Treatment of pathogens in stormwater by antimicrobial-modified filter media

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ABSTRACT
Novel filter media incorporated with antimicrobial agents are potentially effective solutions to improve pathogen removal efficiency in current filtration units in WSUD systems. A laboratory study was conducted to develop new filter media through surface modification of granular activated carbon (GAC) and zeolite with a range of antimicrobial agents (i.e. quaternary ammonium compounds and heavy metal compounds). In total, 16 types of new media were successfully developed. Their efficiency for pathogen removal was assessed in columns using *E. coli* as a model microorganism based on two factors: type of filter media and flow rate. CuO-treated GAC (G-CuO) could provide 84-95% *E. coli* removal at low flow rates. Cu^{2+}-treated GAC (G-Cu^{2+}) could provide comparable removal at both high and low flow rates, while the metal leaching from this media was found to be less than the value specified by Australian Drinking Water Guideline.

KEYWORDS
E. coli; filtration; inactivation; nutrients; pathogens; stormwater

INTRODUCTION
Today the world is facing significant water shortages. At the same time, urbanization has resulted in significantly increased surface water runoff, which has changed urban hydrology and degraded receiving-water quality. One vision is to harness the potential of stormwater to address these issues. However, one concern with stormwater harvesting is the notoriously variable and usually high concentrations of pathogens in urban stormwater (CWP, 2000). Without adequate treatment, these pathogens impose significant human health risks to users of both recreational water bodies and stormwater harvesting systems (Haile et al, 1999; Hipp et al, 2006). Water Sensitive Urban Design (WSUD) technologies, such as wetlands, biofilters and porous pavements, have successfully reduced traditional pollutants in stormwater including sediment, nutrients and heavy metals. However, the capacity of these technologies for pathogen removal is limited and the treated water does not meet the requirements for harvesting and some recreational uses (Bratieres et al, 2008; Smith andPerdek, 2004). A promising solution is to improve the pathogen removal efficiency of current filtration units in WSUD systems through the development of antimicrobial agents-treated filter media.

Surface modified zeolite and GAC with known antimicrobial agents have been tested for pathogen removal with promising results. For example, Bowman showed that despite the instability of hexadecyltrimethylammonium bromide-modified zeolite at low ionic strength (Li et al, 1998), this media removed most of bacteria and viruses in sewage effluent

(Bowman, 2003). Dimethyloctadecyl [3-(trimethoxysilyl) propyl]ammonium chloride-treated zeolite provided a permanent antimicrobial surface (Abbaszadegan et al. 2006). Heavy metal compounds modified-media (especially Ag, Cu and Zn) have also been proved to be promising for pathogen removal. For example, Cu-Zeolite and Zn-zeolite reduced *E. coli* concentration in sewage by 3.4 and 2.4 log respectively within 6 h (Milan et al, 2001). Aluminum hydroxychloride modified very fine GAC particles removed *E. coli* by 6 log (Pal et al, 2006).

Despite all the efforts given in this field, it is hard to use available literature findings because of the vast differences in evaluating new media among the aforementioned studies. Furthermore, the available data based on synthetic water spiked with seed cultures is only of limited use when attempting to extrapolate to field conditions, partly because of the lower removal efficiency of native microorganisms than those that are spiked (Hijnen et al, 2005) and limited interaction time provided between microorganisms present in the natural water environment. In addition, there is no reported study to apply the technology for pathogen treatment in stormwater. This is especially important considering the distinct characteristics of stormwater (as compared with other water sources), such as intermittent dry-wet events, peak flow, highly variable concentrations of pollutants and flow rates (including pathogens, TSS, salinity, TOC, etc.), etc.

In response, this study aims to develop the most suitable combinations of antimicrobial and base filter materials for stormwater treatment by examining the performance of a range of newly developed media exposed to the same conditions. This paper comprises 18 types of modified and unmodified media and assesses their performance (in triplicates) using natural stormwater runoff. The effect of a range of factors including type of base media, type of antimicrobial agents, and detention time were examined for *E. coli* and nutrients treatment.

METHODS

Selected antimicrobial agents and base media - The antimicrobial agents used in this study and their sources are: aluminum hydroxychloride (AHC) (23-24% Al_2O_3, 83-83% basicity), Omega Chemicals, Australia; zinc sulfate heptahydrate, copper chloride, iron chloride, and hexadecyltrimethylammonium chloride (QAC), Merck Chemicals, Australia; dimethyloctadecyl [3-(trimethoxysilyl) propyl]ammonium chloride (Si-QAC), Sigma-Aldrich. The based materials selected were granular activated carbon (GAC, Acticarb GC1200N), Activated Carbon Technologies Pty Ltd, Australia; and zeolite, Zeolite Australia with the particle size ranged between 0.3-0.6 mm.

Modification of GAC and Zeolite - A range of small scale experiments was conducted to optimize the amount of each antimicrobial agent on each base material. In total, 16 types of new media were developed according to the following procedures:

- *Z-Cu^{2+}, Z-Fe^{3+}, Z-Zn^{2+}, Z-Zn^{2+}/Cu^{2+}/Fe^{3+}, G-Cu^{2+}:* Sodium chloride pretreated zeolite was modified with Cu^{2+}, Fe^{3+}, Zn^{2+} through ion exchange with each individual metal solution, and with Zn^{2+}/Cu^{2+}/Fe^{3+} through ion exchange with Zn^{2+} solution, Cu^{2+} solution, and Fe^{3+} solution in series (Milan et al, 2001). GAC was modified with Cu^{2+} by soaking particles in copper chloride solution for 24 h with continuous stirring.
- *Z-$Fe(OH)_3$:* soaking media in iron solution for 1 h followed by in situ precipitation at pH 11.
- *Z-$Zn(OH)_2$, G-$Zn(OH)_2$:* in situ precipitation of $Zn(OH)_2$ at neutral pH (Pal et al, 2006).
- *G-AHC: in situ* precipitation of AHC at neutral pH (Pal et al, 2006).

- *Z-CuO, G-CuO*: in situ deposition of $Cu(OH)_2$ at elevated pH followed by thermal decomposition at 400°C for Z-CuO while at 200°C for G-CuO.
- *Z-TiO$_2$, G-TiO$_2$*: sol-gel process followed by annealing at 350°C (Lopez et al, 2010).
- *Z-Si-QAC, G-Si-QAC*: Slurry of the base media was treated with a Si-QAC solution (1%) slowly. The pH of the slurry was then adjusted to 3 using 1 M HCl. The mixture was then left in oven at 60°C for 3 h. The particles were then separated and dried at 40°C overnight followed by a final washing and drying (Tienteh Chen, 2006; Isquith et al, 1972).
- *Z-QAC:* technology described by (Li et al, 1998).

The metal content of the modified filter media was analysed using method USEPA 6010/6020 in a NATA-accredited laboratory to indicate the level of antimicrobial agents coated onto the media. For Si-QAC and QAC modified media, Total Kjeldahl Nitrogen (TKN) analysis was conducted using method APHA 4500-Norg.

Evaluation of modified and unmodified media - In total, 54 columns were constructed with the modified and unmodified media (3 replicates for each configuration). Each column (29.5 mm diameter and 350 mm long) had one piece of fine screen mesh at the bottom and was packed with 70 mL of filter media (around 100 mm in depth) (Figure 1). These columns were dosed with natural stormwater collected from the outlet of sedimentation basin immediately after or during storm events (water quality shown in Table 1). The inflow and outflow were sampled and analysed for nutrients and heavy metals (by NATA-accredited lab) and *E. coli* (Colilert MPN). The effect of detention time on removal efficiency was assessed by controlling the flow rate (i.e. restricting the outlet); two sampling runs at different flow rates were conducted (as shown in Table 1). Finally, bacteria growth inhibition on filter media was qualitatively examined by flushing each column with 160 mL deionized water at a flow rate of 1 ml/min two weeks after sampling run 1 and monitoring the level of viable *E. coli* in the effluent.

Table 1 General qualities of collected stormwater and flow rate used for each sampling run

		Run 1	Run 2
Water quality	T (°C)	21	12.4
	TDS (g/L)	0.4	0.478
	pH	7.1	7.35
	Electric Conductivity (µs/cm)	626	747
	Turbidity (NTU)	30.4	20.7
	E. coli (MPN/100ml)	2965	14264
Flow rate (ml/min)		10	1
Detention time (min)		6.8	68

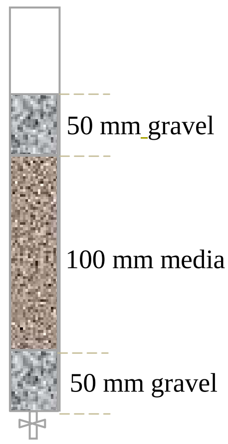

Figure 1: Test column

Data Analysis. The distributions of the removal rates for each media type were presented in the form of boxplots. Groups of data were also compared using a 2-factor univariate ANOVA (factors: flow rate and media type). Following a significant finding (i.e. if $p<0.05$), posthoc tests were then performed using either the Tukey test when population variances were equal or Games-Howell test if population variances were not equal. Adjustment for multiple testing was not considered, for the reasons listed in (Perneger, 1998).

RESULTS AND DISCUSSION

Optimization of the coating process - The level of antimicrobial agents on the modified media are shown in Table 2. Metal contents on hydroxide or oxide modified media (0.25-0.6 wt%) is equal or less than half of those on heavy metal ion modified zeolite (1.1-1.6 wt%), suggesting that the former coating may occur predominately on the surface of zeolite particles, thereby limiting their pathogen removal capacity. Except for Cu^{2+}, the content of coating materials on GAC was significantly higher than that on zeolite. Copper content, for example, on G-CuO was 2.9 times that on Z-CuO, while TKN content on G-Si-QAC was 3.4 times that on Z-Si-QAC, suggesting the dominant advantages of large surface area of GAC particles for antimicrobial agent immobilization.

Table 2 Level of antimicrobial agents on modified media

Antimicrobial agents	Zeolite		GAC	
	Media type	Content of antimicrobial agents (mg/kg media)	Media type	Content of antimicrobial agents (mg/kg media)
Nil	Z0	<5 (Cu), 30 (Zn), 3600 (Fe), 9 (Ti), 17 (TKN)	G0	46 (Cu), 73 (Zn), 93 (Fe), 79 (Al)
Cu^{2+}	Z-Cu^{2+}	13,000 (Cu)	G-Cu^{2+}	5,900 (Cu)
Zn^{2+}	Z-Zn^{2+}	11,000 (Zn).		
Fe^{3+}	Z-Fe^{3+}	12,000 (Fe)		
$Zn^{2+}/Cu^{2+}/Fe^{3+}$	Z-$Zn^{2+/}Cu^{2+}/Fe^{3+}$	4800/2400/8800 (Zn/Cu/Fe)		
$Zn(OH)_2$	Z-$Zn(OH)_2$	2,500 (Zn).	G-$Zn(OH)_2$	3,700 (Zn).
$Fe(OH)_3$	Z-$Fe(OH)_3$	5,700 (Fe)		
CuO	Z-CuO	5,600 (Cu)	G-CuO	16,000 (Cu)
TiO_2	Z-TiO_2	260 (Ti)	G-TiO_2	290 (Ti)
Si-QAC	Z-Si-QAC	220 (TKN)	G-Si-QAC	750 (TKN)
QAC	Z-QAC	250 (TKN)		
AHC	-	-	G-AHC	3,400 (Al)

***E. coli* removal efficiency of new media** – The *E. coli* removal efficiency was investigated over 18 types of media and two sampling runs with different detention time (i.e. 6.8 min in Run 1 and 68 min in Run 2).

Type of media. Statistical analysis showed that media type is a significant factor influencing *E. coil* removal ($p<0.001$) (Figure 2). Posthoc tests identified that with exception of G-CuO, G-Cu^{2+} significantly outperformed all the other GAC-based media (mean 94.2% with CV of 4% vs 56% with CV of 27%). The measured copper concentration in the infiltrate of G-Cu^{2+} (0.31-0.81 mg/L) is below the guideline value specified by Australian Drinking Water Guideline (NHMRC & NRMMC, 2004), and this amount is negligible compared with the copper concentration in the column (i.e. 5.9 mg/g media which is equivalent to 3717 mg/L of media), possibly suggesting that the removal mainly occurred in the column. Z-Cu^{2+} (100% with CV of 0%) provide similar level of *E. coli* removal as G-Cu^{2+}. However, the copper leaching from the former media was quite high. Further experiments demonstrated that the leaching is actually quite correlated with the electric conductivity (EC) level in the water source. Therefore, the highly variable EC level in natural stormwater (e.g. 74-1810 µs/cm;

Perscom Fuchs database) hinders its direct application for stormwater treatment, suggesting either further optimization of the coating process. Similar challenges were observed for the zinc compounds when modifying zeolite, although they showed reasonably good but highly variable removal. Inactivation of microorganisms by copper is thought to involve site-specific Fenton mechanisms (Maier et al, 2009). Copper may also bind to proteins interfering with the normal function of enzymes, resulting in cell death.

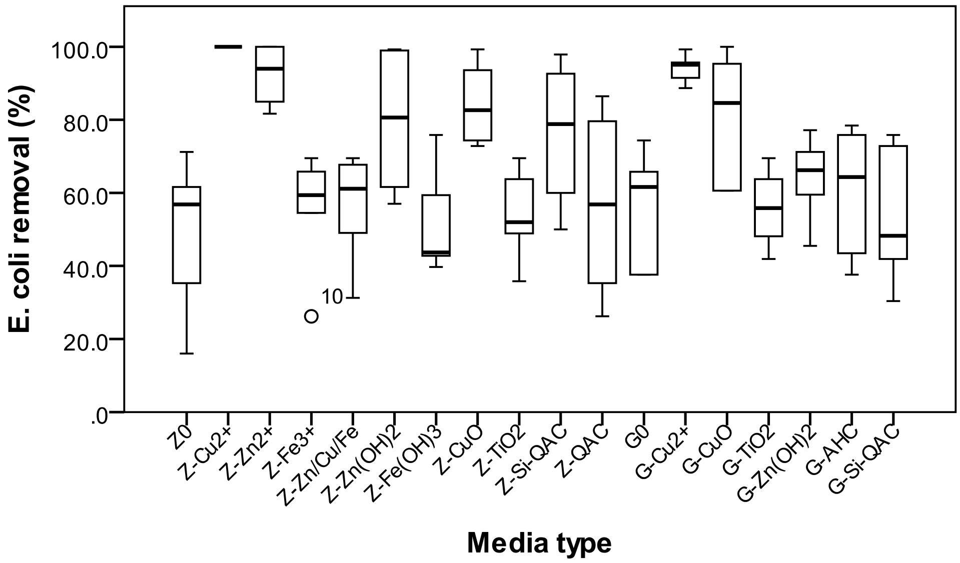

Figure 2 *E. coli* removal rates for all media types (3 replicates) over two sampling runs (Run 1 and 2)

Media Z-CuO, Z-Si-QAC, and G-CuO showed slightly better removal than the base media. However, the large variance among replicates made the conclusion rather weak, suggesting more replicates or repeated dosing in future experiments. It may also mean non-uniform treatment of the media during the preparation processes. Nevertheless, Z-CuO and Z-Si-QAC deserve further investigation due to their good stability (e.g. copper leaching of 0.24 mg/L in Run 2 for Z-CuO, while Z-Si-QAC showed no detectable leaking).

All the other antimicrobial agents modified media prepared in this study provided a similar level of removal efficiency to that of the base media, suggesting unsuitability for stormwater treatment or further optimization of the coating process.

Flow rate. Overall, flow rate is a significant factor for *E. coli* removal ($p<0.05$), with the effect being more obvious for certain media types than the others (Figure 3). Both base media, for example, showed significantly enhanced *E. coli* removal rates at lower flow rates, suggesting better adsorption of microorganisms in these columns. For Z-Si-QAC and G-CuO, the large variance observed in Run 1 was reduced significantly when using a lower flow rate (i.e. Run 2).

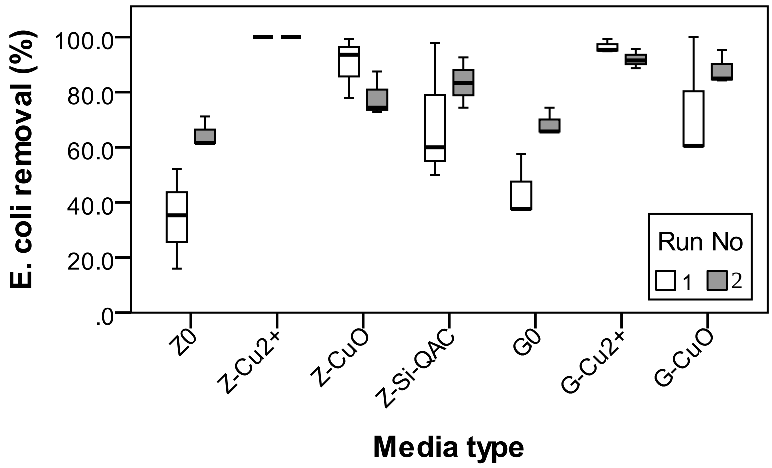

Figure 3 *E. coli* removal rates for selected media types (3 replicates) over Run 1 (flow rate 10 ml/min) and Run 2 (flow rate 1 ml/min)

Nutrients removal efficiency of new media – For the good performers, such as G-Cu^{2+}, G-CuO, Z-Cu^{2+}, etc, their effect on nutrients removal was also assessed in terms of TN and TP removal (Figure 4). It is observed that zeolite-based new media had no significant negative effect on nutrients removal (both TN and TP). Base and modified GAC provided about 80% TN removal. However, base GAC was found to leach phosphorus significantly resulting in over 500% leaching of TP, possibly a result of the actual filter media material leaching from the column. Modification of GAC with Cu^{2+} seems to lower the level of leaching, possibly because the coating process helped reduce material leaching. The most significant effect on inhibiting leaching was observed on CuO modified GAC (i.e. G-CuO), which can even effectively remove TP from stormwater.

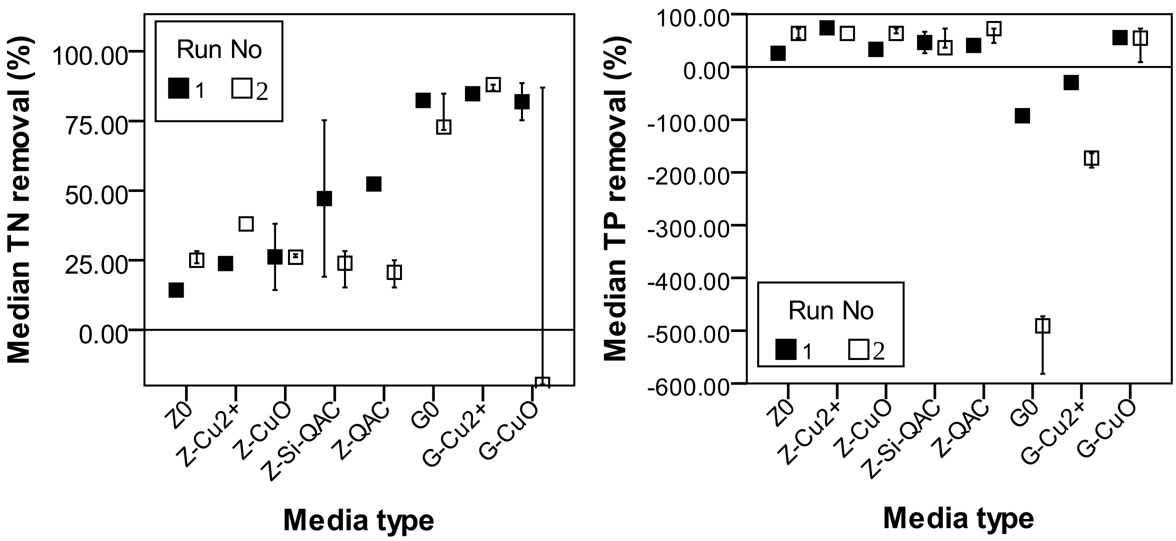

Figure 4. Mean and 95% confidence interval of removal for TN (left) and TP (right) relative to filter media over Sampling Run 1 and 2

Bacterial growth in new media–Copper ion modified media were found to be the most effective on inhibiting growth or survival of *E. coli* since no or quite low level of *E. coli* was detected in the effluent (e.g. <35MPN/100mL) (Figure 5). Si-QAC modified zeolite and CuO modified GAC showed large variance for this effect. For Z-Si-QAC, for example, one replicate completely inhibited the growth of *E. coli* while the other two replicates showed no such effect. This pattern is consistent with the *E. coli* removal performance of these columns during Run 1.

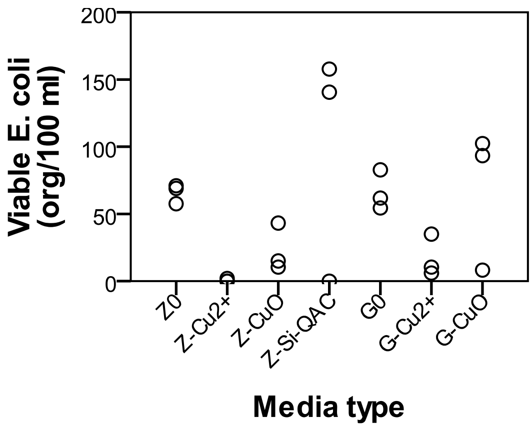

Figure 5. Viable *E. coli* cells in the effluent of selected columns during a flushing process of the columns with deionized water two weeks after sampling Run 1

CONCLUSIONS

This study identified several promising alternative filter media for native pathogen treatment in stormwater. However, to deliver a long-lasting, cost effective antmicrobial media for stormwater treatment universally without deteriorating the removal of other pollutants is still challenging. Future work will include

- Optimize the coating processes to improve the stability of the new media;
- Investigate the removal processes in the new media;

Large variances in *E. coli* removal were observed among the replicate columns packed with Z-Si-QAC and G-CuO, which might be caused by the differences in flow rates among replicates and/or the non-uniform treatment of the media. More replicates in future studies might be a solution. These media have also been shown to impose negligible negative effect on nutrients removal with several coatings on GAC, effectively inhibiting phosphorous leaching.

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REFERENCES

Abbaszadegan M, Monteiro P, Ouwens RN, Ryu H, Alum A. (2006). Removal and inactivation of Cryptosporidium and microbial indicators by a quaternary ammonium chloride (QAC)-treated zeolite in pilot filters. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering*, **41**, 1201-1210.

Bandyopadhyaya R, Sivaiah MV, Shankar PA. (2008). Silver-embedded granular activated carbon as an antibacterial medium for water purification. *J. Chem. Technol. Biotechnol.*, **83**, 1177-1180.

Bowman RS. (2003). Applications of surfactant-modified zeolites to environmental remediation. *Microporous Mesoporous Mater.*, **61**, 43-56.

Bratieres K, Fletcher TD, Deletic A, Zinger Y. (2008). Nutrient and sediment removal by stormwater biofilters: A large-scale design optimisation study. *Water Res.*, **42**, 3930-3940.

Chen T. 2006. Porous inkjet printing substrate containing polymer-grafted mineral oxides.*United States Patent No. 20060093761*

CWP. (2000). Microbes and Urban Watersheds: Concentrations, Sources, & Pathways. *Watershed protection techniques*, **3**, 554-565.

Haile RW, Witte JS, Gold M, Cressey R, McGee C, Millikan RC, Glasser A, Harawa N, Ervin C, Harmon P, Harper J, Dermand J, Alamillo J, Barrett K, Nides M, Wang GY. (1999). The health effects of swimming in ocean water contaminated by storm drain runoff. *Epidemiology*, **10**, 355-363.

Hijnen WAM, Brouwer-Hanzens AJ, Charles KJ, Medema GJ. (2005). Transport of MS2 phage, Escherichia coli, Clostridium perfringens, Cryptosporidium parvum and Giardia intestinalis in a gravel and a sandy soil. *Environ. Sci. Technol.*, **39**, 7860-7868.

Hipp JA, Ogunseitan O, Lejano R, Smith CS. (2006). Optimization of stormwater filtration at the urban/watershed interface. *Environ. Sci. Technol.*, **40**, 4794-4801.

Isquith AJ, Abbott EA, Walters PA. (1972). Surface-bonded antimicrobial activity of an organosilicon quaternary ammonium chloride *Applied Microbiology*, **24**, 859-863.

Li ZH, Roy SJ, Zou YQ, Bowman RS. (1998). Long-term chemical and biological stability of surfactant modified zeolite. *Environ. Sci. Technol.*, **32**, 2628-2632.

Lopez L, Daoud WA, Dutta D. (2010). Preparation of large scale photocatalytic TiO2 films by the sol-gel process. *Surface & Coatings Technology*, **205**, 251-257.

Maier RM, Pepper IL, Gerba CP, eds. 2009. *Environmental Microbiology*: Academic Press

Milan Z, de las Pozas C, Cruz M, Borja R, Sanchez E, Ilangovan K, Espinosa Y, Luna B. (2001). The removal of bacteria by modified natural zeolites. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering*, **36**, 1073-1087.

NHMRC & NRMMC, ed. 2004. *Australian Drinking Water Guidelines*. Canberra: National Health and Medical Research Council and National Water Quality Management Strategy, Australian Government

Pal S, Joardar J, Song JM. (2006). Removal of E-coli from water using surface-modified activated carbon filter media and its performance over an extended use. *Environ. Sci. Technol.*, **40**, 6091-6097.

Perneger TV. (1998). What's wrong with Bonferroni adjustments. *Br. Med. J.*, **316**, 1236-1238.

Smith JE, Perdek JM. (2004). Assessment and management of watershed microbial contaminants. *Crit. Rev. Env. Sci. Technol.*, **34**, 109-139.