection of both infectious and non-infectious viruses using real-time PCR and BGM cell line at four separate full-scale wastewater treatment utilities at the influent, pre and post-disinfection processes. In total 18 NanoCeram and 18 1MDS filters were compared for the detection of Human Adenovirus F40/41 (HAdV) and Human Enterovirus (HEV). The average

HAdV concentration for the NanoCeram and 1MDS filters for the influent samples are 2.5×10^5 and 4.6×10^5 , 1.7×10^3 and 9.4×10^3 for the pre-disinfection and 8.8×10^2 to 5.6×10^3 viruses/L for post-disinfection samples, respectively. The average HEV concentration using the NanoCeram and 1MDS for the influent was 1.7×10^5 and 1.1×10^5 , 1.1×10^3 and 3.0×10^1 for pre-disinfection, 1.1×10^2 and 8.9×10^1 viruses/L for the post-disinfection. However, a significant difference (p -value < 0.05) was detected, possibly due to the detection limit. Based on the cell culture results, the average log value for the NanoCeram and 1MDS at the influent, pre and post-disinfection samples were 3.69 and 4.14, 1.02 and 1.42, and 0.11 and 0.32 MPN/100L, respectively.

No significant difference (p -value > 0.05) was detected between the filters. During this study, it was demonstrated that the NanoCeram filter is just as reliable as the 1MDS filter for both infectious and non-infectious viruses, but at a much lower cost per unit filter.

Conclusions

- We have determined from our study that for routine monitoring of infectious enteric viruses the NanoCeram performed similar to the 1MDS but costing 1/5 the price (\$260.00 for 1MDS compared to \$50.00 for the NanoCeram per filter). Both filters followed the USEPA Virology sampling and processing protocol.
- There was no significant difference (p -value > 0.05) between the NanoCeram and 1MDS for the detection of infectious viruses at three separate locations throughout wastewater treatment.
- This is the first study to compare the performance of the NanoCeram and 1MDS filter for both infectious and non-infectious viruses throughout wastewater treatment.

References

- Karim, M.R., Rhodes, E.R., Brinkman, N., Wymer, L., Fout, G.S. (2009) New electropositive filter for concentrating enterovirus and noroviruses from large volumes of Water. *Appl. Environ. Microbiol.* 78:2393-2399.
- Polaczyk, A.L., Roberts, J.M., Hill, V.R. (2007) Evaluation of 1MDS electropositive microfilters for simultaneous recovery of multiple microbe classes from tap water. *Journal of Microbiological Methods.* 68:260-266.
- Sobsey, M.D., Glass, S.J. (1980) Poliovirus concentration from tap water with electropositive absorbent filters. *Appl. Environ. Microbiol.* 40:201-210.

Results

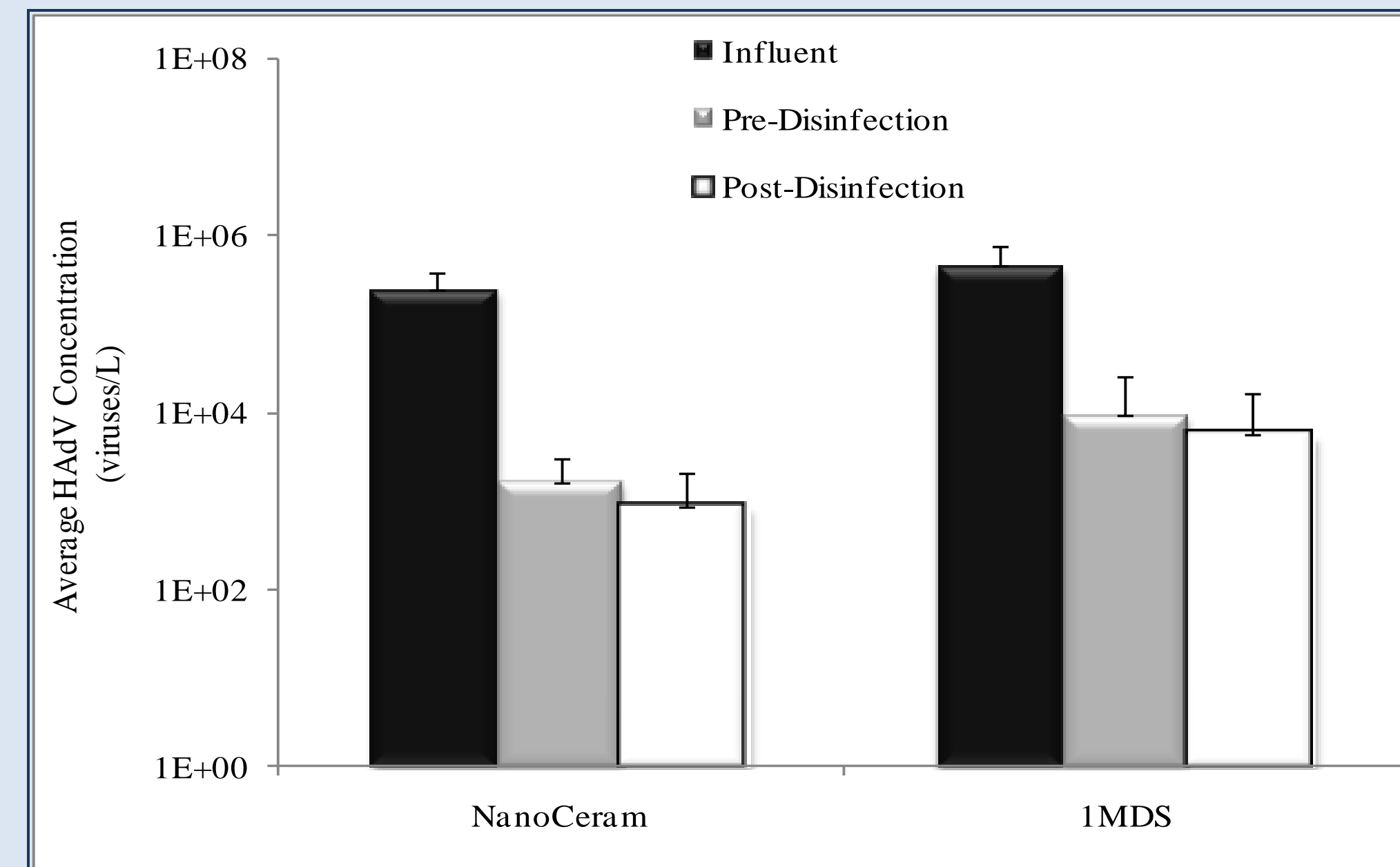


Figure 1: Comparison in average real-time PCR concentration of HAdV for the influent, pre and post disinfection between the NanoCeram and 1MDS cartridge filters for all sampling events. Sample size was $n=6$ for influent, pre and post-disinfection for each filter. Total sample size was $n=36$.

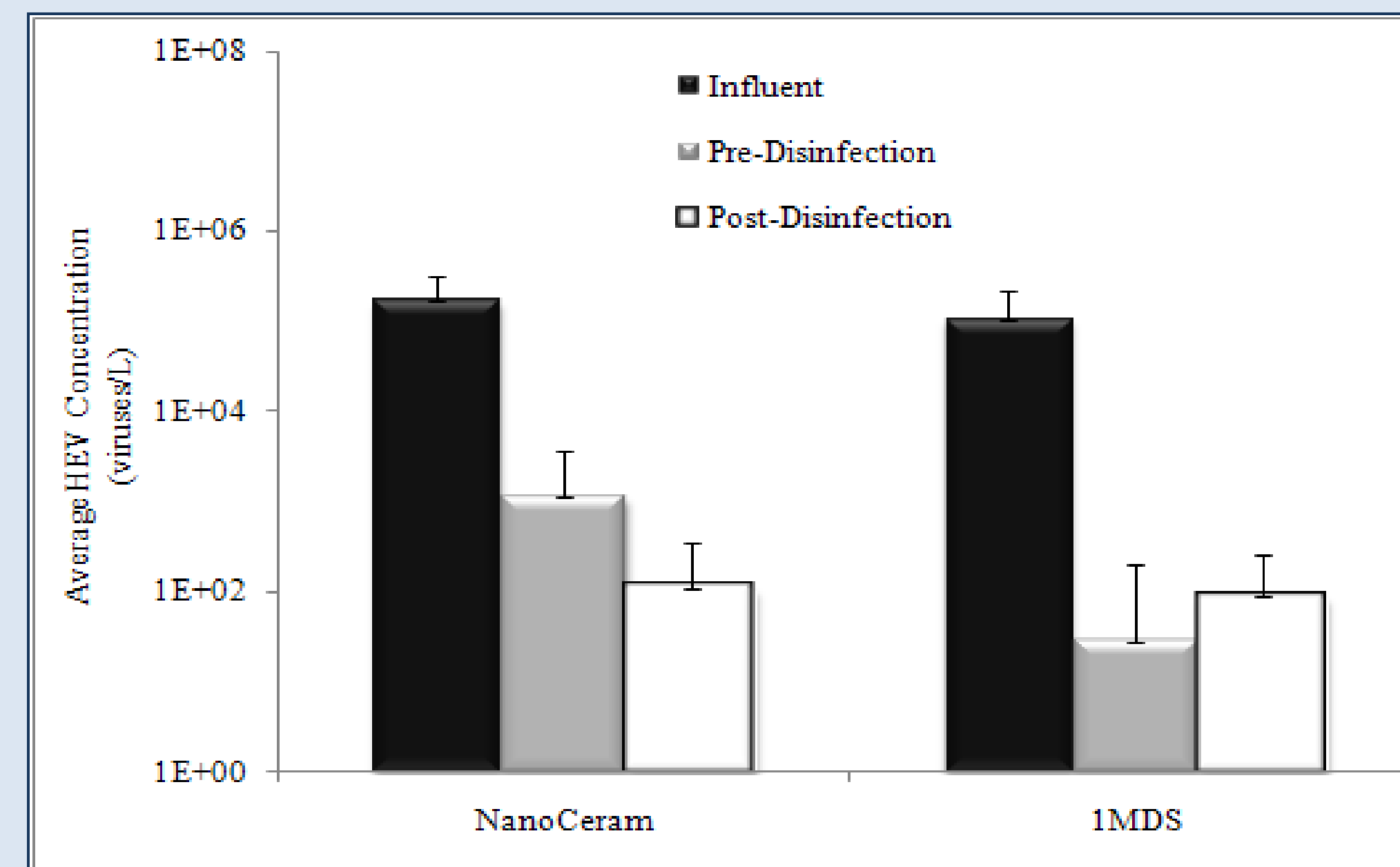


Figure 2: Comparison in average real-time PCR concentration of HEV for the influent, pre and post disinfection between the NanoCeram and 1MDS cartridge filters. Sample size was $n=6$ for influent, pre and post-disinfection for each filter. Total sample size was $n=36$. Detection limit was used for the pre-disinfection concentration for the 1MDS filter.

Table 1: List of viral concentration using real-time PCR and BGM cell culture data for both NanoCeram and 1MDS filters. *ND - Not Detected

WWTP	Date	Sample Point	Filter Type	HAdV-F 40/41 (viruses/L)	HEV (viruses/L)	BGM (MPN/L)	WWTP	Date	Sample Point	Filter Type	HAdV-F 40/41 (viruses/L)	HEV (viruses/L)	BGM (MPN/L)
1	06/24/2009	Influent	NanoCeram	2.8×10^5	4.6×10^4	65	3	09/11/2009	Influent	NanoCeram	3.0×10^5	1.9×10^5	36
		Pre-Disinfection		2.0×10^3	3.3×10^2	0.1			Pre-Disinfection		2.6×10^3	ND	0.11
		Post-Disinfection		2.6×10^2	ND	0.014			Post-Disinfection		1.8×10^3	ND	0.01
		Influent	1MDS	7.7×10^5	3.3×10^4	8.9			Influent	1MDS	4.6×10^5	6.8×10^4	360
		Pre-Disinfection		4.8×10^3	ND	0.53			Pre-Disinfection		4.1×10^3	ND	0.012
		Post-Disinfection		2.3×10^3	4.1×10^2	0.031			Post-Disinfection		2.0×10^3	ND	0.014
2	09/17/2009	Influent	NanoCeram	3.4×10^5	3.9×10^5	680	4	07/28/2009	Influent	NanoCeram	4.1×10^5	2.5×10^5	---
		Pre-Disinfection		1.8×10^3	ND	2.1			Pre-Disinfection		1.4×10^2	6.4×10^3	---
		Post-Disinfection		2.3×10^2	ND	0.012			Post-Disinfection		1.4×10^2	6.1×10^2	---
		Influent	1MDS	3.5×10^5	3.0×10^5	230			Influent	1MDS	1.0×10^5	1.6×10^5	---
		Pre-Disinfection		2.2×10^3	ND	0.13			Pre-Disinfection		4.5×10^2	ND	---
		Post-Disinfection		7.2×10^2	ND	0.014			Post-Disinfection		1.1×10^1	1.2×10^2	---
	09/24/2009	Influent	NanoCeram	3.0×10^5	1.9×10^5	---		08/05/2009	Influent	NanoCeram	4.2×10^4	1.2×10^3	6
		Pre-Disinfection		2.6×10^3	ND	---			Pre-Disinfection		3.5×10^3	9.2×10^1	0.014
		Post-Disinfection		1.8×10^3	ND	---			Post-Disinfection		2.9×10^3	4.4×10^1	0.014
		Influent	1MDS	4.6×10^5	6.8×10^4	---			Influent	1MDS	9.1×10^5	3.0×10^2	84
		Pre-Disinfection		4.1×10^3	ND	---			Pre-Disinfection		4.5×10^4	ND	2.1
		Post-Disinfection		2.0×10^3	ND	---			Post-Disinfection		2.9×10^4	ND	0.43

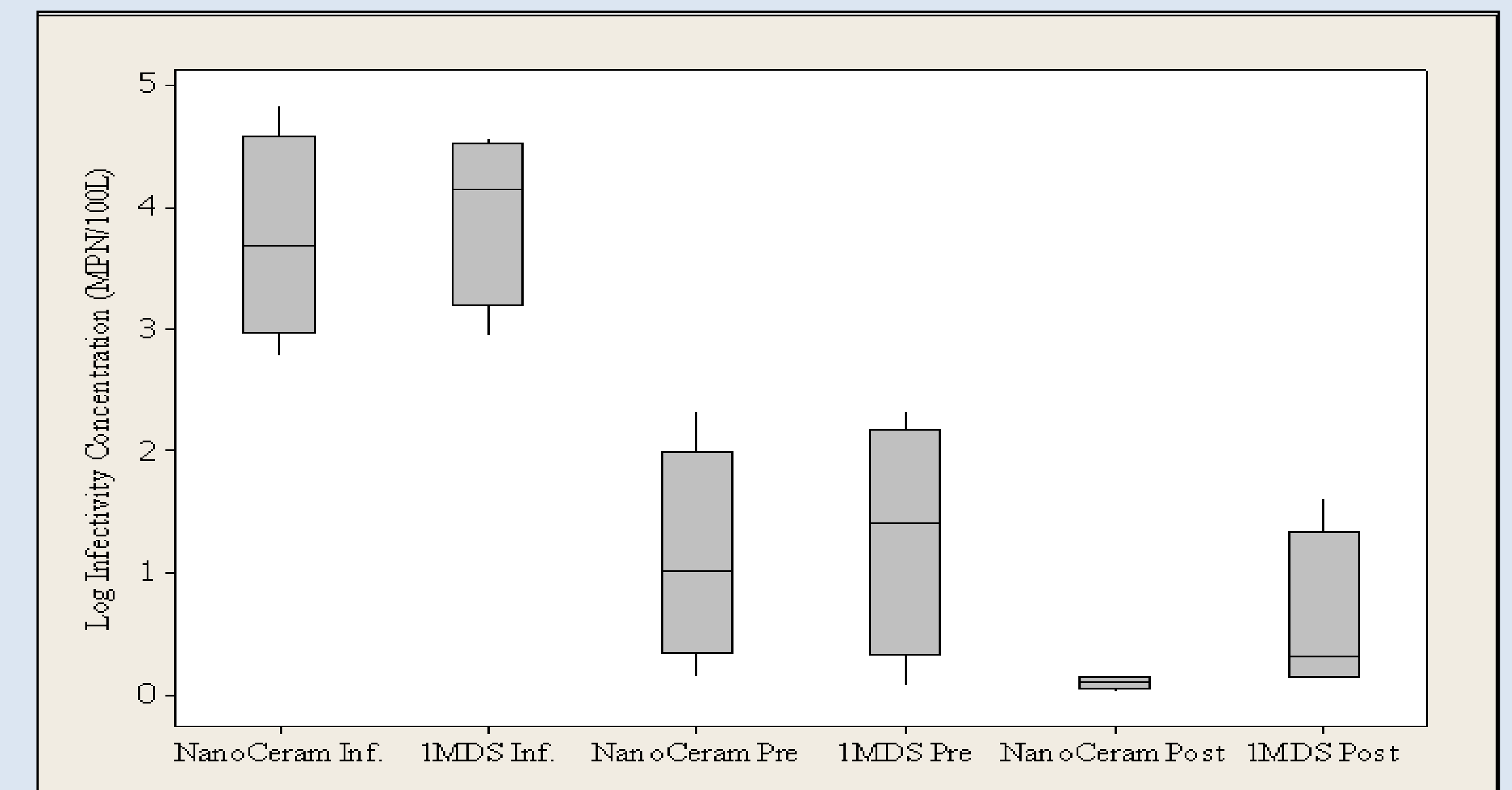


Figure 3: Comparison of virus infectivity using BGM cell line between the NanoCeram and 1MDS filters for influent, pre and post disinfection samples. Values are expressed in MPN/100L. Inf. – Influent, Pre – Pre-Disinfection and Post – Post-Disinfection. Influent ($n=8$), Pre-Disinfection ($n=8$) and Post-Disinfection ($n=8$), total samples ($n=24$).

Methods

Table 2: List of the WWTPs sampled from

WWTP	Location	Wastewater Treatment Process (Biological Treatment)	Average Flow (MGD)	Capacity (MGD)	Disinfection	# of Samples Collected (NanoCeram/1MDS)
1	East Lansing, MI	Activated Sludge	12.5	19.0	Chlorine	3/3
2	Lansing, MI	Activated Sludge	17.0	20.0	UV	6/6
3	Imley City, MI	Oxidation Ditch	0.2	0.4	UV	3/3
4	Romeo, MI	Rotating Biological Contactors	0.8	2.2	Chlorine	6/6

Table 3: List of enteric virus primer and probes, gene regions, reaction conditions and references used in this study

Virus Type	Gene Region	Primers/Probes	Sequence (5' to 3')	Reaction Condition (temp (°C), time)	Reference
HAdV40/41	Hexon	Forward	ACCCACGATGTACCCAGAC	95, 10s denaturation	Xagorarakis et al. 2007 - Modified from Jiang et al. 2005
		Reverse-1	ACTTTGTAGAGTACCGGTTTC		
		Reverse-2	CACCTTGTAGAGTAAACCGGTTC		
		Probe	CGACGGCAGAAACCCGAGCT		
HEV	5'- Untranslated Region	Forward	ACATGGTGTGAAGAGTCTATTGACCT	95, 15s denaturation	Diersen et al. 2007
		Reverse	CCAAGTAGTCGGTCCGC		
		Probe	TCCGGCCCTGAATCGGCTAAT		
		Probe	TCCGGCCCTGAATCGGCTAAT		



Figure 4: 1MDS filter used to sample the wastewater at each location throughout this study.



Figure 5: NanoCeram electropositive filter used to compare with the 1MDS.

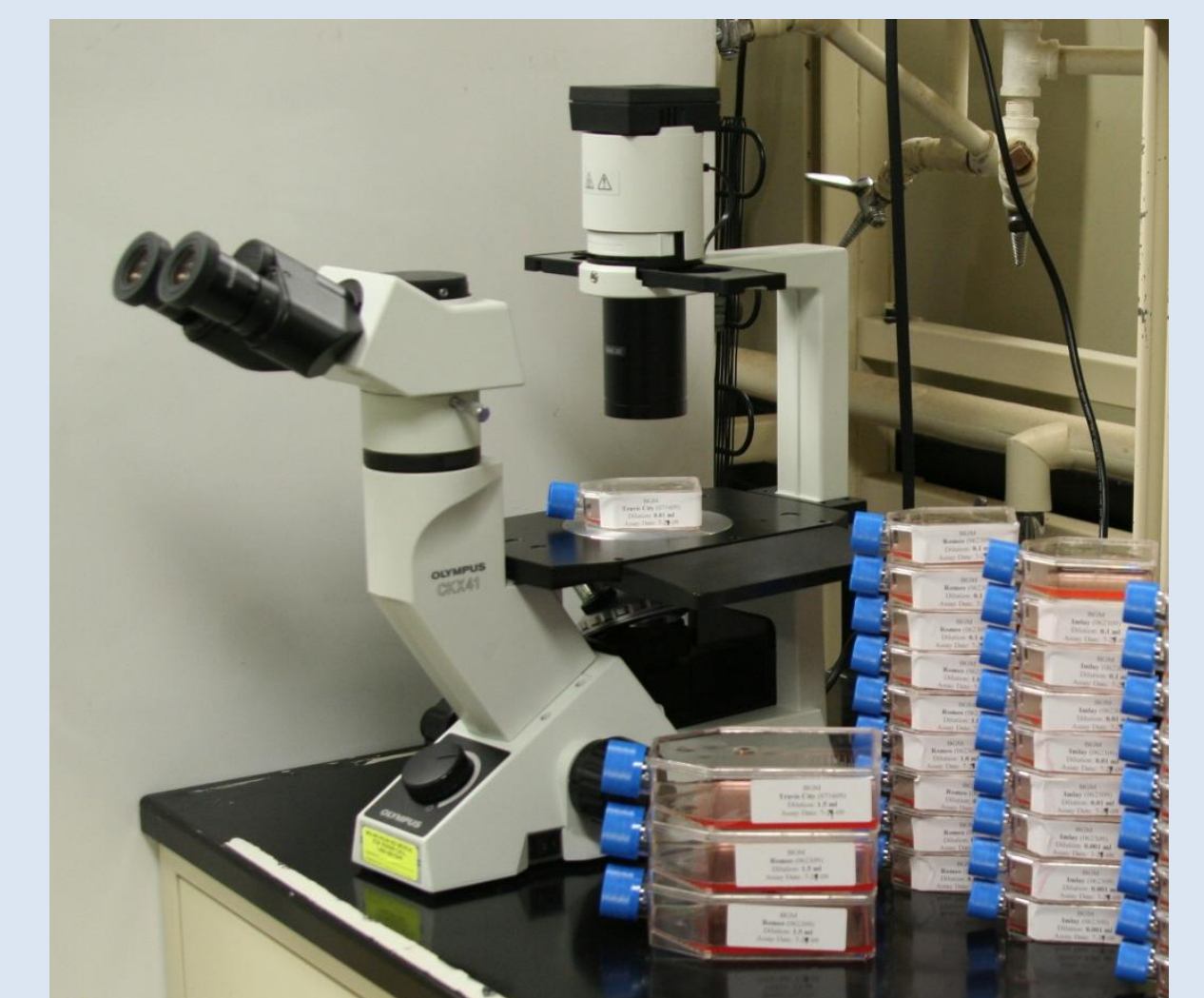


Figure 6: Inverted microscope used to check the BGM cell line for infectivity of the wastewater samples. Both NanoCeram and 1MDS filter samples were compared to determine if a significant difference was observed for capturing infectious viruses at all three sampling locations throughout the treatment process.