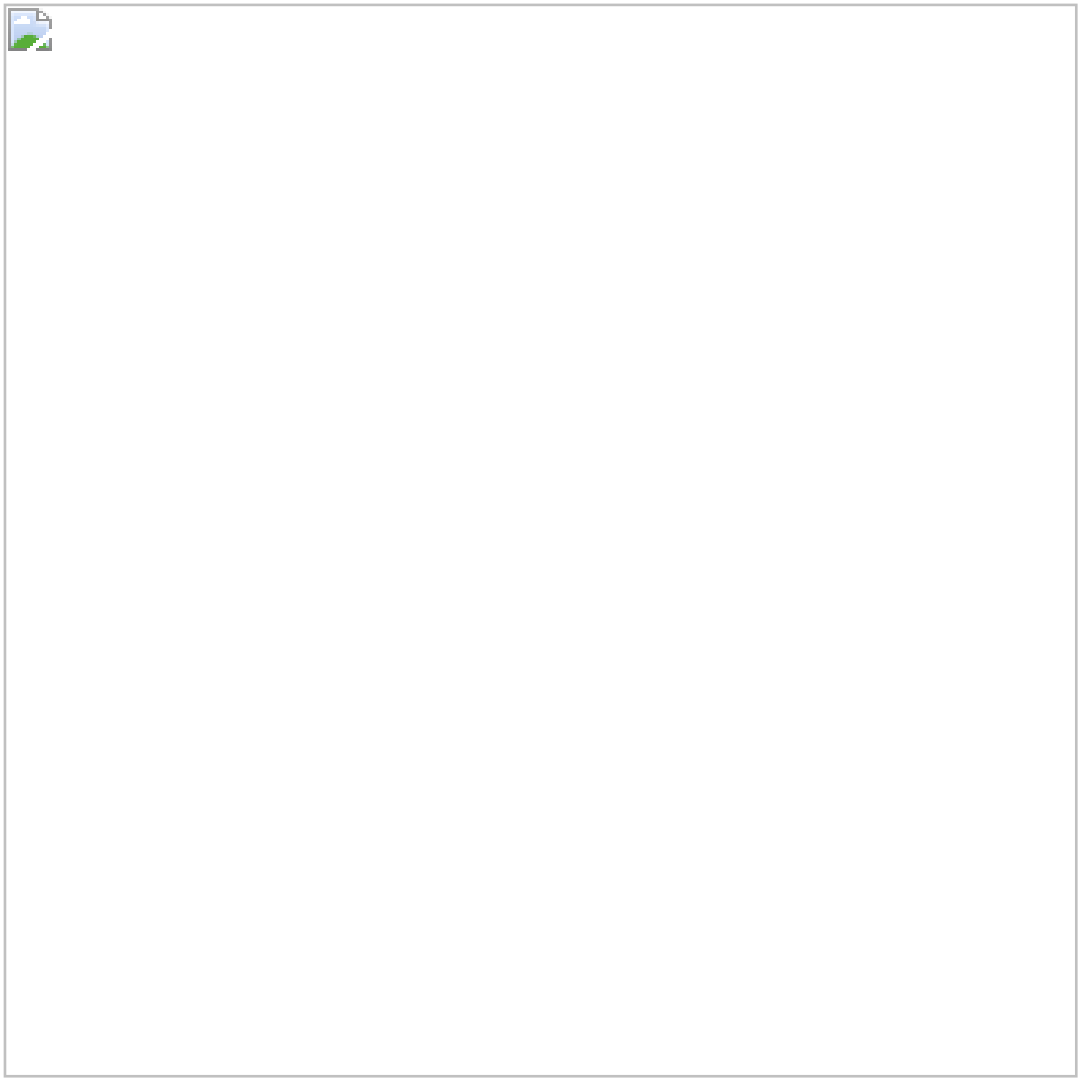

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Source: Air, Soil and Water Research, 1(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/ASWR.S825

Evaluation of Stormwater Biofilter Media for *Escherichia coli* Removal in a Laboratory Microcosm

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Abstract: Recreational water sites such as beaches along lakes, rivers, or oceans, are one of the most popular activities in many parts of the world. Recently rainfall and runoff due to rainfall events has been associated with increasing microbial levels in recreational water. This runoff can lead to beach closures and potentially unsanitary conditions at popular swimming beaches. The impact of stormwater on beach water quality has led to a myriad of option for controlling stormwater. Some of these include grass buffer partitions, stormwater detention basins, media filters, catch basin inserts, and infiltration units. Biofilters, or infiltration units are gaining popularity as a treatment option for stormwater around the Great Lakes basin, but we are aware of no studies that have looked at the indicator organism (i.e. *Escherichia coli*, or *E.coli*) removal potential of these infiltration units and the media used in them. The overall objective of this study was to evaluate the performance of a stormwater biofilter medium in removing the indicator organism *E.coli* in a laboratory system. When several laboratory biofilter system were challenged with *E.coli* concentrations of 2.82E3 and 2.85E5 *E.coli*/100mL of simulated stormwater in a 1.25 cm rain event, the systems were able to remove between 83 and 100% of the *E.coli* in this influent. During a subsequent 1.25 cm rain event with *E.coli*-free water, the biofilter was able to retain 68%–100% of the *E.coli* originally inoculated into the system. The results of this study indicate that these systems hold promise for mitigation of *E.coli* from storm water near recreational beaches. These findings will assist beach managers, engineers, and municipal stake holders evaluate the usefulness of biofilter infiltration as a storm water management tool in order to decrease *E.coli* input into beach areas.

Keywords: beach health, biofilter, *E.coli*, infiltration, rainfall, stormwater, water quality

Introduction

Recreational water sites such as beaches along lakes, rivers, or oceans, are one of the most popular activities in many parts of the world. Fecal material from swimmers, domestic animals (dogs, cattle, and horses), as well as waterfowl (geese, gulls, and ducks), all lead to increases in microbial loading at beaches (Winfield and Groisman, 2003; Kleinheinz et al. 2003). Recently rainfall and runoff due to rainfall events has been associated with increasing microbial levels in recreational waters along numerous beaches including several coastal areas in Wisconsin (Ackerman and Weisberg, 2003; Kinzelman, Racine County, WI Health Department, personal communication, 2003). This runoff can lead to beach closures and potentially unsanitary conditions at popular swimming beaches (Sampson et al. 2006; Kleinheinz et al. 2006).

Heavy rainfall was implicated in increasing bacterial contamination at beaches in several areas of the country (Ackerman and Weisberg, 2003; Haack et al. 2003). Some beaches are automatically closed or restricted after a large rainfall event, even without microbiological testing of water (Ackerman and Weisberg, 2003; Kinzelman, Racine County, WI Health Department, personal communication, 2003). On the Santa Monica Bay beaches in southern California, health departments typically issue warnings for the public to avoid recreational water contact for 3 days following a rainfall event (Ackerman and Weisberg, 2003). This public health warning is based on 5 years of fecal coliform data taken daily and after rainfall events. Although storms are fairly infrequent in southern California, rainfall events did precipitate microbial contamination exceedences due to storm water runoff (Schiff et al. 2003). In Milwaukee, WI automatic beach closures occur following heavy rainfall events due to finding a positive correlation between rainfall and *E. coli* concentrations over many years (Kinzelman, Racine County, WI Health Department, personal communication, 2003).

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The impact of stormwater on beach water quality has led to a myriad of option for controlling stormwater. Some of these include grass buffer partitions (Guber et al. 2007), stormwater detention basins (Hogan and Walbridge, 2007), media filters (Barrett, 2005, catch basin inserts (Morgan et al. 2005), and infiltration units (Birch et al. 2005) to name just a few. In general, these systems attempt to limit input of both chemicals, nutrients, and metals into water systems. The studies we are aware of have evaluated the success of the stormwater mitigation strategies in terms of the removal of total P, COD, Nitrogen, TSS, Pb, water volume, turbidity, and heavy metals (Hogan and Walbrudge, 2007; Guber et al. 2007; Furumai et al. 2005, Brezonik and Stadelmann, 2002; Bechet et al. 2006). In a few non-biofilter studies the transport of indicator organisms such as *Escherichia coli* (*E.coli*) and Enterococcus have been evaluated. There are a few evaluations of biofilter (infiltration media units) performance using metals, solids, nutrients, and organochlorine pesticides, hydraulic properties, (Birch et al. 2005; Hatt et al. 2006; Furumai et al. 2005; Bechet et al. 2006). However, we are aware of no studies that have looked at the indicator organism (i.e. *Escherichia coli*, or *E.coli*) removal potential of infiltration units and the media used in them. While there is certainly a multitude of evidence to support the effectiveness of these systems in removing many environmental contaminants, it is clear that more work is needed to evaluate their usefulness in removing biological contaminants associated with stormwater.

The overall objective of this study was to evaluate the performance of a stormwater biofilter medium in removing the indicator organism *E.coli* in a laboratory system. The results of this study will be used to assess the feasibility and design of full-scale stormwater management systems with the overarching goal of increasing beach water quality in Door County, WI (Lake Michigan).

Materials and Methods

Laboratory biofilter set-up

The laboratory biofilter column (Fig. 1) was borosilicate glass, 5 cm in diameter, contained a Teflon valve at the bottom to regulate flow, and was filled with varying amounts of biofilter media (Table 1) and/or sand (United States Department of Agriculture Classification – Medium to very coarse sand with a permeability of 0.006 cm/hr). These simulated biofilter columns of media were set-up at a depth of 15 or 30 cm. The biofilter media to sand mix was either 1:1 (Biofilter Media:Sand), or 1.5:1 (Biofilter Media:Sand) depending on which trial and as indicated in the results. The ratio of biofilter media to sand was recommended by the manufacturer of the biofilter media (Miller Engineers and Scientists, Sheboygan, Wisconsin, U.S.A.).

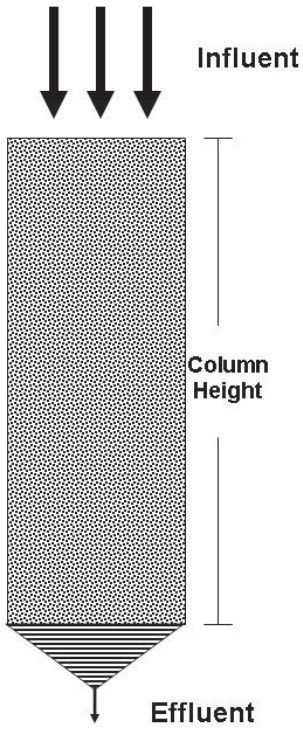

Figure 1. Schematic of the biofilter column used in this study.

The water flow rates and *E.coli* concentrations were chosen to simulate real-world values found during several years of *E.coli*-stormwater relationships in Door County Wisconsin (Kleinheinz, 2007). The mixtures of Biofilter media were selected due to their impending use in re-engineered beaches along Wisconsin's Lake Michigan shoreline.

Preparation of E.coli inoculum

The *E. coli* inoculum water was prepared using the LS 232 strain of *E.coli* from the University of Wisconsin—Oshkosh's Environmental Microbiology Laboratory. This strain of *E.coli* was recovered from a swimming beach in 2005. The aforementioned *E.coli* was grown for 18–24 hours in nutrient broth. The resulting culture was then centrifuged at

Table 1. Characteristics of the biofilter media.

pH		7.9 (slurry)	
Dry Density		70.2 pcf	
Permeability cm/hr		0.037 cm/sec	
Permeability inch/hr		0.073 cm/sec	
Influent pH[A]	5.6	5.6	5.6
Trial Number	1	2	3
Influent TSS[B] (mg/L)	0	100	300
Effluent TSS[B] (mg/L)	47	19	62
Percent TSS[B] Reduction	n/a	87	82
Effluient Nitrate (mg/L)	<0.110	<0.110	<0.110
Effluent Nitrite (mg/L)	0.02	0.04	0.012
Effluent Phosphorus (mg/L)	3.95	2.14	2.11

[A]water in = pH adjusted distilled water.
[B]TSS=Total Suspended Solids.

12,000 × g for 10 minutes, the supernatant discarded, and the resulting pellet of *E.coli* resuspended in 0.85% NaCl. This centrifugation and washing procedure was repeated three times to yield an essentially nutrient-free inoculum. The starting inoculum was either 2,820 *E.coli*/100mL of water for the first trial or, 285,000 *E.coli*/100mL of water for the second trial. These concentrations of *E.coli* were chosen and typical and 'worst-case' *E.coli* loadings from stormwater in Door County, WI (Kleinheinz, 2005). *E.coli* analysis using the Colilert® test (described below) was used for this enumeration.

Water sample collection after passing through column

In order to determine the effect of the biofilter medium on the removal of *E.coli*, an all-in-one, 1.25 cm of water rain event was simulated using 250 mL of *E.coli* inoculum. As stated previously, this was representative of rain events in Door County, Wisconsin, U.S.A. (Kleinheinz, 2007). This inoculum was allowed to wash through the laboratory system using only gravity flow. The first effluent from the column, indicated as First 1% on the graphs, was the first 2mLs of sample collected out of the column. The next approximately 75 mLs was collected (25%–50% samples), the next 75 mLs was collected (50%–75% samples), and the final 2 mLs of effluent (Final 1% samples). This initial inoculum was meant to simulate the 'first-flush' from a rain event. After the *E.coli* inoculum had washed completely through the column, and no further effluent was observed, 250 mL of *E.coli*-free water was washed through the column in an identical manner to the *E.coli* inoculum. This was intended to represent additional rainfall and determine how much, if any, of the trapped *E.coli* could be washed from the column.

All water samples were collected into sterile, polystyrene collection bottles (IDEXX Corp., Portland, ME). Upon collection, samples were immediately placed in a cooler at 4 ºC until *E. coli* analyses of samples were conducted. Samples were analyzed within 4 hours of collection. The University of Wisconsin – Oshkosh Environmental Microbiology lab was utilized for all analysis; it is a Wisconsin State Certified Laboratory with a Quality Assurance plan on file with the WI Department of Agriculture, Trade and Consumer Protection (DATCP).

Amount of *E.coli* removed from the column was calculated by:

E.coli Removal = ((Influent) − (Effluent)) × Dilution

Sample analysis

The defined substrate test, Colisure® (IDEXX Corp., Portland, ME), was used to analyze all samples for *E. coli* (American Public Health Association, 1999). Incubation and microbial enumeration from samples were conducted following the manufacturer's recommendations. All results were reported as most probable number (MPN) of *E. coli* per 100 mL of water. Positive and negative controls were prepared in accordance with the laboratory's quality assurance plan.

Graphical and statistical analysis

Graphing was performed with Microsoft Excel 2004. Statistical analysis was performed using Systat version 11.0.

Results and Discussion

Overall, *E. coli* was substantially reduced under all operational conditions using the proposed biofilter media. The 2.82E3/100mL loading of *E.coli* coupled with a biofilter column height of 15 cm (1:1 ratio of biofilter media to sand) showed 93%–100% removal of *E.coli* from a 2 cm of inoculum added to the column (Fig. 2). Subsequent washing of the column with another 2 cm of *E.coli*-free water still showed 89%–100% of the *E.coli* being removed in the column (Fig. 2). When the ratio of biofilter media to sand was increased to 1.5:1, the removal from the initial 2 cm inoculum increased to 99%–100% (Fig. 3, Table 2). Subsequent washing of the column with another 2 cm of *E.coli*-free water still showed 76%–100% of the *E.coli* being removed in the column. Two and four days after completing this study another 2 cm wash of biofilter column with *E.coli*-free water revealed 99.9% removal of *E.coli*, and no detectable *E.coli*, respectively.

Furthermore, the 2.82E3/100mL loading of *E.coli* coupled with a biofilter column height of 30 cm (1:1 ratio of biofilter media to sand) demonstrated 93%–100% removal of *E.coli* from a 2 cm of inoculum added to the column (Fig. 4). Subsequent washing of the column with another 2 cm of *E.coli*-free water still demonstrated 77%–100% of the *E.coli* being removed in the column (Fig. 4). When the ratio of biofilter media to sand was increased to 1.5:1, the removal from the initial 2 cm inoculum increased to 83%–100% (Fig. 5, Table 3). Again, subsequent washing of the column with another 2 cm of *E.coli*-free water still showed 68%–100% of the *E.coli* being removed in the column. Two and four days after completing this study another 2 cm wash of biofilter column with *E.coli*-free water revealed 99.9% removal of *E.coli*, and no detectable *E.coli*, respectively.

In another trial with a loading of 2.85E5/100mL *E.coli* coupled with a biofilter column height of 15 cm (1:1 ratio of biofilter media to sand) showed 68%–100% removal of *E.coli* from a 2 cm of inoculum added to the column (Fig. 6). Subsequent washing of the column with another 2 cm of *E.coli*-free water still showed 80-100% of the *E.coli* being removed in the column (Fig. 6). When the ratio of biofilter media to sand was increased to 1.5:1, the removal from the initial 2 cm inoculum increased to 94%–100% (Fig. 7, Table 3). Subsequent washing of the column with another 2 cm of *E.coli*-free water still showed 78%–100% of the *E.coli* being removed in the column. Two and four days after completing this study another 2 cm wash

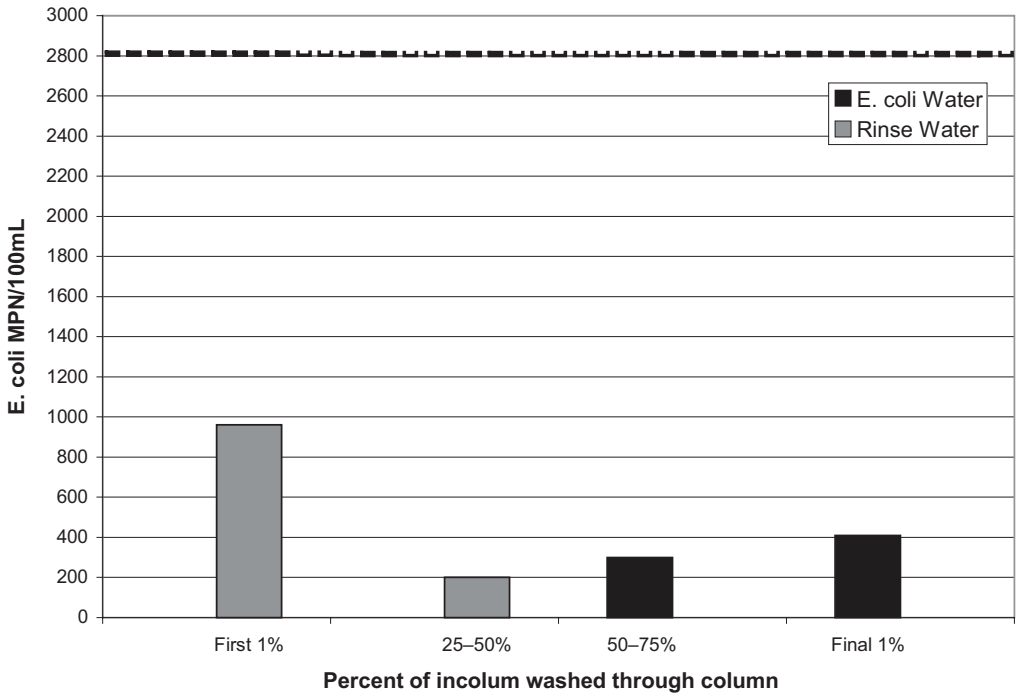

Figure 2. *E.coli* MPN/100mL from various portion of the effluent a15 cm test column and 1:1 (Biofilter mix:sand) ratio. Starting *E.coli* inoculum for this trial is represented by the dashed line and was 2.83E3 *E.coli*/100mL.

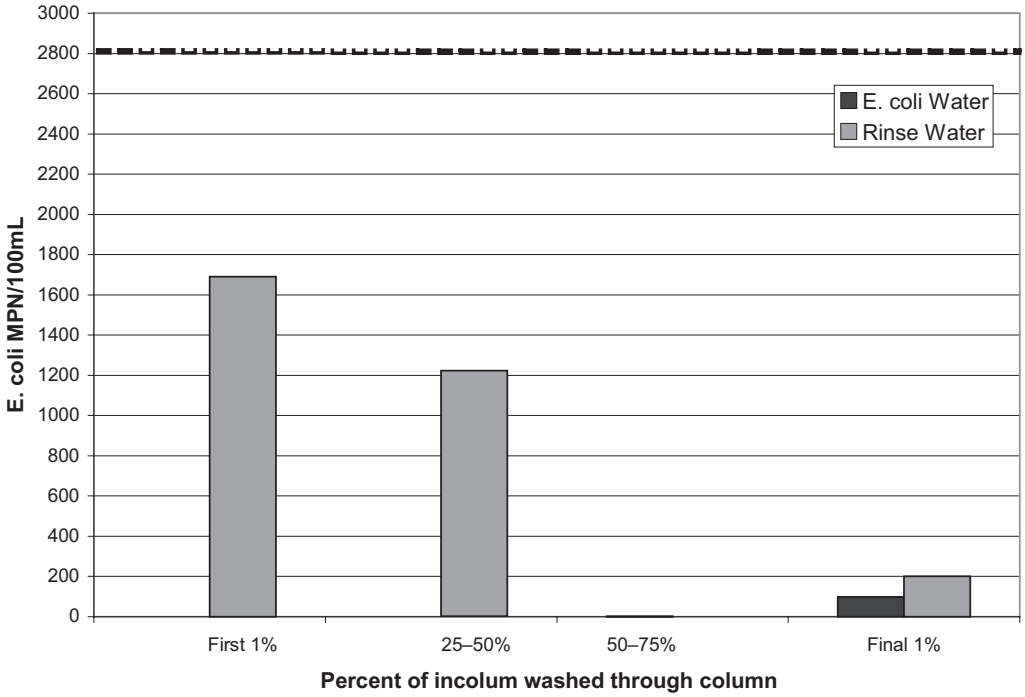

Figure 3. *E. coli* MPN/100mL from various portion of the effluent a 15 cm test column and 1.5:1 (Biofilter mix:sand) ratio. Starting *E.coli* inoculum for this trial is represented by the dashed line and was 2.83E3 *E.coli*/100mL.

of biofilter column with *E.coli*-free water revealed 99.4% removal of *E.coli*, and no detectable *E.coli*, respectively.

The 2.85E5/100mL loading of *E.coli* coupled with a biofilter column height of 30 cm (1:1 ratio of biofilter media to sand) showed 83%–100% removal of *E.coli* from a 2 cm of inoculum added to the column (Fig. 8). Subsequent washing of the column with another 2 cm of *E.coli*-free water still showed 73%–100% of the *E.coli* being removed in the column (Fig. 8). When the ratio of biofilter media to sand was increased to 1.5:1, the removal from the initial 2 cm inoculum increased to 81%–100% (Fig. 9, Table 3). Subsequent washing of the column with another 2 cm of *E.coli*-free water still showed 70%–100% of the *E.coli* being removed in the column. Two and four days after completing this study another 2 cm wash of biofilter column with *E.coli*-free water revealed 99.4% removal of *E.coli*, and no detectable *E.coli*, respectively.

At the lower *E.coli* loading, which is typical of many storm water discharge sites (Kleinheinz, 2007), the increased column depth did not appreciably increase *E.coli* removal indicating that the 15 cm bed depth was sufficient for the majority of *E.coli* removal at this loading. Increased bed depth and changing of the ratio of biofilter media to sand did not greatly reduce or increase the *E.coli*

Table 2. Percent removal of *E.coli* from laboratory microcosm during low *E.coli* loading. The initial incolumn concentration was 2.82E3 *E.coli* /100mL.

Mixture ratio (Biofilter media: sand)	Column height (cm)	Mean removal of *E.coli* from initial incolum	Maximum removal of *E.coli* from initial incolum	Mean *E.coli* found from clean water wash	Maximum *E.coli* found from clean water wash
1:1	15	93%	100%	89%	100%
1:1	30	93%	100%	77%	100%
1.5:1	15	99%	100%	76%	100%
1.5:1	30	83%	100%	68%	100%

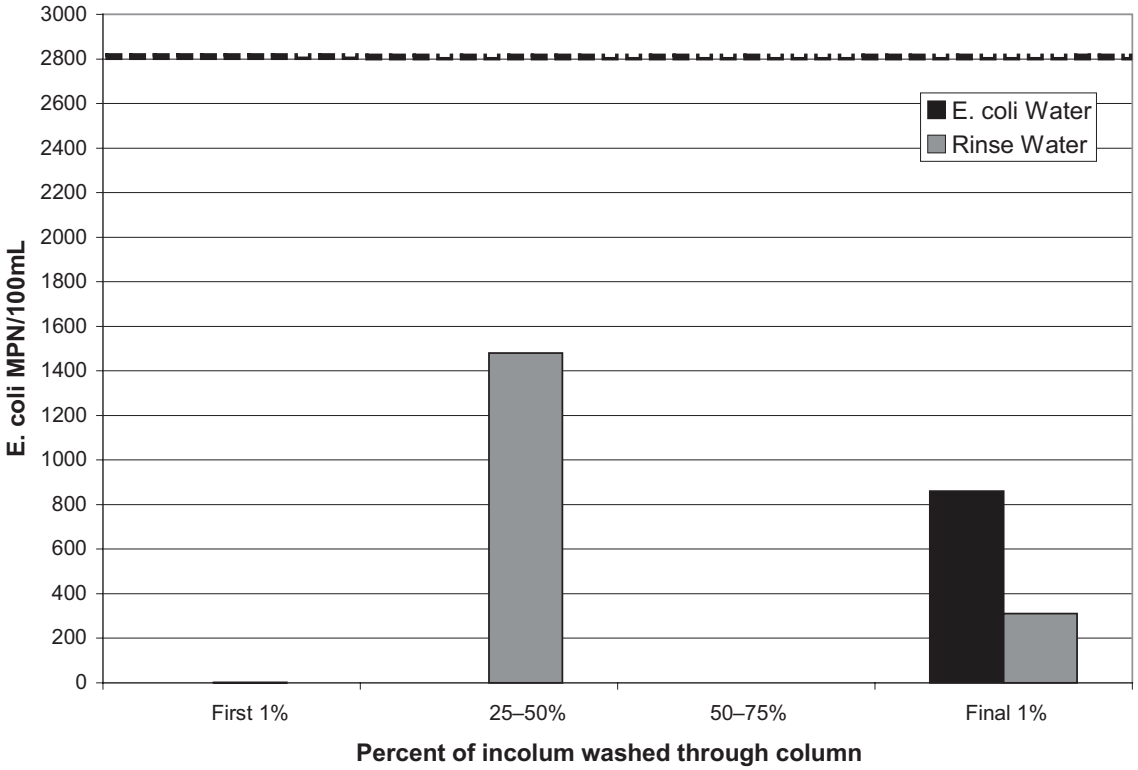

Figure 4. *E.coli* MPN/100mL from various portion of the effluent a 30 cm test column and 1:1 (Biofilter mix:sand) ratio. Starting *E.coli* inoculum for this trial is represented by the dashed line and was 2.83E3 *E.coli*/100mL.

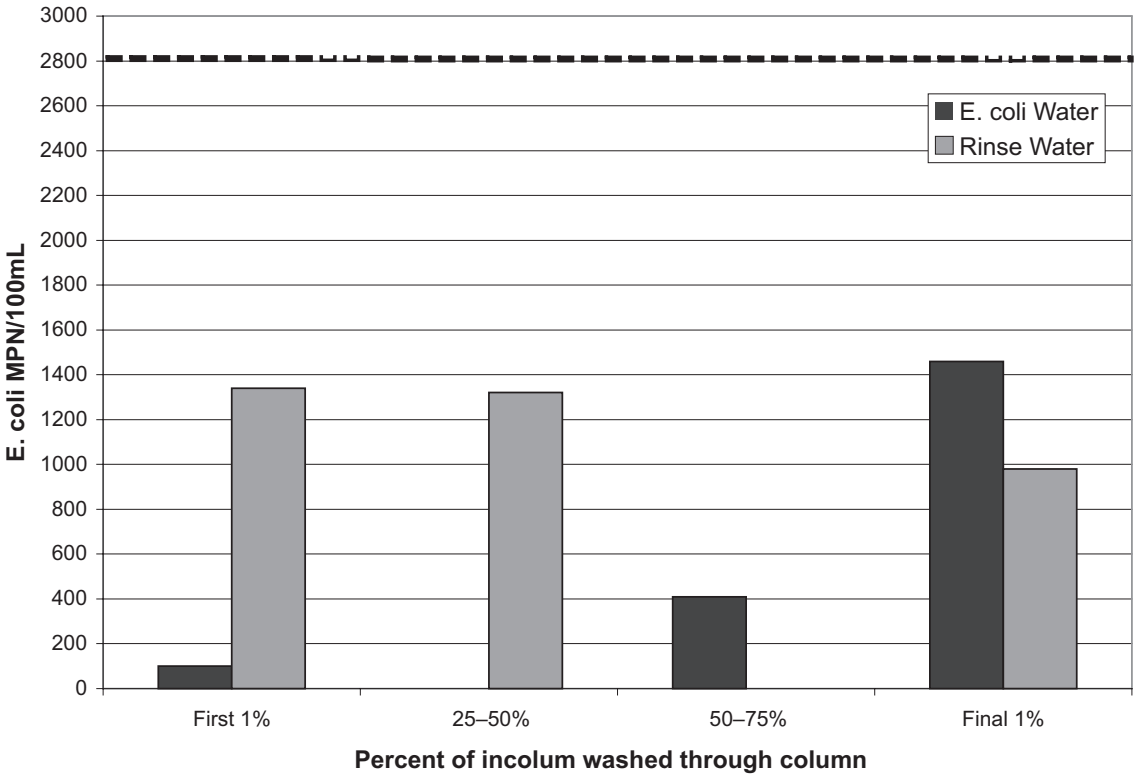

Figure 5. *E.coli* MPN/100mL from various portion of the effluent a 30 cm test column and 1.5:1 (Biofilter mix:sand) ratio. Starting *E.coli* inoculum for this trial is represented by the dashed line and was 2.83E3 *E.coli*/100mL.

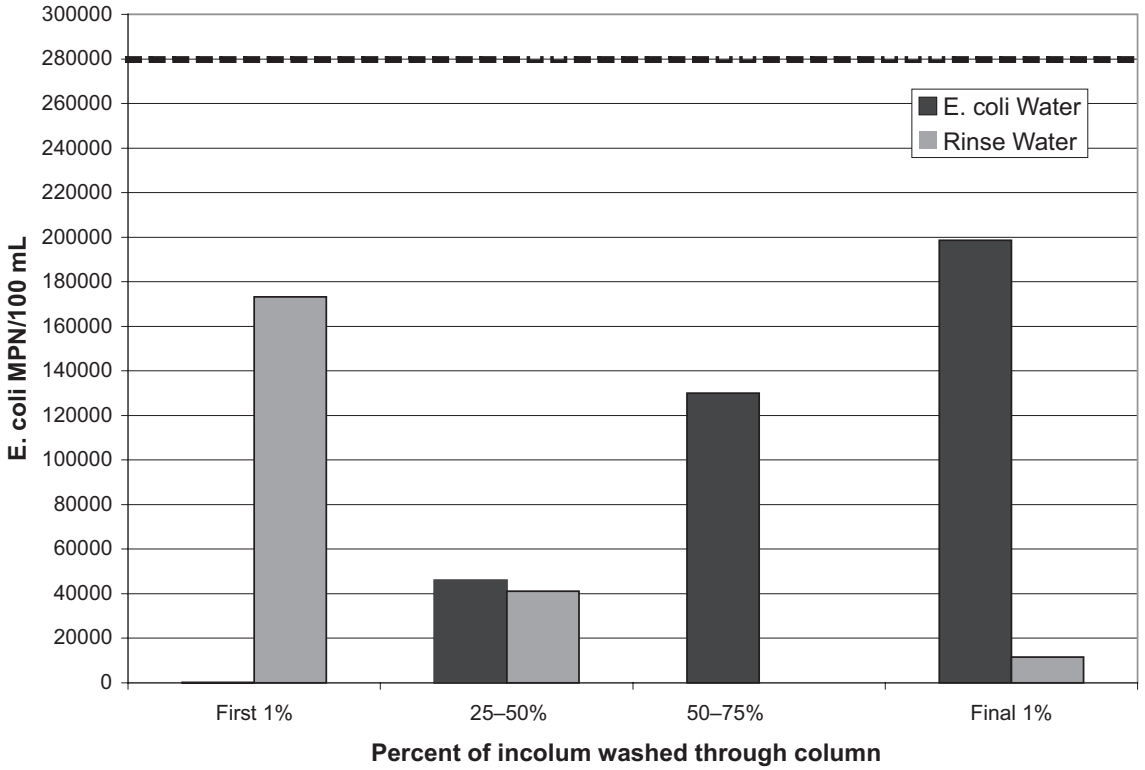

Figure 6. *E.coli* MPN/100mL from various portion of the effluent a 15 cm test column and 1:1 (Biofilter mix:sand) ratio. Starting *E.coli* inoculum for this trial is represented by the dashed line and was 2.85E5 *E.coli*/100mL.

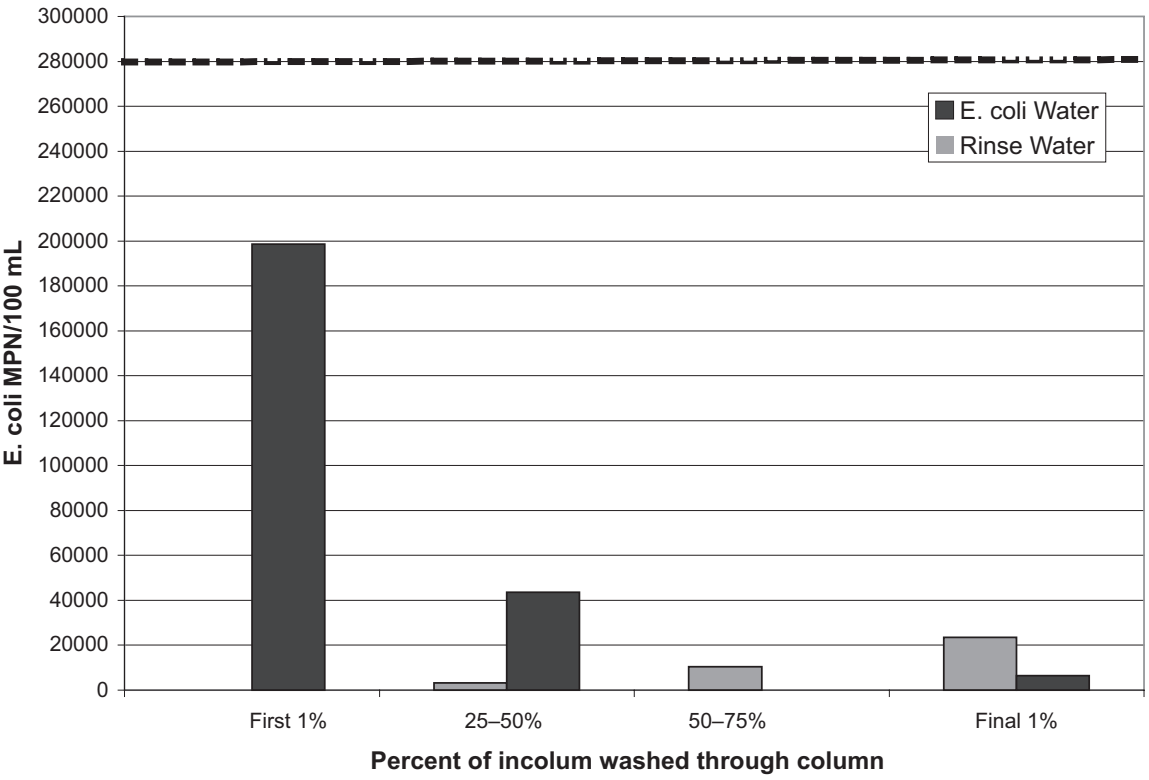

Figure 7. *E.coli* MPN/100mL from various portion of the effluent a 15 cm test column and 1.5:1 (Biofilter mix:sand) ratio. Starting *E.coli* inoculum for this trial is represented by the dashed line and was 2.85E5 *E.coli*/100mL.

Table 3. Percent removal of *E.coli* from laboratory microcosm during high *E.coli* loading. The initial incolumn concentration was 2.85E5 *E.coli*/100mL.

Mixture ratio (Biofilter media: sand)	Column height (cm)	Mean removal of *E.coli* from initial incolum	Maximum removal of *E.coli* from initial incolum	Mean *E.coli* found from clean water wash	Maximum *E.coli* found from clean water wash
1:1	15	68%	100%	80%	100%
1:1	30	83%	99%	73%	86%
1.5:1	15	94%	100%	78%	100%
1.5:1	30	81%	100%	70%	100%

removal. At the higher *E.coli* loadings, which is typical of severe storm water discharge (Kleinheinz, 2007), the increased column depth did increase *E.coli* removal at the 1:1 ratio of biofilter mix to sand, but not at the 1.5:1 ratio. Additionally, the higher loadings did lead to increased breakthrough of *E.coli* during the initial inoculum washing, however, the system was still able to remove the majority of *E.coli* during even these elevated loadings. Remarkably, the system was very resilient when challenged with additional *E.coli*-free wash water and demonstrated continued filtration efficiency. Overall, the two ratio's of biofilter mix to sand—either 1:1 or 1.5:1, appeared to function in a similar manner in terms of *E.coli* removal. The overall efficiency of the system at the two loadings provides a good indication of the efficiency of these systems under very different loading scenarios. The fact that very low *E.coli* was able to be washed from the column two days after the studies (all concentrations and rations of media), and no detectable *E.coli* was recovered four days after the study appears to indicate that *E.coli* can not survive or multiply in the media. One-gram samples of the media analyzed four days after the study also indicated that no detectable *E.coli* was present.

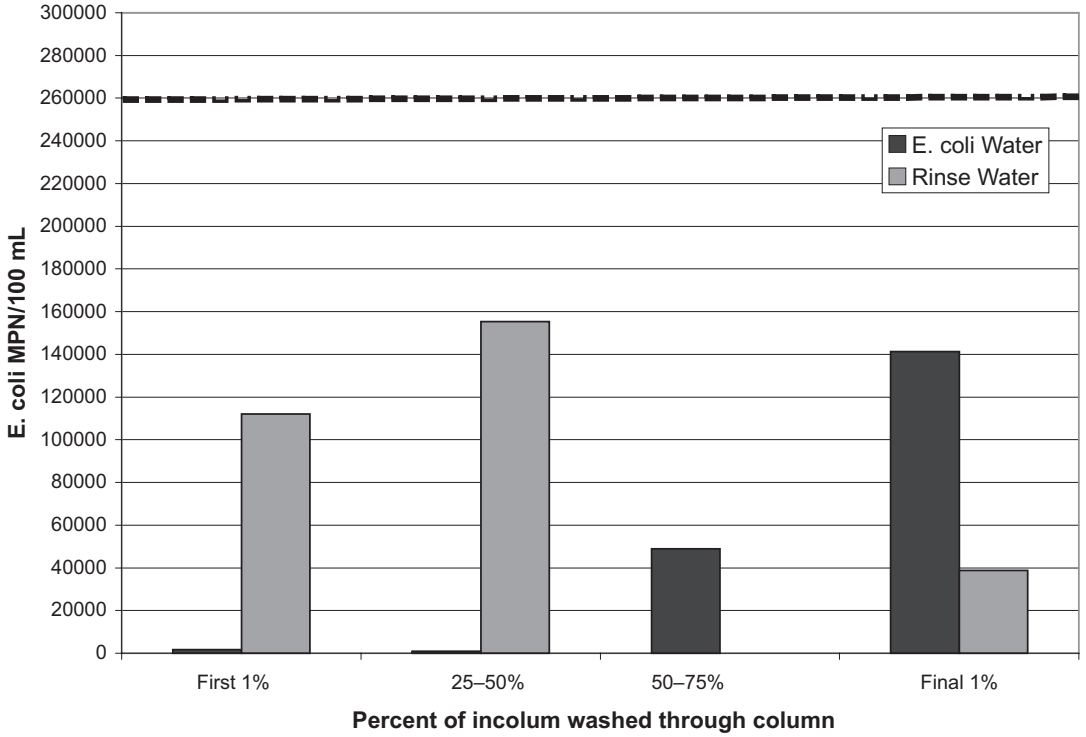

Figure 8. *E.coli* MPN/100mL from various portion of the effluent a 30 cm test column and 1:1 (Biofilter mix:sand) ratio. Starting *E.coli* inoculum for this trial is represented by the dashed line and was 2.85E5 *E.coli*/100mL.

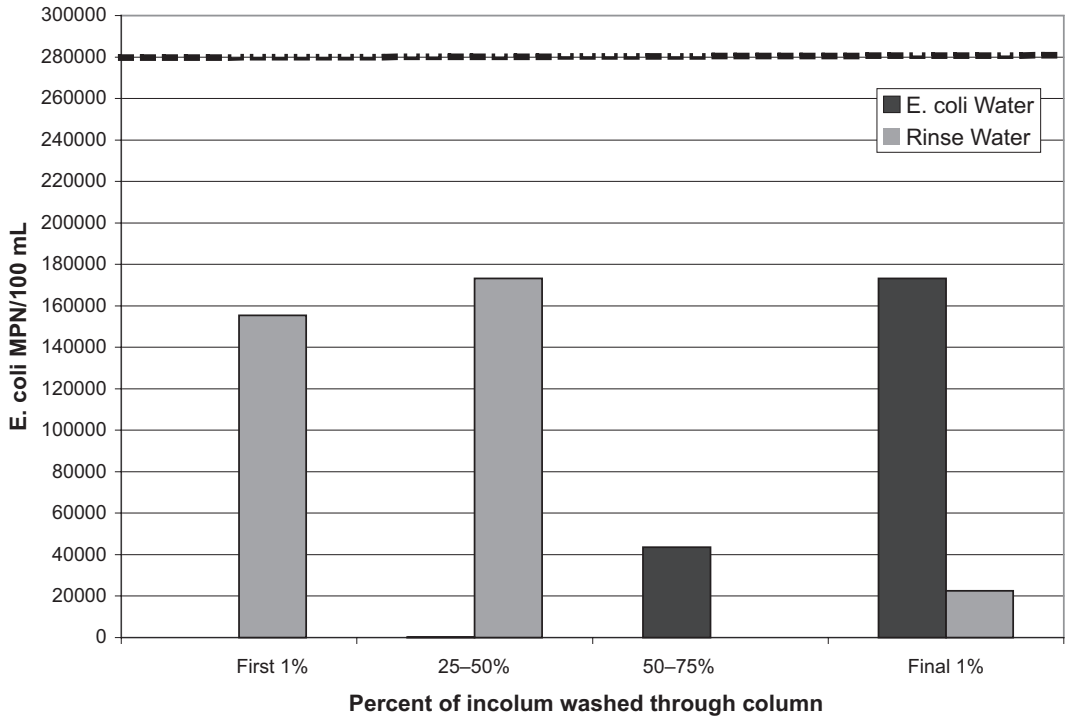

Figure 9. *E.coli* MPN/100mL from various portion of the effluent a 30 cm test column and 1.5:1 (Biofilter mix:sand) ratio. Starting *E.coli* inoculum for this trial is represented by the dashed line and was 2.85E5 *E.coli*/100mL.

Overall, this study provides valuable insight into the potential *E.coli* removal capabilities of a biofilter unit designed for *E.coli* mitigation from stormwater discharged in proximity to swimming beaches. To our knowledge this is the first attempt to investigate the *E.coli* removal capabilities of infiltration beds (i.e. biofilters) intended for storm water *E.coli* mitigation. There is obviously much more research that should be conducted into the effectiveness of these units, different media mixes, and the field evaluation of these units that can not be replicated in this simple laboratory unit. The best evaluation of this technology would be the detailed evaluation of full-scale units installed in a location that receives high concentrations of *E.coli* from stormwater. The evaluation of *E.coli* concentrations on a seasonal and year-to-year basis along with an evaluation of media condition, removal efficiency, and the impact on adjacent beach water quality would provide a very complete assessment of this technology. Certainly, there are additional engineering concerns such as filter maintenance and colmation effects that need to be elucidated, however, the results of this study indicate that these systems hold promise for mitigation of *E.coli* from storm water near recreational beaches. These findings will assist beach managers, engineers, and municipal stake holders evaluate the usefulness of biofilter infiltration as a storm water management tool in order to decrease *E.coli* input into beach areas.

Acknowledgements

The funding for this project was provided by the University of Wisconsin – Oshkosh Environmental Microbiology Research Laboratory. Special thanks to Miller Engineers & Scientists of Sheboygan, Wisconsin for providing the biofilter media and advice on mixture ratios.

References

Ackerman, D. and Weisberg, S.B. 2003. Relationship between rainfall and beach bacterial concentrations on Santa Monica Bay beaches. *J. Water Health*, 1:85–90.

American Public Health Association. 1999. Standard Methods for Examination of Water and Wastewater. 20th Ed. Edited by L.S. Clescerl, A.E. Greenberg, A.D. Eaton. Washington, D.C.

Barrett, M.E. 2005. Performance comparison of structural stormwater best management practices. *Water Environ. Res.*, 77:78–86.

Bechet, B., Durin, B., Legret, M. and Le Chloirec, P. 2006. Colloidal speciation of heavy metals in runoff and interstitial waters of a retention/infiltration pond. *Water Sci. Technol.*, 54:307–14.

Birch, G.F., Fazeli, M.S. and Matthai, C. 2005. Efficiency of an infiltration basin in removing contaminants from urban stormwater. *Enriron, Monit. Assess.*, 101:23–38.

Brezonik, P.L. and Stadelmann, T.H. 2002. Analysis and predictive models of stormwater runoff volumes, loads, and pollutant concentrations from watersheds in the Twin Cities metropolitan area, Minnesota, MN. *Water Res.*, 36:1743–57.

Furumai, H., Jinadasa, H.K., Murakami, M., Nakajima, F. and Aryal, R.K. 2005. Model description of storage and infiltration functions of infiltration facilities for urban runoff analysis by a distributed model. *Water Sci. Technol.*, 52:52–60.

Guber, A.K., Karns, J.S., Pachepsky, Y.A., Sadeghi, A.M., Van Kessel, J.S. and Dai, T.H. 2007. Comparison of release and transport of manure-borne *Escherichia coli* and entercocci under grass buffer conditions. *Lett. Appl. Microbiol.*, 44:161–7.

Haack, S.K., Fogarty, L.R. and Wright, C. 2003. *Escherichia coli* and Enterococci at beaches in the Grand Traverse Bay, Lake Michigan: sources, characteristics, and environmental pathways. *Environ. Sci. Technol.*, 37:3275–82.

Hatt, B.E., Siriwardene, N., Deletic, A. and Fletcher, T.D. 2006. Filtermedia for stormwater treatment and recycling: the influence of hydraulic properties of flow on pollutant removal. *Water Sci. Technol.*, 54:263–71.

Hogan, D.M. and Walbridge, M.R. 2007. Best management practices for nutrient and sediment retention in urban stormwater runoff. *J. Envrion. Qual.*, 36:386–95.

Kinzelman, J., Ng, C., Jackson, E., Gradus, S. and Bagley, R. 2003. Enterococci as indicators of Lake Michigan recreational water quality: comparison of two methodologies and their impacts on public health regulatory events. *Appl. Environ. Micro.*, 69:92–6.

Kleinheinz, G. 2007. A multi-tiered approached to E.coli assessments at Door County, WI beaches and the utilization of these assessments in sustainable beach redesign options. Society for Environmental Toxicology and Chemistry 28th Annual Meeting, November 12–15, Milwaukee, WI.

Kleinheinz, G. 2005. Microbial Source Tracking Options. Great Lakes Beach Association/State of the Lake Annual Meeting, November 2–3, Green Bay, WI.

Kleinheinz, G.T., McDermott, C.M., Leewis, M.C. and Englebert, E. 2006. Influence of sampling depth on *Escherichia coli* concentrations in beach monitoring. *Water Research*, 40:3831–7.

Kleinheinz, G.T., McDermott, C.M. and Sampson, R. 2003. Recreational Water: Microbial Contamination and Human Health. Special "Water" Volume of the. *Transactions of the Wisconsin Academy of Sciences*, 90:75–86.

Sampson, R., Swiatnicki, S., McDermott, C. and Kleinheinz, G. 2006. The effects of Rainfall on E.coli and total coliform levels at 15 Lake Superior Beaches. *Water Resource Management*, 20:151–9.

Schiff, K.C., Morton, J. and Weisberg, S.B. 2003. Retrospective evaluation of shoreline water quality along Santa Monica Bay beaches. *Mar. Environ. Res.*, 56:245–53.