The fate of antimicrobials entering the aquatic environment is an increasing concern for researchers and regulators, and recent research has focused on antimicrobial contamination from point sources, such as wastewater treatment facility outfalls. The terracumulation of antimicrobials and mobility in diffuse pollution pathways should not be overlooked as a contributor to the spread of bacterial resistance and the resulting threat to human drug therapy. This review critically examines recent global trends of bacterial resistance, antimicrobial contaminant pathways from agriculture and water treatment processes, and the need to incorporate diffuse pathways into risk assessment and treatment system design.

Slow sand filters are used in rural regions where source water may be subjected to antimicrobial contaminant loads from waste discharges and diffuse pollution. A simple model was derived to describe removal efficiencies of antimicrobials in slow sand filtration and calculate antimicrobial concentrations sorbed to the schmutzdecke at the
end of a filtration cycle. Input parameters include water quality variables easily quantified by water system personnel and published adsorption, partitioning, and photolysis coefficients. Simulation results for three classes of antimicrobials suggested greater than 4-log removal from 1 μg/L influent concentrations in the top 30 cm of the sand column, with schmutzdecke concentrations comparable to land-applied biosolids. Sorbed concentrations of the antimicrobial tylosin fed to a pilot filter were within one order of magnitude of the predicted concentration.

To investigate the behavior of antimicrobial contaminants during multi-stage filtration, five compounds from four classes of antimicrobials were applied to a mature slow sand filter and roughing filter fed raw water from the Santiam River in Oregon during a 14-day challenge study. Antimicrobial removal efficiency of the filters was calculated from 0.2 mg/L influent concentrations using HPLC MS/MS, and sorption coefficients ($K_d$, $K_{oc}$, $K_{om}$) were calculated for schmutzdecke collected from a mature filter column. Sulfonamides had low sorption coefficients and were largely unaffected by multi-stage filtration. Lincomycin, trimethoprim, and tylosin exhibited higher sorption coefficients and limited mobility within the slow sand filter column. The lack of a significant increase in overall antimicrobial removal efficiency indicated biodegradation is less significant than sorption in multi-stage filtration.
Antimicrobial Contaminant Removal
by Multi-Stage Drinking Water Filtration

By

Stephen J. Rooklidge

A DISSERTATION
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degree of

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APPROVED:

Redacted for privacy

Major Professor, representing Bioresource Engineering

Redacted for privacy

Head of the Department of Bioengineering

Redacted for privacy

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Stephen J. Rooklidge, Author
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CONTRIBUTION OF AUTHORS

Dr. John Bolte contributed the Runge-Kutta integration code of the LETA slow sand filter model, and Mr. Erick Burns assisted in the mathematical application of theoretical considerations during the LETA model development. Dr. Peter Nelson and Dr. Robin Collins made intellectual contributions to this manuscript. Dr. Jennifer Field contributed to the development of the HPLC MS/MS method used to analyze antimicrobial residue in surface water.
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DEDICATION

This research work is dedicated to the memory of Dr. J. Ronald Miner. His kindness, understanding, and professionalism made all the lives he touched better for having known him.
CHAPTER 1

GENERAL INTRODUCTION
Research Summary

The presence of antimicrobial residues in environmental soil and aquatic systems has recently become an issue of concern because increased bacterial resistance may occur from exposure to low concentrations of the contaminants. Human consumption of antimicrobials in contaminated drinking water may diminish the efficacy of drugs used to treat infectious diseases. Rural water treatment systems are especially vulnerable to trace organic contamination from source water because treatment methods rarely include oxidation or adsorption processes common in larger municipal water treatment systems. Until the fate and removal efficiencies of antimicrobial compounds are determined in water and wastewater treatment processes, environmental and human health risks are difficult to assess.

This research study focused on removal of antimicrobial contaminants in roughing and slow sand (multi-stage) filtration drinking water treatment; methods commonly used in rural regions and developing nations. Laboratory experiments were conducted to investigate antimicrobial sorption on schmutzdecke and sand media phases of the treatment process. A mathematical model of antimicrobial removal was developed for slow sand filtration considering media characteristics, source water organics concentration, headloss, and equilibrium photolysis and sorption coefficients. This model may be used to assess ionizable organic contaminant removal in slow sand filtration and evaluate risk to water treatment systems using contaminated source water.
Antimicrobial removal efficiency studies were performed at the slow sand filter research facility of the City of Salem, Oregon. Representative antimicrobials include the sulfonamide, macrolide, lincosamide, and diaminopyrimidine classes of compounds. Each of the antimicrobial compounds were applied to pilot-scale roughing and slow sand filters, and removal of aqueous concentrations was assessed for at least twenty pore volume detention cycles using an HPLC MS/MS method developed for this research project.

The research described in this text is the first investigation toward a deeper understanding of antimicrobial behavior in multi-stage filters that will ultimately assist water treatment purveyors using slow sand filtration to make effective decisions regarding capital investments necessary to remediate trace antimicrobial contaminants found in surface waters.

Research Objectives

1. Review of potential human and environmental health risks from antimicrobial contaminants and resistant bacteria introduced into the aquatic environment from diffuse pollution pathways;

2. Development of a simple predictive model for removal of antimicrobial contaminants in slow sand filters;

Objective 1

An extensive and critical review was conducted to investigate proliferation of antimicrobial resistant strains of bacteria, antimicrobial contamination in the aquatic environment, and potential contributions from diffuse pollution pathways. Future research directions are identified, and the concept of *terraccumulation* is introduced to describe land-applied animal manure and waste treatment facility biosolids as a reservoir of organic contaminants contributing to diffuse pollution. The use of *degradation efficiencies* to evaluate complete wastewater treatment system removal of organic contaminants is proposed.

Objective 2

The primary goal of developing a predictive mathematical model was to estimate antimicrobial removal of slow sand filters (SSF) during the biological maturation stage and filtration cycle until accumulation of headloss requires the sand/water interface (schmutzdecke) to be scraped and cleaned before returning to service. Model development of antimicrobial removal in slow sand filters would not be applicable to rural water systems unless the variables were easily and inexpensively obtained. Research focused on parameters that are accessible to water operators with limited resources: sand media characteristics; volatile solids for estimates of influent organic matter concentrations; non-volatile solids for estimates of inorganic matter; and filter
headloss as an indicator of biological layer maturation. A basic predictive antimicrobial removal model was developed using photolysis and sorption coefficients available from literature. The model results were compared to data acquired during a pilot study using source water from the Santiam River in western Oregon.

Objective 3

Initial laboratory experiments determined antimicrobial sorption behavior in the schmutzdecke and sand phases of a slow sand filter treatment system. Pilot-scale studies of antimicrobial contaminant removal efficiencies in roughing and slow sand filters were conducted at the City of Salem, Oregon's Geren Island water treatment facility. Various antimicrobials were fed to the pilot filters, and aqueous concentrations were analyzed by high performance liquid chromatography-tandem mass spectrometry (HPLC MS/MS). Previous research at this site on metals removal (Alarcon, 1997), operational strategies (Raju, 1998), pretreatment alternatives (Rooklidge and Ketchum, 2002a) and filter amendments (Rooklidge and Ketchum, 2002b) have been performed since the city initiated its research program in 1995.

Pilot Study Site Description

The city of Salem, Oregon is located at 44.943°N Latitude, 123.034°W Longitude on the Willamette River, and was established as the state capitol of Oregon in 1866. The city
uses the North Santiam River as its primary source for drinking water, and the river has an average annual discharge of $6.9 \times 10^9$ m$^3$/yr (Bastasch, 1998). A large portion of the Santiam River watershed lies within a state-designated scenic waterway and national forest. The water is of exceptionally good quality (Table 1), with average turbidity < 2 nephelometric turbidity units (ntu). The Santiam River is a major tributary of the Willamette River, with its confluence at River Mile 109.0, and drains a watershed of approximately 1984 km$^2$ (Bates et al., 1996). Detroit Dam, completed in 1953, is located upstream at River Mile 60.9 on the North Santiam River, and is operated by the Army Corps of Engineers for power production, flood control, and recreation. Logging within the watershed is estimated to have occurred at a rate of 0.25-0.5% of land area per year between 1955 and 1990, with the majority of this activity below 1200-m elevation due to wilderness restrictions at higher elevations (Jones and Grant, 1996).

The water treatment facility for the City of Salem was originally built in 1955, and is located on Geren Island (formerly Stayton Island) at River Mile 31 approximately 30 miles below Detroit Dam, near the town of Stayton, OR (Figure 1). Geren Island is approximately 90 hectare with 8-hectare of slow sand filters providing a total capacity of 400 ML/d. An infiltration gallery was originally built in 1937, and chlorine disinfection and fluoridation are provided after filtration. The facility produces drinking water for approximately 155,600 residents, and sells wholesale water to nearby cities and water districts.
Table 1

Range of raw water parameters of the North Santiam River.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkalinity (mg/L as CaCO₃)</td>
<td>17 - 22</td>
</tr>
<tr>
<td>Calcium (mg/L as CaCO₃)</td>
<td>7.0 - 7.8</td>
</tr>
<tr>
<td>Hardness (mg/L as CaCO₃)</td>
<td>16.5 - 18.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.1 - 7.3</td>
</tr>
<tr>
<td>Silica (mg/L)</td>
<td>15 - 16.2</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>9 - 14</td>
</tr>
<tr>
<td>Total Solids (mg/L)</td>
<td>30 - 50</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>0.5 - 4</td>
</tr>
<tr>
<td>Total Organic Carbon (mg/L)</td>
<td>&lt; 2.5</td>
</tr>
</tbody>
</table>

Figure 1  Location of Geren Island water treatment facility near Stayton, OR.

Slow Sand Filtration History

The use of sand media to filter drinking water dates to the early nineteenth century, with installations in Paisley and Greenock, Scotland as the earliest known references to successful design uses of the technology. Current construction of slow sand filter
systems are based on the design refined by Simpson for the London-area Chelsea Water Company in 1829 (Baker, 1948). By 1852, drinking water supplied for public consumption from the river Thames near Saint Paul’s cathedral was required to be filtered (Huisman and Wood, 1974). Water suppliers in Germany, Switzerland, and Hungary also began filtering surface water sources in the 1850’s, and the European cholera epidemic of the early 1890’s proved conclusively the benefits of filtered water supplies on the health of urban populations (Hazen, 1913).

Municipalities in the United States built slow sand filter systems at several Northeastern seaboard locations in the 1870’s, and by the turn of the century twenty treatment plants had been built in nine states (Hazen, 1913). The advent of mechanical rapid sand filtration slowed the expansion of slow sand installations, and relegated the use of these systems to water purveyors capable of accessing large tracts of land necessary for filter construction. Rural areas in North America and many developing nations are the primary benefactors of slow sand technology due to the passive nature of the treatment process. The lack of a need for constant pumping to maintain the filtration rate through the filter, the ease of operation, and the possibility of using non-mechanical maintenance procedures make slow sand filtration an appropriate technology for small and rural communities (Visscher et al., 1987). Although initially popular in Europe, SSF are used extensively in India, Africa, and South America, and the quality of effluent from many of these filter systems continues to be excellent (Mullen, 1989).
The last decade has produced exemplary research in slow sand filtration, and many of the parameters necessary for adequate design have been thoroughly investigated. Research on the use of various sand filter media (McMeen and Benjamin, 1996), sand media characteristics (Lehmann, 1996; Di Bernardo and Rivera, 1996), filtration rates (Van der Hoek et al., 1996), resanding strategies (Kors et al., 1996), solids penetration (Ellis and Aydin, 1995), and computer modeling (Ojha and Graham, 1994; Shiba, 1996) have led to a substantial increase in the understanding of processes necessary for adequate treatment.

The initial raw water quality is an important design consideration when communities consider slow sand filtration as a potential treatment method. High levels of suspended solids in the source water limits the use of passive treatment systems (Visscher et al., 1987; Huisman and Wood, 1974; Martin and Martin, 1991), and regional climate considerations are important factors for passive filtration due to raw water temperature fluctuations and algal populations (Seger and Rothman, 1996; El-Taweel and Ali, 1999).

Pretreatment of raw water sources allows the use of passive filtration (Galvis et al., 1993) or thermal pasteurization (Burch and Thomas, 1998) treatment techniques by decreasing particulate matter and organic contaminants prior to filter application. The addition of Ranney collection systems (Logsdon et al., 2002), filter fabric in the media (Graham et al., 1996), and roughing filters (Wegelin, 1996) have widened the use of slow sand filters to include regions previously forced to implement more complex treatment methods. Pretreatment techniques and filter amendment research, such as enhanced filter ripening
using organic and synthetic polymers (Jellison et al., 2000), granulated activated carbon addition (Page et al., 1996), pre-ozonation (Cable and Jones, 1996; Yordanov et al., 1996), benzoate addition (Eighmy et al., 1988), and multiple-stage coagulation (Carlson and Gregory, 2000) have broadened the application of passive filtration in regions that can afford to implement these types of infrastructure improvements.

Slow Sand Filter Characteristics and Treatment Mechanisms

Raw water enters top of the filter, and the supernatant flows by gravity through the filter column. The water flows through a layer of sand, travels through a layer of larger gravel, and flows through a perforated pipe underdrain collection grid to the treated water storage and distribution system (Figure 2). The uninterrupted use of slow sand filters throughout the filtration cycle, proper filter design, and the diligence of filter operating personnel reduce the threat of finished drinking water contamination.

Figure 2  Schematic of a common slow sand filter design.
Common slow sand filter design criteria are listed in Table 2 (Hendricks et al., 1991), and the resulting estimates of effluent water quality are presented in Table 3 (Visscher et al., 1987). The recommended influent turbidity limit for optimum slow sand filter operation is less than 10 ntu, and many engineers consider source waters that regularly exceed this limit as unacceptable candidates for slow sand filter treatment. Raw water sources that occasionally exceed turbidity limits are also regarded with apprehension unless the slow sand filter is augmented with a pretreatment method that reduces influent turbidity to acceptable levels.

### Table 2

Common slow sand filter design criteria.

<table>
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<th>Design Criteria</th>
<th>Recommended</th>
</tr>
</thead>
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<tr>
<td>Hydraulic Loading Rate</td>
<td>0.1 - 0.2 m/hr</td>
</tr>
<tr>
<td>Filter Bed Area</td>
<td>5 - 200 m² per filter</td>
</tr>
<tr>
<td>Depth of Sand Bed:</td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>0.8 - 0.9 m</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.5 - 0.6 m</td>
</tr>
<tr>
<td>Sand Size:</td>
<td></td>
</tr>
<tr>
<td>Effective Diameter</td>
<td>0.15 - 0.30 mm</td>
</tr>
<tr>
<td>Uniformity Coefficient (UC)</td>
<td>&lt; 5, preferably &lt; 3</td>
</tr>
<tr>
<td>Gravel Support Depth</td>
<td>0.3 - 0.5 m</td>
</tr>
<tr>
<td>Supernatant Depth</td>
<td>1.0 m</td>
</tr>
</tbody>
</table>

Water entering the top of a slow sand filter has been modeled in earlier research as being subjected to many of the same forces acting on particulate contaminants as rapid sand filters. The concept of a sand media bed being viewed as a group of individual collectors produced equations of transport and capture mechanisms acting on the contaminant...
particle by interception, sedimentation, and diffusion (Yao et al., 1971). Interception occurs when the contaminant particle attaches to the sand media after collision. Therefore, the larger the particle that flows through the interstitial void between the media grains the more likely it is that collision will occur.

Table 3


<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment Performance</th>
</tr>
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<tbody>
<tr>
<td>Color</td>
<td>30 - 100% reduction</td>
</tr>
<tr>
<td>Turbidity</td>
<td>&lt; 1 NTU</td>
</tr>
<tr>
<td>Fecal Coliform</td>
<td>1 - 3 log units</td>
</tr>
<tr>
<td>Total Organic Carbon</td>
<td>15 - 25% reduction</td>
</tr>
<tr>
<td>Trihalomethone Precursors</td>
<td>&lt; 25% reduction</td>
</tr>
<tr>
<td>Enteric Viruses</td>
<td>2 - 4 log units</td>
</tr>
<tr>
<td>Giardia Cysts</td>
<td>2 - 4 log units</td>
</tr>
<tr>
<td>Organic Matter</td>
<td>60 - 75% COD reduction</td>
</tr>
<tr>
<td>Heavy Metals</td>
<td>30 - 95% reduction</td>
</tr>
</tbody>
</table>

Sedimentation occurs when the contaminant particle collides with the sand media under vertical influence from the force of gravity. The probability of collision with media grains increases as velocity of the fluid slows and retention time within the filter increases. Diffusion is the effect of thermal molecular motion on the particles causing a random movement as the contaminants pass through the filter. Diffusion is an important mechanism for particles < 1 μm average diameter, and fluid temperature affects the rate of particle motion along the streamline.
Huisman and Wood (1974) used the concept of media grain upper region surface areas as small collectors that can be idealized as a large sedimentation basin with an area equal to the sum of grain surface areas within a sand bed. Haarhoff and Cleasby (1991) provide an excellent review of the diverse physical mechanism models that have been used to estimate slow sand contaminant removal efficiency for a variety of sand media grain sizes, but many models are limited because the lack of consideration for biological growth within the filter bed. As raw water bacteria, diatoms, algae, and vegetative matter are deposited in the filter, removal efficiencies stray from the purely physical model estimates, and the need for empirical pilot study assessments on individual raw water sources becomes imperative. Modeling of headloss accumulation and contaminant removal efficiency by idealizing the biofilm growth at the schmutzdecke as a filter “layer” (Ojha and Graham, 1996) has advanced understanding of the biological role, but empirical factors and estimates of microbiological interactions within the sand media continue to be a vital part of theory affirmation.

Collision of contaminant particles with stationary sand media by transport processes can only be an effective removal mechanism if the contaminants attach to the media or settle within the interstitial media voids. Decades of research indicate the attachment of particles is enhanced by the formation of an adsorptive layer of biofilm on media grains. This biofilm is formed from populations of algae, diatoms, protozoa, rotifers, and bacteria (Huisman and Wood, 1974). Also present are extracellular polysaccharides exuded by microorganism populations inherent in surface water sources (Cunningham et al., 1991).
that decrease with media depth in a mature filter (Duncan, 1988). This extracellular polymer formation has been suggested as a source of destabilization for bacteria and clay entering the filter bed (Bellamy et al., 1985). The extracellular activity of the bacterial populations includes hydrolytic enzyme formation necessary for the cleavage of natural organic matter to subunits available for bacterial transport and utilization (Visser, 1985). Adsorption of solid contaminants also decreases with filter depth (Ellis and Aydin, 1995), and filter biological maturity has been shown to be crucial for organic matter adsorption (Woudneh et al., 1997).

The combination of biological and physical removal mechanisms in slow sand filters provides excellent effluent quality from raw water sources high in organic material. However, when any of the described filter removal processes are jeopardized the contaminant removal efficiency of the filter decreases. As the filter efficiency is compromised, the possibility of contaminants passing through the filter media into the available drinking water supply increases.

The importance of the schmutzdecke biological layer on the overall filtering process is illustrated by its removal when scraping the top centimeter of media during maintenance, which immediately lessens the headloss and returns the flow rate to design levels. After cleaning of the schmutzdecke from the slow sand filter, a ripening period is required to allow the biofilm growth to reach maturity, and the effluent is wasted until turbidity and biological test results are within established limits because the overall contaminant
removal efficiency is diminished during the filter ripening period following cleaning. A decrease in pH through the filter indicates metabolic activity is occurring (Collins et al., 1989). The length of filter ripening periods can be altered by factors such as ambient and raw water temperature, raw water bacterial populations, and available nutrient supply (Huisman and Wood, 1974). In high mountainous or spring-fed cold raw water sources with low organic content and moderate alkalinity, biofilm development can be too slow to achieve optimum contaminant removal in an economical time period (Lehmann, 1996).

The presence of natural organic matter in surface waters complicates prediction of colloidal particle stability in attempted treatment removal processes because of the variety of adsorption mechanisms that can occur in aquatic environments. Humic matter may specifically bind to the edges of clay minerals and oxide surfaces by ligand exchange at moderate to low solution pH (Parfitt et al., 1977). Kretzschmer and Sticher (1997) found hematite colloid mobility was greatly enhanced in the presence of small additions of humic acid, but the humic-coated colloid mobility was sharply decreased by the subsequent addition of CaCl₂. This behavior was explained by diffuse double-layer compression around the charged surfaces and the increased complexation of colloid-bound humic acid functional groups with free calcium ions. Earlier investigations of alum addition by Black and Hannah (1961) led to a substantial increase in the use of synthetic and natural polymers as coagulants by water purveyors having source waters subject to erratic fluctuations in turbidity.
Spikes in turbidity levels at treatment facility raw water intake structures are often associated with rain events in the watershed. An increase of tributary flows can cause upstream landslides and erosion in unstable terrain that influences the suspended and colloidal dispersions in the surface discharge. The most common method of pretreatment in the United States to remove colloidal clay from raw water is the addition of chemical coagulants to destabilize the clay and allow flocculation and sedimentation of the clumped particles prior to filtration (Figure 3).

![Treatment train of a chemically pretreated slow sand filter system.](image)

Chemical methods require a continuous supply of coagulants and close monitoring by skilled operations personnel. By 1968, estimates of sludge production from the use of coagulants reached one million tons per year, and the waste disposal problem associated with the use of these materials has not abated. Dewatering of coagulant sludge prior to landfill disposal is a mechanized process that may be beyond the abilities of rural water treatment facilities. Discharging dilute sludge waste to settling ponds is regarded as a temporary disposal method that requires extensive land area (Russell and Peck, 1998).
Developing nations that rely on slow sand filtration for treatment in rural communities have also experienced high turbidity in source waters and treated effluent due to the non-settling character of clay present and the filter's inability to adequately capture colloidal material, but chemical coagulants are often difficult and expensive to obtain. The abandonment of slow sand filtration for rapid sand filters preceded by chemical coagulation and flocculation, as has historically occurred in wealthier nations, may not be a reasonable option for economically-challenged countries. The effectiveness of slow sand filters for the production of safe water in rural regions cannot be disputed, and erratic raw water turbidity caused by colloidal clay is not a problem solely inherent among wealthier nations. Therefore, passive pretreatment systems that are effective and inexpensive to operate have been researched and developed to mitigate SSF treatment system deficiencies.

Roughing Filter Pretreatment

Roughing filters (RF) are generally designed in a similar manner as slow sand filters, with the exceptions of having larger media grain size and faster filtration rates. Unlike slow sand filters, sedimentation of particles is the primary removal mechanism within the RF media bed, and the flocculating environment within the interstitial region of the media grains enhances particle collision without raising the velocity of the fluid to the point of causing excessive particle shear and sediment mobilization. Roughing filter design represents natural processes found in gravel riverbeds that filter suspended material from
water destined for underground aquifers. The crushed gravel in the roughing filter provides a large surface area for the flowing water to contact; allowing flocculation from particle collision in the circuitous route through the rock and sedimentation among the media grains due to the short settling distance within the interstitial media voids. The addition of RF pretreatment (multi-stage filtration) maintains the simplicity of the SSF treatment process while achieving suspended solids and turbidity removal acceptable for drinking water.

John Gibb’s filter design of 1804 used decreasing sizes of gravel prior to slow sand filtration of water for the city of Paisley, Scotland (Baker, 1948), and the expansion of coarse gravel filtration in SSF systems continued in Great Britain until the introduction of rapid sand filtration in the 1930’s. Several European cities continue the use of roughing filtration to the present day for aquifer recharge, but this practice is usually halted during seasonal periods of high water turbidity (Wegelin, 1996). Common placement of roughing filter pretreatment for slow sand filters is illustrated in Figure 4.

![Figure 4: Multi-stage filtration treatment process flow.](image-url)
Roughing filters are usually comprised of individual flow-through filter cells or compartments that contain decreasing sizes of gravel in the direction of flow. Passive filter systems in common use near rivers or canals include Dynamic Filtration (Figure 5). This design allows for gravity flow from source water through a bed of crushed gravel (6 mm average diameter), with the overflow being wasted back to the river channel.

![Figure 5](image-url) Example of a common dynamic roughing filter design.

A drawback to dynamic filtration is the need to remove and clean the gravel media when the filter has become clogged during high turbidity events, and this limitation suggests use in raw water sources that experience turbidity peaks of less than a few hours duration. The substantial filtration rate of 5 m/hr in these filters is not designed to alter water quality of low turbidity, but instead is intended to protect downstream filter systems from intermittent peaks of high solids concentrations; effectively shutting off the water supply as the filter media clogs. The filter is then cleaned after each event and returned to service.
Research on dynamic filtration by Latorre et al. (1996) on the Cuaca River in Colombia established the applicability of this pretreatment method on highly polluted clay-bearing rivers. Filtration rates between 2 and 4 m/hr produced suspended solids removal efficiencies of 83-87% and turbidity removal of 50-52%, but it was also estimated that iron present in the raw water may have enhanced particle flocculation. The overall removal efficiencies of the filter were not significantly altered by changes in flow velocities less than 5 m/hr, but recommended flow velocity for the tested system was below 4 m/hr.

Vertical-flow filtration can be used downstream of a dynamic filter to further increase solids removal and protect the slow sand filter from solids breakthrough. Modern designs based on the filters built to treat water supplied to the city of Paris, France in 1899 use a minimum of three filter cells with each subsequent cell containing smaller sizes of gravel than the previous cell (Baker, 1948). Media size fractions range from average diameters of 20 mm in the first cell down to 4 mm in the last, and can operate in either upflow or downflow configurations (Figure 6). The gravel media is submerged under a supernatant water level, and filtration rates vary from 0.3 to 1.0 m/hr.

Downflow RF research by Galvis et al. (1993) in Colombia and Pardon (1989) in Peru has shown the contaminant removal efficiencies of this type of filter adequately pretreat water subject to high turbidity. Filter velocities between 0.1 and 0.8 m/hr were tested on natural river water in Colombia with an average of 50 ntu, and removal efficiencies were
between 55% and 45%. Challenge tests using sludge were conducted with turbidities greater than 200 ntu and the removal rates achieved 90% for the lowest filtration rate to 70% for the highest flow velocity (Galvis et al., 1993).

![Diagram of downflow (a) and upflow (b) vertical roughing filters.](image)

**Figure 6**  Schematic of downflow (a) and upflow (b) vertical roughing filters.

Upflow roughing filters are also equipped with decreasing sizes of gravel in the direction of water flow, and must have drainage chambers in each filter cell to facilitate regular cleaning. Filter freeboard must be sufficient to produce gravity hydraulic flushing capable of flow velocities greater than 90 m/hr (Wolters et al., 1989). Galvis et al. (1996) reported 97% suspended solids removal and 80% turbidity removal for raw water with average SS of 100 mg/L and turbidity of 95 ntu. Comparisons to horizontal roughing
filters during the research in Cali, Colombia proved an advantage is gained in hydraulic filter cleaning because the majority of large solids are kept in the lower chamber near the maintenance drains. Flushing water and operational labor were lower in the upflow filters due to this inherent design advantage. Various filter media materials have been used in research studies to make the pretreatment method applicable to many regions. Examples of media research includes blast furnace slag (El-Taweel and Ali, 1999), coconut fibers, and burnt rice husks (Frankel, 1981). The use of organic media includes the limitation of having to dispose of the media after the filtration cycle is complete.

Horizontal-flow roughing filter (HRF) installation has been a particularly successful pretreatment method for suspended solids removal in rural areas of developing nations that have a limited availability of skilled operators (Wegelin, 1996). The success of Wegelin’s initial modeling of horizontal roughing filtration using kaolin suspensions (1987) and Collins et al. (1994) evaluations of roughing filtration design variables led to greater understanding of applied research results in the countries of China, Ghana, Switzerland, Thailand, Tanzania, Peru, Sudan, Bolivia, and Colombia.

The raw water enters the filter by an intake weir and flows horizontally through decreasing sizes of media contained in separate compartments (Figure 7). Flow velocities range from 0.3-1.5 m/hr, and the average media diameter ranges from 20 mm in the first cell to 4 mm in the last. Overall filter length normally ranges from 5-7 meters, with a filter cell length ratio of 3:2:1. Typical media depth designs are less than 1 meter
to facilitate ease of media replacement, and drainage chambers are provided with the same freeboard and flushing velocities as vertical filters. The solids storage capacity for HRF is very large, and adequate filter service life can be years before filter clogging necessitates media removal (Collins et al., 1994).

Figure 7 Horizontal flow roughing filter.

Research in Colombia used modified HRF by separating each cell as an individual compartment in series, but this modification produced no better turbidity removal results than vertical filtration (Galvis et al., 1996). An original HRF pretreatment filter in Bolivia was replaced with an upflow system pretreated with chemical coagulants due to turbidity fluctuations between 150 and 1300 ntu that the initial HRF could not remove (Ingallinella et al., 1998). This alteration of passive roughing filters by the addition of coagulants is regarded as "direct" roughing filtration. Ahsan et al. (1996) found a coagulant addition of 1 mg(AI^{3+})/L as alum decreased raw water influent from 200 ntu to an horizontal roughing filter effluent of less than 3 ntu at filtration rates of 5-7 m/hr. This dosage clogged the HRF at rates far higher than filters without coagulant addition (3-5
days), and cleaning process modifications must be designed before this type of treatment can be reliably used in the field. However, the combination of passive filter pretreatment with coagulant additions of short duration expands the use of slow sand filters to source waters with high turbidity.

Limestone Contactors

Limestone contact basins have been used for many years as a post-filtration treatment alternative for corrosion control by increasing the buffering capacity of water in the storage and distribution system (Letterman et al., 1986). Limestone contactors are closed-to-atmosphere tanks or boxes filled with crushed limestone that are designed to allow water to flow through unimpeded while the carbonate constituents of the media dissolve. The dissolution products raise the pH and alkalinity of the water, and impart no contaminants if the media is relatively pure. Reference to early marble contactor use in Germany described treating slow sand filter effluent by raising the pH from 5.8 to 7.0 in an installation treating thirty to forty thousand cubic meters of effluent per day, and was completely overhauled every eight years (Cox, 1933). This reference also mentions an application of a chalk contactor in Egypt in 1912, but the original citation is unavailable. The use of passive calcite limestone contactors has expanded to include pH adjustment of acidic drainage residues from abandoned mines (Faulkner and Skousen, 1994). Investigations in British Columbia, Canada have proven the efficacy of limestone contactors for use with alum in pretreatment flocculation (Benjamin et al., 1992).
Crushed limestone as roughing filter media was used in early studies by Wegelin in Tanzania (1983) with influent water of approximately 60 ntu and 100 mg/L suspended solids. These bench-scale experiments were followed by a 15 meter long HRF experiment that produced effluent of less than 20 ntu from a natural water source, and the remaining turbidity was attributed to residual color in the source water. The reference is vague about the actual media used in the pilot study, but limestone gravel is the only media of roughing filter size mentioned. The use of limestone amendments to roughing filtration increased clay sedimentation (Rooklidge et al., 2002) and enhanced effluent corrosion control characteristics (Rooklidge and Ketchum, 2002a).

Pilot Study Roughing and Slow Sand Filter Construction

The pilot study horizontal roughing filter was manufactured from exterior-grade plywood supported by a beam and pier-block foundation (Figure 8), and sealed with epoxy at wood connections and fastener points to form a watertight box. The exterior dimensions of the entire box construction was 0.9 meters high, 2.44 meters wide, and 2.44 meters long, and had a sealed wall separating the parallel filter compartments for an interior individual filter length to width ratio of 2.12:1. The first compartment was filled with gravel media and the second contained granulated activated carbon as the final treatment step before pilot effluent was introduced back to the municipal water system. The filter was covered by thin plywood sheeting to prevent falling debris from impacting the filter media, but the cover was not tight enough to restrict atmospheric CO₂ conversion.
The crushed basaltic river rock was provided by Morse Bros. (Stayton, OR), the high-calcium limestone donated by Ash Grove Cement Co. (Portland, OR), and the media was graded and washed prior to filter installation. The roughing filter had longitudinal filter cells holding various rock gradations (Table 4) separated by steel screen. The first cell of the roughing filter had an initial layer of calcite limestone (Table 5) with basalt river rock of the same gradation making up the remaining cell. The other cells contained smaller filter media at a final cell length ratio of 0.45:2.55:2:1, and the entire pilot filter system is shown in Figure 9.

Table 4

Roughing filter media size range.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Size (mm)</th>
<th>Porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>30 - 20</td>
<td>0.53</td>
</tr>
<tr>
<td>Second</td>
<td>30 - 20</td>
<td>0.51</td>
</tr>
<tr>
<td>Third</td>
<td>20 - 12</td>
<td>0.49</td>
</tr>
<tr>
<td>Fourth</td>
<td>12 - 9</td>
<td>0.47</td>
</tr>
</tbody>
</table>
Table 5

Calcite amendment mineral analysis.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCO₃</td>
<td>98.3</td>
</tr>
<tr>
<td>MgO</td>
<td>0.46</td>
</tr>
<tr>
<td>SiO₂</td>
<td>0.62</td>
</tr>
<tr>
<td>Al₂O₃</td>
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</tr>
<tr>
<td>Fe₂O₃</td>
<td>0.23</td>
</tr>
<tr>
<td>SO₃</td>
<td>0.14</td>
</tr>
<tr>
<td>Na₂O</td>
<td>0.04</td>
</tr>
<tr>
<td>K₂O</td>
<td>0.03</td>
</tr>
<tr>
<td>MnO</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Figure 9  Photograph of pilot roughing and slow sand filter system installed at Geren Island.
Pilot slow sand filters were built from 3.05-m tall black polyethylene tanks with a 1.2-m internal diameter, and provided with 2-cm PVC influent and overflow piping. The 0.6-m deep crushed river rock support media complied with AWWA B100 standards, and was installed in layers of decreasing average size from 20-mm at the bottom to 1.5-mm at the sand/gravel interface. The initial sand media depth in the filters was 1.0 meter, with a uniformity coefficient of 2.26, effective size of 0.30 mm, and N2-B.E.T. surface area of 1.95 m²/g. The sand media was washed during production, and the assembled filter was allowed to run to waste for three weeks prior to the pilot antimicrobial challenge study.

Earlier qualitative studies by Rooklidge (2001) investigated removal of penicillin-G from a calcite-amended roughing filter, using a presence/absence microbial inhibition assay test. Aqueous penicillin-G was fed to the filter at an initial concentration of 4 ppb. Triplicate samples were taken from the influent, calcite layer effluent, and RF effluent throughout the detention time of the filter. Results indicated a decrease of antimicrobial bioactivity occurred in the roughing filter, but no quantitative experiments were performed. However, penicillin-G was not used in this research because the antibiotic may be hydrolyzed in wastewater treatment systems, and the compound was not present in a recent investigation of U.S. surface waters (Kolpin et al., 2002). This study used antimicrobials identified in surface water that included receiving waters of feedlot operation waste, and the classes of antimicrobials described below have been discovered as contaminants in aquatic systems worldwide (Kolpin et al., 2002; Golet et al., 2002; Calamari et al., 2003).
Pilot and Laboratory Study Antimicrobials

This research study examined roughing and slow sand filter removal efficiency of five compounds from four classes of antimicrobials: sulfonamides; lincosamides; macrolides; and diaminopyrimidines. The chemical structure of each antimicrobial used during the pilot filter challenge study and sorption experiments is illustrated in Figure 10. The antimicrobials used for experimentation were all of HPLC-grade quality and purchased from MP Biomedicals (Aurora, OH). Storage of the compounds was according to the manufacturer’s recommendations, and laboratory stock solutions were made monthly by dissolving 15 mg of each antimicrobial in 30 mL of methanol and refrigerating at 4°C.

Sulfonamides are broad-spectrum antimicrobials inhibiting both gram-positive and gram negative bacteria, and cross-resistance among the entire class of compounds is considered complete. The representative sulfonamides used for this study included two veterinary medications and one drug prescribed for human use. Sulfamethazine (CAS no. 57-68-1) is used in veterinary applications for control of livestock and poultry coccidiosis, and sulfamethoxazole (CAS no. 723-46-6) is commonly combined with trimethoprim (CAS no. 738-70-5) to treat human pneumonia and urinary tract infections (Fullerton, 1998). Lincomycin (CAS no. 154-21-2) is prescribed to treat human infections caused by gram-positive organisms, and has veterinary applications for growth promotion in swine and poultry. Tylosin (CAS no. 1401-69-0) is used to promote growth in cattle and treat poultry respiratory infections (VPB, 1997).
Figure 10  Pilot and laboratory study antimicrobial chemical structures.
Conclusions

- Slow sand filtration is still a viable method of water treatment most suitable for raw water sources low in turbidity and suspended solids. Pretreatment can expand the treatment method to include source waters with higher turbidity.

- Roughing filtration prior to slow sand filtration (multi-stage filtration) has been shown to be an efficient and effective treatment technique for source waters subjected to high sediment loads.

- Multi-stage filtration is used in rural regions that may be subjected to antimicrobial contamination from upstream waste discharges or diffuse pollution.

- Few organic contaminant studies and no quantitative antimicrobial removal efficiency studies have been conducted on multi-stage filtration systems.

- To keep the continued use of multi-stage filtration applicable to systems burdened with trace organic pollutants, research must be conducted that investigates the fundamental removal processes and efficiencies of these filtration techniques.
CHAPTER 2

ENVIRONMENTAL ANTIMICROBIAL CONTAMINATION FROM TERRACCUMULATION AND DIFFUSE POLLUTION PATHWAYS

Stephen J. Rooklidge

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Introduction

Amending farm fields with animal manure and processed biosolids from water and wastewater treatment plants (WWTP) is a common agricultural practice, and contaminants sorbed to these amendments may concentrate in soil over time. This *terraccumulation* of sorbed compounds provides a reservoir of pollutants that can mobilize in soil and leach to groundwater or be carried by seasonal erosion to surface water (Novotny, 2003; Pedersen et al., 2003). Antimicrobials contained in manure and biosolids may enhance selection of resistant bacteria by entering the aquatic environment through pathways of diffuse pollution (USEPA, 2002); adding to selective pressure exerted by discharge of antimicrobials from point sources such as WWTP outfalls (Levy, 1998). Surface water and shallow groundwater are commonly used for drinking water, and antimicrobials are now found to pollute many aquatic sources. The potential exists for proliferation of resistant bacteria and human consumption of antimicrobials below therapeutic levels to decrease efficacy of drug therapies used against infectious diseases (Witte, 2000; Levy, 2002).

Antimicrobial chemotherapy has been an important medical development since the first investigations of antibacterial dyes by Ehrlich in the beginning of the twentieth century. However, by the late 1940's, bacteria resistant to antimicrobials were soon recognized as serious problems in clinical environments, such as hospitals and care facilities (Martin, 1998). Hospitals are expected to experience the most radical change of resistance
because abundance of pathogenic organisms and antimicrobial compounds produce a ripe breeding ground for bacteria insensitive to even the most effective course of therapy.

Until the mechanisms of bacterial resistance are fully understood, the economic impact of increased human infection rates can only be estimated in clinical situations. McGowan (2001) investigated economic costs associated with treating clinical patients having antimicrobial resistant bacterial infections, and his conclusions anticipated the impacts beyond the US $4 to 5 million estimated by the Institute of Medicine (1998). Bacterial resistance forces the research community to develop methods of altering antimicrobial structures that allow the compounds to avoid inactivation, yet structural modifications alone are not enough to avert bacterial resistance. The expanding use of household antibacterial products and agricultural antimicrobials foster cross-resistance to drugs specific for human therapy, and may have the greatest consequences to children and the elderly (Levy, 2001; Shea, 2003).

Many excellent reviews of the current state of knowledge are available that summarize bacterial resistance development and transfer (Witte, 2000; Teuber, 2001; White et al., 2002; McDermott et al., 2003), but the importance of environmental pollution pathways to the proliferation of resistant bacteria has only recently received attention. The extent of bacterial resistance caused by trace antimicrobials in the environment has yet to be clearly defined, and contributions from agriculture are debated and often contradictory (Witte, 1998; Aarestrup and Wegener, 1999; Piddock et al. 2000; Schwarz et al., 2001). The benefits of reducing antimicrobial resistance are difficult to quantify in
environmental studies because the ability for controlled comparison is limited and diversity in natural systems present unique scientific obstacles. Levy (2002) suggested the problems of clinical and environmental bacterial resistance were best approached by the scientific community addressing each defined issue individually, instead of disparate scientific fields jumping between emerging priorities. Boxall et al. (2003) proposed a method to prioritize research of veterinary drugs entering soil and aquatic systems by using data collected from environmental sampling and pathway mobility experiments, but relevant data was insufficient to investigate many currently used antimicrobials.

The examples of contamination routes illustrated in Figure 11 are pathways between the use and disposal of antimicrobials. This figure is a variation of a complex web focusing on routes of contamination related to human food and water consumption (Jørgensen and Halling-Sørensen, 2000). The cycle of contamination and consumption connects each component of the web to form a pool of resistant bacteria and antimicrobial pollution exerting selective pressure that may threaten the future effectiveness of human therapeutic drugs.

The focus of this review is to examine how common agricultural and water treatment practices contribute to antimicrobial contamination in environmental systems. Each pollution pathway presents scientific and engineering challenges and opportunities that are still largely unexplored, but evaluation of human health risks from rising trends of resistance must include antimicrobial use, abuse, disposal, and persistence in
environmental systems (Kümmerer, 2003). The results of careless antimicrobial administration, inadequate treatment of contaminated drinking water, and the ultimate effects of antimicrobial use in agriculture may present global consequences in the future. Although comprehensive action by the research community may be difficult to justify before economic benefits are completely elucidated, recent scientific results from diverse fields presented in this review indicate the extent of antimicrobial contamination and resistant bacteria is not limited by geographic region or economic circumstance.

![Diagram of antimicrobial pollution pathways](image)

**Figure 11** Antimicrobial pollution pathways associated with food and water consumption.

**Global Trends of Antimicrobial Resistance**

Widespread use of a new antimicrobial is commonly followed by emergence of bacteria no longer sensitive to the compound, and this response drives the search for new and effective therapeutic drugs. Bacterial resistance to antimicrobials can occur in several
ways. Exposure to antimicrobials may initiate intrinsic processes or mutations within a microorganism that reduce susceptibility to destructive mechanisms of the compound, and bacteria can acquire resistance from other cells by direct transfer of genes coded for resistance. Selective pressure from antimicrobial exposure fosters dominance by resistant bacteria, but introduction of resistant strains to a sensitive population may disseminate resistance prior to any antimicrobial exposure (Lipsitch and Samore, 2002).

The loss of bacterial sensitivity to antimicrobial therapies results in a greater reliance on synthetic antimicrobials new to the market. McCaig et al. (2003) identified a significant decrease in effectiveness of older antibiotics, such as ampicillin and cephalosporin, and a greater use of quinolone and macrolide antimicrobials that were once considered a last line of defense against resistant bacterial strains. *Streptococcus pneumoniae* is estimated to cause one million deaths a year, but this microbe's susceptibility to fluoroquinolones has significantly decreased in New York, U.S. (Quale et al., 2002). A Pennsylvania study found no fluoroquinolone resistant *Campylobacter jejuni* in the state health care system between 1982 and 1992, but resistant isolates were discovered in over 40% of tested patients by 2001 (Nachamkin et al., 2002). The dramatic three-year rise of fluoroquinolone resistant *E. coli* in a Dutch hospital indicated the problem of antimicrobial resistance is not unfamiliar to Europe (van Belkum et al., 2001), and these findings were supported by Finnish travelers returning to their home country with a 20% rise of fluoroquinolone resistant *C. jejuni* within five years (Hakanen et al., 2003).
The presence of enterococci isolates resistant to vancomycin did not appear significant in a recent Croatian study (Andrasevic et al., 2002), but 22% of methicillin resistant S. aureus and 38% of S. pneumoniae isolates also proved resistant to penicillin. S. aureus has been shown to be susceptible to vancomycin resistance transfer from enterococci, and reduced effectiveness of multiple antimicrobials is not uncommon (Huycke et al., 1998). McGeer and Low (2003) investigated percentages of bacteria resistant to macrolide and penicillin by geographic region, and McCormick et al. (2003) used mathematical models to predict dual drug resistance of S. pneumoniae isolated at eight sites around the U.S. could exceed 41% by 2004.

The encouraging slow rise of beta-lactam resistant S. pneumoniae and fluoroquinolone resistant E. coli in the British Isles during the 1990’s (Speller et al., 1996; Livermore et al., 2002) and the decrease of methicillin resistant S. aureus in French hospitals may not be reflected among nations of the developing world (Aubry-Damon and Courvalin, 1999). Nigerian students were tested for E. coli isolates resistant to four common antimicrobials and resistance to tetracyclines increased from 35 to 100% between 1986 and 1998 (Okeke et al., 2000). At the beginning of the study only 30% of the isolates were susceptible to antimicrobials, but the last year of the project revealed all isolates were resistant to at least one of the tested drugs.

Okeke et al. (1999) reviewed human social practices that create antimicrobial resistance in developing countries. Resistant bacteria gain competitive advantage over susceptible
populations in the presence of antimicrobials, and inappropriate self-medication is common in developing nations that allow sale directly to the public or untrained personnel dispensing drugs. Ingestion of substandard, counterfeit, improperly stored, or sub-therapeutic doses of antimicrobials leads to ineffective or incomplete treatment that increases the pressure of resistant strain selection, and excreted compounds and metabolites are often distributed into the surrounding environment by inadequate waste disposal or unhygienic sanitary conditions.

Many strategies have been suggested to diminish or delay the impacts of bacterial resistance in clinical environments. Collaboration with pharmaceutical producers to educate clinicians and the public about judicious use of antimicrobials has become an important tool for the health community (Epstein, 1995). Huycke et al. (1998) concluded improved surveillance of multi-drug resistance events would alert clinicians to therapy vulnerability, and Scheld (2003) suggested prescribing antimicrobials only when necessary and after determination of an optimal course of therapy. Fridkin et al. (2002) found decreasing rates of antimicrobial administration in hospital intensive care units curbed the prevalence of vancomycin resistant organisms. Stratton (2003) defended the strategy of changing first line drugs from bacteriostatic to bactericidal to slow the spread of resistance by killing the target organism before mutated strains can develop, but increasing resistance to fluoroquinolones makes clinical use of sufficient doses to destroy resistant strains untenable. The potential exists for older, less prescribed antimicrobials to once again be used in clinical therapy, and even expanded to what is now considered
extra-label administration. This "rotation cycle" may keep antimicrobials effective in the past from achieving a useless status (Walsh, 2000).

Viewed as a strategic effort by the public health community to maintain antimicrobial effectiveness, clinical approaches may hold important implications for reduction of bacterial resistance on a global scale. Education of prudent antimicrobial use, identification of pathways and extent of contamination, and remediation of impacted systems are methods that would assist environmental scientists and engineers to ameliorate effects of antimicrobial contamination before eventual consequences are fully realized.

Antimicrobials in the Environment

Environmental antimicrobial resistance studies have been conducted for several years, but lowered analytical method detection levels have expanded investigations of trace contaminant concentrations to complex environmental matrices. A major nation-wide surface water study by the U.S. Geological Survey indicated pollutant concentrations in 139 streams included a range of antimicrobial compounds used in human and animal therapy, with elevated detection frequencies of sulfamethoxazole, tylosin, and trimethoprim (Kolpin et al., 2002). Two reaches of an Italian river were estimated to have peak oxytetracycline loads flowing at 4 mg/s (Calamari et al., 2003), and Golet et al. (2002) found fluoroquinolone concentrations of 19 ng/L in a Swiss river system.
Evidence of antimicrobial pollution is not restricted to surface water, however, as Sacher et al. (2001) found maximum sulfamethoxazole concentrations approaching 410 ng/L in 10% of tested German groundwater wells.

Bacteria populations exist which are intrinsically resistant to antimicrobials, and exposure to antimicrobials may induce genes coded for resistance that are subsequently transferred to other members of the microbial community. Ash et al. (2002) described sixteen U.S. rivers where over 40% of bacteria resistant to one or more antimicrobials had at least one plasmid coded for resistance, and genes resistant to ampicillin were detected in 70% of the isolated plasmids. This study built upon earlier research that found 21 river sites produced bacteria between 4 and 59% resistant to ampicillin (Ash et al., 1999). Twenty-four percent of surface water Salmonella strains tested in Greece exhibited resistance to at least one of 20 antimicrobials, and 26% were able to transfer resistance to E. coli (Arvanitidou et al., 1997). Almost 54% of coliform isolates collected from a Korean river were resistant to at least one of the tested antimicrobials (Park et al., 2003).

The environmentally pervasive consequences of antimicrobial resistance are even being used to differentiate between human and animal sources of fecal contamination in aquatic environments (Graves et al., 2002). Application of statistical techniques to estimate fecal contributions from diffuse pollution has been successfully used to characterize aquatic systems on the watershed scale (Wiggins et al., 1999; Whitlock et al., 2002) in the same manner as point source identification in marine environments (Choi et al., 2003).
In aquatic and soil systems, as in clinical environments, contributions to selective pressure from antimicrobial contamination have yet to be fully determined because the complexity and abundance of bacteria species precludes identification of a single definitive causal relationship. However, constriction or elimination of pollution pathways may reduce resistance proliferation by limiting introduction of contaminants and resistant organisms to susceptible bacterial populations.

Antimicrobials in Water and Waste Treatment

Toxicological effects of human consumption of trace antimicrobials in drinking water and agricultural products are beyond the scope of this review, but Stuer-Lauridsen et al. (2000) concluded the lack of toxicity data and analysis of environmental contamination were the largest obstacles to obtaining accurate environmental and human health risk assessments. Initial aquatic risk assessments of pharmaceuticals have been completed in three recent studies (Halling-Sørensen et al. 2000; Golet et al., 2002; Calamari et al., 2003), using risk quotients comprised of predicted, measured, or predicted no-effect contaminant concentrations. The predicted concentration of contaminant was calculated by pharmaceutical sales in the geographic region or estimated contributions to aquatic systems from WWTP. Jones et al. (2002) used drug prescription and available toxicity data of the top 25 pharmaceuticals sold in England to estimate risk quotients, and found amoxicillin and oxytetracycline antimicrobial concentrations exceeded no-effect levels. Their models for predicted concentrations assumed no contaminant adsorption during
sewage treatment, and the WWTP outfall was the main point of contaminant entry to the environment. Schowanek and Webb (2002) used a model relying on both deterministic WWTP descriptions and stochastic hydrologic input to predict environmental concentrations of several pharmaceuticals, and contributions from terraccumulation and diffuse pathways may be a future refinement.

Entry of antimicrobials into streams and rivers from human wastewater treatment plant effluent is the most direct route of contamination, and municipalities with hospitals may send both antimicrobials and resistant bacteria discharging to surface receiving waters. *E. coli* in effluent from modern German activated sludge sewage treatment plants were found to be resistant to several antimicrobials (Reinthaler et al., 2003), and selected Swiss WWTP effluent contained up to 87 ng/L of fluoroquinolone (Golet et al., 2003). Military facilities not only provide crowded conditions that encourage resistant strain proliferation, but may produce antimicrobial-laden waste effluents (Gray et al., 1999). The prophylactic use of antimicrobials during bioterrorism threats described by Shepard et al. (2002) could send large concentrations of these pollutants into military or municipal WWTP and eventually to receiving waters used for drinking water sources.

When sludge from modern waste treatment facilities is processed and land-applied as biosolids, the majority of bacteria contained in the waste may be destroyed, but remnants of sorbed contaminants may not have been eliminated during waste treatment. Ozonation of wastewater was recommended to increase the degradation of veterinary and human
antimicrobials (Balcioglu and Ötker, 2003), and Hartig et al. (2001) suggested membrane filtration of tertiary effluent provided enhanced adsorption of sulfonamides to activated carbon. Removal of sulfonamides and fluoroquinolone antimicrobials in wastewater treatment batch reactors was studied by Huang et al. (2002), and strong sludge sorption and inactivation by chlorine provided almost 90% removal. Fluoroquinolone reduction in Swiss WWTP was estimated to be between 88 and 92%, with sludge sorption identified as the primary treatment mechanism (Golet et al., 2003), but Halling-Sørensen et al. (2000) concluded the fluoroquinolone ciprofloxacin was not strongly sorbed to activated sludge. Biodegradation studies have been conducted on fluoroquinolones originating from WWTP outfalls (Kümmerer et al., 2000; Wilson et al., 2003), but few other antimicrobial contaminants have been investigated. These studies may eventually lead to optimized treatment systems compatible with current engineering practices, but rarely have research studies considered sorbed antimicrobial mobilization to surface or groundwater after biosolid land application.

Waste sludge is also generated during drinking water treatment, and the biosolids are deposited in landfills or spread on agricultural soils. There is a scarcity of studies investigating the fate of antimicrobials in drinking water treatment systems. Ternes et al. (2002) investigated removal of non-antimicrobial pharmaceuticals in ozonation and activated carbon drinking water treatment processes, and Huber et al. (2003) confirmed oxidation of pharmaceutical compounds as an effective treatment method. Adams et al. (2002) reported common treatment processes, such as softening and coagulation, have
sulfa drug removal efficiencies far lower than ozonation, activated carbon adsorption, and reverse osmosis. However, these modern treatment techniques are not globally applied—especially in the developing world. Although a review by Heberer (2002) concluded many pharmaceutical compounds are efficiently removed by drinking water treatment processes, removal of contaminants from aqueous solution does not diminish potential effects of sorbed antimicrobials to agricultural soil ecosystems. Figure 12 illustrates pathways contributing to antimicrobial terraccumulation, where water and wastewater treatment biosolids are combined with contaminated irrigation water and manure in agricultural soils.

Figure 12 Sources contributing to antimicrobial terraccumulation in agriculture. Shaded arrows represent potential relative contaminant contributions from various pathways.
Drinking water and wastewater treatment processes need continued research to develop effective organic contaminant remediation methods that restrict introduction of antimicrobial compounds to the environment. Water treatment methods common in rural regions and the developing world are among the last to be adequately improved because many techniques, such as slow sand filtration, were not designed to treat organic pollutants developed during the previous century. These systems are simple and require little operator knowledge to be effective against suspended sediments generated from agriculture or seasonal erosion (Visscher et al., 1987), but high bacterial density in filter biomass increases the likelihood of resistant gene transfer (Schwartz et al., 2003). The waste biomass is not treated before land application, and additional processes are needed to destroy resistant organisms and inactivate antimicrobial and metabolite contaminants. Continued research is warranted because restoration of antimicrobial bioactivity may occur in waste applied to neutral or weakly-acidic soils (Halling-Sørensen, 2000).

Traditional wastewater treatment system removal efficiencies do not consider the fate of sorbed contaminants once the sludge leaves the facility. The use of treatment facility degradation efficiencies in infrastructure design and environmental risk assessment would accurately represent facility contribution to diffuse pathways. Degradation efficiencies are determined by laboratory and field evaluation of the loss of antimicrobial bioactivity during the treatment process. Remaining effluent aqueous concentrations and potentially-mobile sorbed antimicrobials would then be used to refine predicted concentrations in environmental risk models.
Antimicrobials in Agriculture

Feeding antimicrobials to livestock and poultry to reduce disease and promote weight gain has been standard practice in developed countries for several decades. Animal wastes are commonly stored in lagoons and eventually spread on agricultural soils. The Union of Concerned Scientists estimated U.S. livestock producers use approximately 11,200 tonnes of growth-promoting antimicrobials annually. Although the Animal Health Institute concluded less than 40% of antimicrobials produced are similar to those used for human therapy (Wadman, 2001), antimicrobial exposure can initiate bacterial resistance to compounds of dissimilar structures (Courvalin, 2001). Tilman et al. (2002) argued the spread of antimicrobial resistance places the ultimate cost of using agricultural growth-promoting feed additives beyond an economic balance regarded as sustainable. Some farm industry representatives insist restriction of growth-promoting antimicrobial feed would raise meat prices and potentially endanger the consumer, but Evans and Wegener (2003) found no significant change in broiler chicken Campylobacter populations and an actual decrease of Salmonella prevalence after Denmark ceased the use of many poultry and cattle growth-promoters.

Limitations of available feed additives may lead to increased use of water-soluble antimicrobials not specific for livestock. This extra-label use can be illegal in therapeutic applications, but few antimicrobials are produced in water-soluble form and inappropriate administration is difficult to regulate (Almond and Monahan, 2000). Farm water
medication devices are susceptible to potential backflows into drinking water distribution systems. Drinking water trough leakage is another pathway of contamination to the surrounding environment.

Antimicrobial exposure and resistant bacteria transfer from animals to humans are potential threats to farmers and processors. Hamscher et al. (2003) reviewed pathways of air-borne antimicrobial contaminants in pig farms, and Zahn (2001) found air-borne tylosin concentrations of 8 ng/L in a ventilated swine facility, with 80% of the ventilation stream culturable bacteria resistant to tylosin. Studies by Schroeder et al. (2002) examined poultry and livestock E. coli isolates that showed a sulfamethoxazole resistant phenotype evident in turkeys, and 71% of the chickens studied exhibited resistance to streptomycin. Greater than 50% of the chicken carcasses containing Salmonella delivered to Greek hospitals were resistant to at least one antibiotic (Arvanitidou et al., 1998), and 27% of enterococci isolated from Spanish chicken products were resistant to vancomycin (Robredo et al. 2000). Hooper (2001) suggested human population reservoirs of fluoroquinolone resistant E. coli developed from strains selected in food animals.

Using livestock and poultry growth-promoting antimicrobials selectively breeds resistant organisms having the potential to affect humans working in the food-producing industry, and the threat of spreading resistance to human-borne organisms caused many European countries to limit agricultural use of growth-promoting antimicrobials. Engberg et al.
(2001) investigated *Campylobacter* resistance trends to the macrolide and quinolone classes of antimicrobials used in food animal production, and DNA profiling of *C. jejuni* identified common genotypes among humans, livestock, and poultry. Their conclusions identified trends of resistance formation in *Campylobacter* that followed agricultural use more closely than the administration of quinolones in human therapy. Although Padiglione et al. (2000) found no link between application of the growth-promoter avoparcin and the persistence of vancomycin resistant enterococci implied in previous research, Borgen et al. (2000) concluded the prevalence of vancomycin resistant strains in Norwegian broiler farms was a consequence of previous avoparcin use. Bogaard et al. (2001) observed common patterns of *E. coli* resistance between poultry and the farmers producing the birds for consumption.

Aquaculture has relied heavily on antibiotic feed additives in farmed fish production to reduce infection and promote fish growth. Early studies by Jacobsen and Berglind (1988) observed sorbed antimicrobial concentrations greater than 4 mg/kg below a marine fish farm. In a recent study of several U.S. land-locked fish farms, Thurman et al. (2002) detected sulfa and tetracycline antibiotics in greater than 14% of samples, and it was assumed these occurrences were due to medicated feed additives.

Development of analytical methods and quantification of antimicrobial contaminants in agriculture are ongoing research efforts, and a comprehensive review is provided by Thiele-Bruhn (2003). Haller et al. (2002) measured sulfonamide concentrations
approaching 20 mg/kg in cattle and pig manure pits. Thirteen U.S. hog farm lagoons showed tetracycline concentrations approaching several hundred µg/L, and chlortetracycline contamination was found in hog lagoon and surface water samples (Meyer et al., 2000). Liquid manure spread on German farm fields was found to contain 4 mg/kg tetracycline, and pasture soil samples revealed 0.86 mg/kg tetracycline in the top 10 cm of soil more than a year after application (Hamscher et al., 2002). Rabølle and Spliid (2000) observed no significant oxytetracycline mobility and weak tylosin mobility in laboratory experiments of Danish agricultural soils, and these results were in accord with earlier studies of oxytetracycline persistence in marine sediments (Samuelsen, 1989).

Partitioning to organic matter in soil and sludge may prove a useful mechanism to model mobility of many antimicrobials (Khan and Ongerth, 2002), as Loke et al. (2002) found sorption of tylosin to pig manure was adequately explained by hydrophobic reactions with organic matter. However, tetracyclines are primarily influenced by complexation with divalent cations (Lunestad and Goksøyr, 1990), and persistence in soil interstitial water may require alternative models of contaminant mobility to surface and groundwater supplies (Halling-Sørensen et al., 2003). Field studies by Boxall et al. (2002) found a sulfonamide applied to clay soil mobilized to a surface water pathway, but had low potential for groundwater contamination in sandy soil. Hirsch et al. (1999) concluded antimicrobials in veterinary applications were of minor importance to groundwater contamination because only two tested samples from wells beneath agricultural fields
presented detectable concentrations, but Campagnolo et al. (2002) found tetracycline concentrations greater than 1 μg/L in wells, springs, and streams proximal to poultry farms. Yang and Carlson (2003) detected a substantial increase of the tetracycline class of antimicrobials along the flow path of a western U.S. river, and lincomycin concentrations approaching 250 ng/L were found in rivers near Italian rural communities (Calamari et al., 2003); both studies assumed the contamination was a result of agricultural practices.

Golet et al. (2003) estimated terraccumulation of fluoroquinolone at maximum biosolids application rates would exceed regulatory requirements of the European Union, but the ultimate effects of persistent land-applied antimicrobials on soil bacteria remain inadequately explored. E. coli and enterococci survived more than 60 days in soil amended with pig manure (Cools et al., 2001), and Sengeløv et al. (2003) observed a temporary increase of tetracycline resistance in bacteria isolated from pig manure-amended soils lasted five months. Although Teeter and Meyerhoff (2003) considered tylosin rapidly degraded, significantly higher proportions of tylosin resistant bacteria were isolated from field soils amended with manure containing the growth-promoting antimicrobial (Onan and LaPara, 2003). De Liguoro et al. (2003) suggested tylosin terraccumulation would be minimized by allowing manure maturation greater than five months prior to land application.
Molecular farming of crops to produce pharmaceuticals has grown in interest because of the potential to manufacture inexpensive antimicrobials, but unregulated disposal of plant residues may add to the overall load of contaminants in agricultural soil. The possibility for resistance transfer and contaminant entry to the environment by diffuse pathways could certainly be increased (Kirk, 2001), and consequences to non-target crops are poorly understood. Jjemba (2002) suggested a standardized reporting system of pharmaceutical uptake in plants would aid future research, but antimicrobials have varied mechanisms of sorption that may prevent normalization to a universal distribution coefficient relevant to ecosystems (Tolls, 2001). An aquatic fern had the capacity to uptake sulfonamide contaminants, and this research indicated promise for phytoremediation of antimicrobials (Forni et al., 2002). However, Migliore et al. (2003) observed both toxic and hormetic affects in crop plants exposed to enrofloxacin, and a significant plant conversion of the agricultural drug to its metabolite used in human therapy (ciprofloxacin) adds still another potential pathway to sub-therapeutic consumption of antimicrobials.

Terraccumulation of antimicrobials from contaminated agricultural amendments affects soil and aquatic ecosystems in ways that have only recently seen investigation. There is a general consensus that the spread of bacterial resistance and its concomitant threat to human health is currently considered the most significant potential impact of antimicrobial mobilization through diffuse pathways.
Conclusions

- The proliferation of resistant bacteria and diminishing effectiveness of human therapeutic drugs reach far beyond the geographic origin of antimicrobial compounds, and these consequences present a truly global concern.

- The focus on reducing contaminant concentrations in water destined for human consumption may enhance proliferation of bacteria species insensitive to antimicrobials by spreading inadequately treated waste into disparate geographical regions.

- To determine true antimicrobial contaminant removal from the cycle of pollution, analytical methods quantifying effluent concentrations, degradation, and mobility in amended soils must be applied to wastewater treatment processes. Engineers and water purveyors need to account for all pollution pathways in the design of treatment plants and rehabilitation of older facilities.

- Although terraccumulation and diffuse pathways contribute to antimicrobial residues found in surface and groundwater, recent human and aquatic organism risk assessments rarely include this phenomenon.

- Collection of pharmaceutical sales data by geographic region and regulations curbing extensive agricultural use of growth-promoting antimicrobials have been implemented in several countries, but are unlikely to be instituted in the United States within the near future. Current practices may be altered if local economic benefits of growth-promoting feed additives include impacts along other routes of the diffuse pollution cycle.

- Strategies used by the health community to maintain effectiveness of human drug therapy, such as public education, judicious use of antimicrobials, identification of contaminant pathways, and investigation of resistant bacteria proliferation, are applicable methods for the environmental scientific and engineering communities to examine global antimicrobial contamination before eventual consequences are fully realized.
CHAPTER 3

MODELING ANTIMICROBIAL CONTAMINANT REMOVAL IN SLOW SAND FILTRATION

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Nomenclature

\( V_w \) \hspace{1em} \text{Supernatant volume} \\
\( V_i \) \hspace{1em} \text{Sand column interstitial water volume} \\
\( Q \) \hspace{1em} \text{Filter water flow} \\
\( C_A \) \hspace{1em} \text{Supernatant aqueous antimicrobial concentration} \\
\( C_s \) \hspace{1em} \text{Supernatant sorbed antimicrobial concentration} \\
\( C_M \) \hspace{1em} \text{Sand column aqueous antimicrobial concentration} \\
\( S_T \) \hspace{1em} \text{Total supernatant solids} \\
\( S_V \) \hspace{1em} \text{Volatile supernatant solids} \\
\( f_{IM} \) \hspace{1em} \text{Mass fraction of inorganic matter} \\
\( f_{OM} \) \hspace{1em} \text{Mass fraction of organic matter} \\
\( \rho_s \) \hspace{1em} \text{Sand media density} \\
\( K_p \) \hspace{1em} \text{Organic matter partitioning coefficient} \\
\( K_C \) \hspace{1em} \text{Clay adsorption coefficient} \\
\( K_s \) \hspace{1em} \text{Sand adsorption coefficient} \\
\( K_P \) \hspace{1em} \text{Photolysis rate coefficient} \\
\( n \) \hspace{1em} \text{Sand media porosity} \\
\( \alpha \) \hspace{1em} \text{Filter surface area perpendicular to flow} \\
\( \beta \) \hspace{1em} \text{LETA code photolysis constant} \\
\( U \) \hspace{1em} \text{Interstitial water velocity} \\
\( t \) \hspace{1em} \text{Time} \\
\( x \) \hspace{1em} \text{Length of sand column layer} \\
\( M_S \) \hspace{1em} \text{Antimicrobial sorbed to influent solids} \\
\( M_{SS} \) \hspace{1em} \text{Antimicrobial sorbed in top centimeter of sand column} \\
\( J_T \) \hspace{1em} \text{Net transfer rate from aqueous to sorbed phase}
Introduction

Discoveries of antimicrobial compounds in the aquatic environment have recently become an issue of concern because increased bacterial resistance to human therapeutic drugs may occur from exposure to resistant microorganisms and consumption of antimicrobials in drinking water. Full assessment of human health impact and risk from environmental antimicrobial pollution requires investigation of the fate and removal efficiencies of these compounds in water treatment facilities using contaminated source water.

In veterinary therapeutic applications and prophylactic antimicrobial administration for livestock and poultry growth-promotion, varying percentages of parent compound are excreted through animal manure (Jjemba, 2002). This manure is commonly stored in lagoons until spread on agricultural land as a soil amendment, in a manner similar to land application of biosolids from wastewater treatment facilities. Antimicrobial residue in treatment facility effluent has been regarded as the primary contributor to aquatic contamination in recently published environmental risk models (Golet et al., 2003; Calamari et al., 2003). However, mobility of sorbed antimicrobials in waste sludge applied to agricultural land has yet to be considered with the same scrutiny as manure-bound veterinary antimicrobials.
Land application of animal manure and biosolids may distribute antimicrobial contaminants into surface and groundwater through diffuse pollution pathways (Rooklidge, 2004). Although Hirsch et al. (1999) concluded agricultural antimicrobial use had low impact on the groundwater wells investigated, Campagnolo et al. (2002) discovered tetracycline in wells, springs, and streams near poultry farms and significant increases of antimicrobial concentrations were found downstream of agricultural communities in the U.S. (Yang and Carlson, 2003) and Italy (Calamari et al., 2003). A nationwide reconnaissance of U.S. surface water streams indicated the presence of several classes of antimicrobial compounds, including quinolones, tetracyclines, and macrolides (Kolpin et al., 2002).

Rural water treatment systems in industrial and developing nations are especially vulnerable to organic contaminants because treatment methods may not include remediation techniques common in larger municipal water systems. Slow sand filtration of lake or river water is one of the most effective drinking water treatment techniques available for rural regions (Logsdon et al., 2002). The treatment method has been shown to be successful for herbicide contaminant removal (Woudneh et al., 1997), yet ineffective for removal of certain polar pharmaceuticals (Ternes et al., 2002).

During slow sand filtration, raw water flows by gravity through a column of sand and layer of support gravel and flows out an underdrain collection grid to the treated water storage and distribution system. As particulate matter accumulates on the sand column
and biomass grows at the sand/water interface, permeability is reduced resulting in increasing supernatant volume. Filter media cleaning is required when a critical headloss is reached, and consists of draining the filter and removing the top layer of sand and biomass (schmutzdecke) before the filter is returned to service. Water treatment facilities can generate up to 1000 tonnes/yr of schmutzdecke waste for every hectare of filter area, and removed schmutzdecke may be applied to agricultural soils or washed to reclaim the sand for further use. Antimicrobial contaminant behavior in slow sand filtration, sorption and accumulation in land-applied schmutzdecke, and potential impacts to terrestrial and aquatic systems have yet to be investigated.

The LETA computer model was developed to simulate removal behavior of the tetracycline, quinolone, and macrolide classes of antimicrobials within slow sand filters. These classes are representative of common human and veterinary antimicrobials, and sorption and photolysis coefficients applicable to this model have been previously published. Specific objectives of this research were to: develop a simple predictive model for antimicrobial contaminant removal in slow sand filters using parameters easily monitored by water system operations personnel; estimate schmutzdecke antimicrobial concentrations at the end of the filtration period; and verify results by analyzing extracted schmutzdecke macrolide concentrations from a pilot filter system using high performance liquid chromatography-tandem mass spectrometry (HPLC MS/MS).
Methods

To examine filter treatment mechanisms of antimicrobial contaminants, a numerical model was developed considering four separate but related compartments within a slow sand filter (Figure 13): the first is the supernatant volume, which is assumed to be well-mixed throughout the filtration period and increases with headloss between filter cleanings; the second and third compartments are comprised of the accumulated organic and inorganic matter in the schmutzdecke; and the fourth is the sand column.

![Diagram](image)

Figure 13 LETA model components and state variables of total antimicrobial contaminant mass in supernatant (T); aqueous mass entering the sand column (A); mass lost to photolysis (F), hydrolysis (H), and biodegradation (B); mass sorbed to influent solids represented by organic matter (OM) and inorganic clays (IM); and contaminant sorbed to sand media (S) and biomass growing on the media (P).
For many classes of antimicrobials, the rate of contaminant mass accumulation in the supernatant may be represented as the total of influent aqueous and sorbed antimicrobial (T) less the rates of aqueous contaminant: flowing into the sand column (A); degraded by photolysis (F), hydrolysis (H), and biodegradation (B); and sorbed to influent solids represented by organic matter (OM) and inorganic clays (IM).

The change of aqueous and sorbed antimicrobial mass within the filter supernatant may then be described by advection/reaction equations (1) and (2) similar to the one-box model for sorbing species presented by Schwarzenbach et al. (1993).

\[
\frac{dC_A V_w}{dt} = Q_{in} C_A^{in} - Q_{out} C_A^{out} - r_F - r_H - r_B - J_T
\]  

(1)

\[
\frac{dC_S V_w}{dt} = Q_{in} C_S^{in} - Q_{out} C_S^{out} - r_{se} - r_F - r_B + J_T
\]  

(2)

Where \( C_A \) is the aqueous antimicrobial concentration, \( C_S \) is the concentration of antimicrobial sorbed to solid particulate matter within the raw water entering the filter, \( Q \) is the flow in and out of the supernatant volume \( (V_w) \), \( r_{se} \) is the average settling rate of solids, \( r_H \) is the rate of antimicrobial hydrolysis, \( r_F \) is the rate of photolysis, \( r_B \) is the rate of biodegradation, and \( J_T \) is the net transfer rate from the aqueous to sorbed phase.

The supernatant effluent flows into the sand column, where antimicrobial sorption/desorption occurs on sand media (S) and filter column biomass (P).
Contaminant sorbed to influent solids may eventually leave the filter system with the effluent or be trapped within the filter column. To determine the aqueous antimicrobial concentration at any time and depth of the slow sand filter, the supernatant model equations are combined with a one-dimensional plug flow equation for porous media presented by Schnoor (1996). Antimicrobial partitioning to dissolved matter and contaminant dispersion are neglected in the vertical-flow filter, and a system mass balance about an incremental control volume produces the differential form of equation (3) describing the aqueous concentration of antimicrobial contaminant in the sand media column ($C_M$).

\[
\frac{\partial C_M}{\partial t} = -U \frac{\partial C_M}{\partial x} - r_{PM} - r_{BM} - r_s - r_{HM}
\]  

(3)

Where $U$ is the interstitial water velocity through the sand column, $r_{PM}$ is the rate of partitioning into organic matter, $r_s$ is the rate of adsorption to sand media, and $r_{HM}$ and $r_{BM}$ are the rates of hydrolysis and biodegradation, respectively.

Slow sand filters are estimated to have less than 25% removal efficiency for dissolved organic matter and colloidal clay (Galvis et al., 1998), and removal of antimicrobials sorbed to these phases is expected to be correspondingly low. Distribution of antimicrobials sorbed to suspended solids may be estimated by adapting previous slow sand filter modeling research (Ohja and Graham, 1992, 1994), but the initial development of the LETA model assumes 100% solids removal efficiency at the schmutzdecke.
Antimicrobial persistence in aerobic soil systems is well-documented (Thiele-Bruhn, 2003), and persistence of antimicrobial residues in the aquatic environment suggests biodegradation by planktonic microorganisms would not be significant during the detention time of the filter system. Trace concentrations of antimicrobial residues in filter influent over a 60 to 90-day filtration period may result in microbial utilization of some contaminants, especially if source water biota is already acclimated to the presence of antimicrobials. However, aerobic degradation of many antimicrobials may not be relevant within the ~12-h slow sand filter detention period (Ingerslev et al., 2001; Teeter and Meyerhoff, 2003). Therefore, biodegradation of sorbed antimicrobials was not calculated from the predicted sorbed mass of contaminant, and the model results are regarded as conservative estimates in an idealized filtration system.

Using the simplifying assumptions below, the governing equations are derived with operational parameters and equilibrium coefficients found in published literature. A recent review of veterinary pharmaceutical adsorption behavior (Tolls, 2001) questions the use of classical hydrophobic organic compound assumptions for these contaminants ($K_{ow}, f_{oc}$, etc.), and this study does not consider adsorption and photolysis coefficients as definitive examples relevant to all conditions of surface water filtration. The use of published coefficients for initial model development is appropriate, as this study is the first to apply fundamental organic contaminant adsorption models to slow sand filtration in an attempt to predict antimicrobial removal behavior in the treatment method and identify future research needs.
Simplifying assumptions for removal of the tetracycline, quinolone, and macrolide classes of antimicrobials in the supernatant and sand column phases of the filter include:

1. Influent total solids are a combination of clay particles and organic matter that vary in concentration throughout the filtration period. The two components of the total solids are differentiated by the operational parameters of volatile and non-volatile mass fractions of the total solids concentration.

2. Influent aqueous antimicrobial contaminant concentrations are constant over a 60-day filtration period.

3. Sorption of antimicrobials to the organic and inorganic matter in the supernatant and sand media is a function of the aqueous concentration of antimicrobial, total solids concentration, and solids mass fraction.

4. Instantaneous and linear equilibrium is assumed to exist between the aqueous and sorbed phases of the antimicrobial concentrations.

5. Photolysis of susceptible antimicrobials is only applied to the aqueous phase, and occurs to an effective penetration depth of 10 cm within the supernatant volume for ten hours of each diurnal cycle.

6. Biodegradation of antimicrobials \((r_B, r_{BM})\) does not occur, and filter efficiency and headloss development are not inhibited by the contaminants.

7. Chemical hydrolysis \((r_{H}, r_{HBM})\) is neglected during the filter residence time, and the average settling rate \((r_{Set})\) of organic matter and clay particles is assumed to be negligible compared to the flow velocity within the well-mixed supernatant.

The supernatant water volume increases over the sand column surface area depending upon the accumulating headloss during the 60-day filtration period, and the change of volume corresponds to the difference of supernatant influent and effluent flow.

\[
\frac{dV_s}{dt} = Q_{in} - Q_{out} \tag{4}
\]
The photolysis reaction parameter for susceptible antimicrobial compounds \( (r_F) \) is modeled as the first-order rate of aqueous contaminant mass loss, where \( K_F \) is the photolysis coefficient and \( \beta \) is the LETA program constant used to satisfy assumption number 5.

\[
r_F = \beta K_F V_w C_A
\]  

(5)

The LETA model is designed to be used with a variety of ionizable antimicrobials that may have preferential interaction with only one of the two idealized organic and inorganic components of the influent solid matter. Therefore, the antimicrobial mass transfer rate term between aqueous and sorbed phases \( (J_T) \) is comprised of the two terms of equation (6) to represent mass sorbed to clay and organic matter.

\[
J_T = \frac{d}{dt} \left( K_C f_{IM} S_T V_w C_A + K_P f_{OM} S_T V_w C_A \right)
\]  

(6)

Where \( K_P \) is the organic matter partitioning coefficient, \( K_C \) is the clay adsorption coefficient, and \( f_{OM} \) is the solids mass fraction of organic matter estimated by the volatile solids concentration \( (S_v) \) divided by the total influent solids concentration dried at 105°C \( (S_T) \). Volatile solids are defined as the mass of solids lost at 550°C, and the fraction of inorganic matter \( (f_{IM}) \) is calculated using the mass remaining after volatilization \( (7) \).
Neglecting the settling, hydrolysis, and biodegradation terms; substitution of the transfer rate term, photolysis term, and \( Q_{\text{out}} \) from rearranged equation (4) into equation (1) presents the concentration of supernatant aqueous antimicrobial contaminant in equation (8), where \( N \) is defined by equation (9).

\[
\frac{dC_A}{dt} = (C_A^{\text{in}} - C_A)(V_wN)^{-1}Q_{\text{in}}
\]

\[
- \left[ \beta K_p + K_p \left( \frac{df_{OM}}{dt} S_T + f_{OM} \frac{dS_T}{dt} + f_{OM} S_T \frac{dV_w}{dt} V_w^{-1} \right) \right]
\]

\[
K_c \left( \frac{df_{IM}}{dt} S_T + f_{IM} \frac{dS_T}{dt} + f_{IM} S_T \frac{dV_w}{dt} V_w^{-1} \right)]C_A N^{-1}
\]

\[
N = 1 + K_p f_{OM} S_T + K_c f_{IM} S_T
\]

The concentration of influent sorbed contaminant is also split into two terms to represent the antimicrobial concentration sorbed to organic matter and clay.

\[
C_s^{\text{in}} = K_p f_{OM} S_T C_A^{\text{in}} + K_c f_{IM} S_T C_A^{\text{in}}
\]

Substituting terms of equations 4, 6, and 10 into the sorbed mass advection/reaction equation (2), and rearranging terms after applying the product rule, presents the equation for change of the supernatant sorbed contaminant concentration.
\[ \frac{dC_s}{dt} = (K_p f_{OM} S_f C_{A}^{in} + K_C f_{IM} S_f C_{A}^{in} - C_s)Q_{in} V^{-1} \]

\[ + K_p \left( \frac{df_{OM}}{dt} S_f C_A + f_{OM} \frac{dS_f}{dt} C_A + f_{OM} S_f C_A \frac{dC_A}{dt} + f_{OM} S_f C_A \frac{dV}{dt} V^{-1} \right) \]

\[ + K_C \left( \frac{df_{IM}}{dt} S_f C_A + f_{IM} \frac{dS_f}{dt} C_A + f_{IM} S_f C_A \frac{dC_A}{dt} + f_{IM} S_f C_A \frac{dV}{dt} V^{-1} \right) \] (11)

The filter media organic matter and sand adsorption reactions illustrated in the LETA model (P and S of Figure 13, respectively) are defined by the partitioning and adsorption terms of equation (3), and the differential form of the change of aqueous antimicrobial concentration within the sand column is presented by equation (12).

\[ \frac{\partial C_M}{\partial t} = -U \frac{\partial C_M}{\partial x} - K_p f_{OM} \rho_s \left( \frac{1-n}{n} \right) \frac{\partial C_M}{\partial t} - K_s f_{IM} \rho_s \left( \frac{1-n}{n} \right) \frac{\partial C_M}{\partial t} \] (12)

Where \( K_s \) is the adsorption coefficient of the sand media, \( \rho_s \) is the sand density, \( n \) is the sand media porosity, and the mass fractions of organic and inorganic matter vary throughout the depth of the sand column. The sand media interstitial velocity is defined by equation (13), where \( \alpha \) is the filter surface area perpendicular to flow.

\[ U = (Q_{in} - \frac{dV}{dt})(n \alpha)^{-1} \] (13)

The terms are rearranged and presented in the difference approximation of equation (14) used in the LETA model. The initial antimicrobial concentration at the top of the sand column is the aqueous concentration in the supernatant (\( x = 0, C_M = C_A \)).
An estimate of contaminant mass accumulating in the solids deposited on the schmutzdecke ($M_s$) is determined by the sorbed mass exiting the supernatant over the 60-day filtration period, calculated by integration of equation (15).

\[
\frac{dM_s}{dt} = \left( Q_{in} - \frac{dV_w}{dt} \right) C_s
\]  

(15)

The accumulating solids and the initial 0.01 meter of sand are removed during maintenance at the end of each filtration period. To account for the total sorbed antimicrobial removed, the results of equation (15) are added to the integration of the difference equation (16), which approximates the antimicrobial mass sorbed within the first centimeter of sand ($M_{ss}$) as the difference between the mass entering the sand column and mass at the 0.01-m depth.

\[
\frac{dM_{ss}}{dt} \approx \left( C_A - C_{M_{ss0}} \right) \left( Q_{in} - \frac{dV_w}{dt} \right)
\]  

(16)

Where the aqueous antimicrobial concentrations and water velocities are assumed to vary slowly relative to the time of travel through the schmutzdecke sand layer.
The final equations (4, 8, 11, 13, 14, 15, and 16) are simultaneously solved to produce the supernatant volume, aqueous concentration of the antimicrobial, and schmutzdecke contaminant mass accumulation at any time over a 60-day filtration period. The supernatant differential equations are solved using a fourth-order Runge-Kutta computer routine written in Visual Basic, with input parameters entered in the Excel spreadsheet (Microsoft, Redmond, WA) of the LETA program. Each sand column depth layer (Δx) is represented by a dedicated difference equation solved simultaneously with the supernatant equations to produce the concentration of contaminant at any time and position in the filter.

Temporal variation of the aqueous supernatant concentration is greatest during the initial loading of the filter because supernatant dilution volume is least at that time. Analysis of temporal discretization was performed for this period, and a series of incrementally smaller time steps (Δt) showed a convergence of supernatant concentrations at values less than 0.01 days (Appendix A). The lack of dispersion or diffusion considerations in the plug-flow sand column indicates the model will be insensitive to spatial discretization. Therefore, time steps of 0.002 days and depth layers of 0.1 m were used.

Initial sorption coefficients for natural organic matter, clay, and sand media are represented by parameters derived in earlier studies of tetracyclines, quinolones, and macrolides on similar materials (Table 6), within a range of pH values relevant to natural surface water.
Model operational parameters were derived from a 60-day antimicrobial challenge experiment on a pilot slow sand filter at the City of Salem, Oregon water treatment facility previously described by Rooklidge and Ketchum (2002a). The pilot filter was a 3-m tall polyethylene tank with a 1.2-m internal diameter (Poly Processing Co., French Camp, CA) fed river water at a rate of 0.15 m/hr from a constant-head reservoir. The 1-m deep basaltic sand media had a uniformity coefficient of 2.26, effective size of 0.30 mm, density of $10^{6.41}$ g/m$^3$, and porosity of 0.35. Operational parameters included supernatant headloss accumulation over a 60-day filtration cycle monitored daily; influent total and volatile solids analyzed from daily grab samples by Standard Methods (APHA, 1998); and mass fraction of organic matter within the sand column as a function of media depth, estimated by schmutzdecke volatilization and trends observed by Ellis and Aydin (1995). Initial investigation of the LETA model used linear interpolation between daily observed data points.

The macrolide antimicrobial tylosin was used to verify schmutzdecke sorption concentrations during the 60-day challenge experiment. Prior to the challenge study,
schmutzdecke samples were collected to investigate matrix interference and tylosin extraction efficiency. The pilot filter was run to waste for 60 days, drained, and 13 schmutzdecke samples were collected in a 20-cm center sample grid pattern to a filter bed depth of 1 cm. The schmutzdecke was dried and refrigerated at 4 °C until the sorbed organic material was extracted using the Soxhlet Method described by Aboul-Kassim and Williamson (2003). Extraction efficiency was determined by extracting 5 g schmutzdecke that had been dried, spiked with 15 µg of tylosin and 3 mM sodium azide in 30 mL of river water, and shaken for 24 hours. The efficiency was calculated by the difference between the initial concentration, aqueous concentration after 24 hours, and extracted concentration.

Tylosin tartrate (MP Biomedicals, Aurora, OH) was dissolved in 50 mL methanol and further diluted in distilled water brought to neutral pH with NaOH. The antimicrobial was injected into the filter influent pipe by peristaltic pump from 4-L clean amber glass bottles for a 1 µg/L final influent concentration. The tylosin was fed to the filter for 60 days, and schmutzdecke samples were collected on the final day and extracted as before. Comparison of predicted and extracted tylosin concentrations accumulating throughout the 60-day pilot study could not be conducted by intermittent sampling without impacting filter integrity, so extracted concentrations were only analyzed at the end of the filtration period. Extracts were dried under nitrogen, reconstituted in distilled water with 10% 2-propanol, and placed in glass vials for analysis by HPLC MS/MS.
A Luna C8(2) 3 µ 4.6 mm x 150 mm column was used (Phenomenex, Torrance, CA) with a Waters 2690 HPLC system (Milford, MA) and Quattro Micro mass spectrometer (Manchester, UK). Quantification of the tylosin mass transition (m/z 916 > 772) was calculated using MassLynx 4.0 software, which multiplied the peak area of analyte by the concentration/area ratio of an internal standard. Sulfadimethoxine was used as the internal standard during analysis (20 µg/L) because of the compound's stability and similar elution time. Tylosin concentrations were calculated from 6-point linear calibration curves using distilled water ranging from 0.5-500 µg/L, with coefficients of determination > 0.995. Recovery from standards made in distilled water ranged from 93-110%. The sample injection volume was 350 µL at a mobile phase flow rate of 0.35 mL/min. The mobile phase consisted of 2-propanol (A) and distilled water with 0.6% formic acid for a pH of 2.5 (B). The mobile phase gradient was 10% mobile phase A increased to 100% by 13 minutes, kept at 100% for 2 minutes, decreased to 10% by 18 minutes, with another 7 minutes of equilibration time.

Results and Discussion

Using the filtration period daily data or linear interpolation between observations, and the contaminant parameters listed in Table 6, the modeled concentration of aqueous tetracycline at various depths of the filter column throughout the 60-day simulation is presented in Figure 14a, and quinolone response exhibited similar behavior. The same filter and influent parameters were again used as LETA inputs, with the exception of
appropriate sorption and photolysis coefficients, to generate the results of macrolide response during the filtration period (Figure 14b). Under the conditions of simulation, tetracyclines, quinolones, and macrolides were predicted to have greater than 4-log removal in the top 40 cm of the slow sand filter, with very low mobility exhibited in the sand column. These results are in agreement with the Rabølle and Spliid (2000) sandy soil column studies, that found greater than 98% removal when aqueous oxytetracycline and tylosin concentrations fell below the analytical detection limits in a 30 cm column.

Figure 14  Aqueous tetracycline and quinolone (a) and macrolide (b) concentrations (g/m³) at various depths of the sand column during a 60-day simulation period.
At the end of the simulated filtration period, quinolone sorption to the removed layer of schmutzdecke (8.9 mg/kg) was comparable to concentrations found in municipal wastewater sludge (Golet et al., 2003). Antimicrobial concentrations estimated by the LETA model may be great enough to inhibit schmutzdecke growth and affect slow sand filter performance because similar quinolone concentrations have been found to alter the diversity of surface water algal species (Wilson et al., 2003). Simulated schmutzdecke sorption of tetracyclines (7.5 mg/kg) was the same magnitude concentration as land-applied wastewater sludge observed by Hamscher et al. (2002). The results for tetracyclines were above concentrations found to be toxic to freshwater cyanobacteria and algae (Halling-Sørensen, 2000), but three orders of magnitudes below terrestrial toxicity of certain soil fauna (Baguer et al., 2000). Macrolide sorption to the schmutzdecke was estimated to be 2.3 mg/kg at the end of the simulated filtration cycle. This value is one to two orders of magnitude below concentrations found in swine waste applied to agricultural soils (Campagnolo et al., 2002), but may be toxic to freshwater algae (Liguoro et al., 2003).

Tylosin Extraction Results

Figure 15 illustrates the mass of tylosin accumulation in the schmutzdecke predicted by the LETA model and concentrations of tylosin extracted from schmutzdecke samples after 60 days. The tylosin concentrations extracted from the pilot filter schmutzdecke
ranged from 0.03 to 0.25 mg/kg, and the samples averaged 0.10 mg/kg ($n = 13$). Matrix interference peaks in the pre 60-day schmutzdecke extractions were < 1% of the lowest post 60-day extraction peaks, and extraction efficiency of the spiked pre 60-day schmutzdecke samples was between 17-22% ($n = 3$). Extraction of tylosin from soil and manure matrices using various methods has ranged from 7-82% (Bewick, 1979; Teeter and Meyerhoff, 2003; Liguoro et al., 2003), and decreased extraction efficiencies after sample storage have been observed (Rabølle and Spliid, 2000). Tylosin is composed of a mixture of different structured compounds (A, B, C, D), with tylosin-A comprising 90% of the antimicrobial (Loke et al., 2002), however, abiotic degradation of the pilot study analyte (tylosin-A) to other forms was not considered during this study.

![Predicted tylosin schmutzdecke concentration (mg/kg) and measured concentrations in extracted samples collected on day 60.](image)

The one to two order magnitude difference between the predicted and extracted concentrations may be explained by antimicrobial extraction efficiency, the conservative assumption of complete solids capture, and neglect of biodegradation occurring in the
schmutzdecke throughout the filtration cycle. These considerations are areas of future investigation and research, but the conservative estimates of the LETA model appeared suitable for initial environmental risk assessments.

LETA Model Sensitivity Analysis

Altering each of the sensitivity parameters by 10 and 30% percent of the initial values generated the percent change of effluent aqueous concentration for the tetracycline, quinolone, and macrolide classes of antimicrobials at the end of a 60-day filtration period (Table 7). Under simulation conditions, clay adsorption had a negligible affect on effluent concentrations, and increasing the $K_F$ effective penetration depth to 50 cm produced less than 5% change in effluent concentrations (data not shown). Therefore, photolysis and clay adsorption were not considered significant removal mechanisms for these antimicrobials during slow sand filtration.

<table>
<thead>
<tr>
<th></th>
<th>Tetracyclines</th>
<th>Quinolones</th>
<th>Macrolides</th>
</tr>
</thead>
<tbody>
<tr>
<td>$%\Delta$</td>
<td>-10</td>
<td>+10</td>
<td>-30</td>
</tr>
<tr>
<td>$K_F$</td>
<td>+1</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>$K_P$</td>
<td>+6</td>
<td>-5</td>
<td>+18</td>
</tr>
<tr>
<td>$K_S$</td>
<td>+200</td>
<td>-63</td>
<td>+3919</td>
</tr>
<tr>
<td>$Q_{in}$</td>
<td>-69</td>
<td>+186</td>
<td>-99</td>
</tr>
<tr>
<td>$n$</td>
<td>-44</td>
<td>+84</td>
<td>-81</td>
</tr>
</tbody>
</table>
Partitioning to organic matter was far more influential for macrolides than the tetracycline and quinolone classes of antimicrobials, but sand adsorption was the dominant removal process in the treatment system for all antimicrobials. Effluent concentration response from decreasing flow rates indicated larger filter areas should be considered during treatment system design to produce a minimum flow velocity, and this alternative is well within current design parameters for slow sand filtration (Huisman and Wood, 1974). Enhanced removal efficiency was also observed for slight changes in sand media porosity; indicating the potential for increased antimicrobial removal during filter maturation.

This study used sorption and photolysis coefficients available in published literature, but there is a lack of extensive research investigating antimicrobial biodegradation and sorption/desorption behavior under environmental conditions relevant to drinking water treatment. Few studies have been conducted on antimicrobial mobility from land-applied wastewater sludge, and at the time of this writing published research results have yet to be found investigating contributions to antimicrobial diffuse pollution from land application of drinking water treatment waste products.

The LETA model was developed as an initial investigatory tool for examination of slow sand filter antimicrobial removal efficiencies and evaluation of contaminated waste products generated from drinking water filtration. However, the model does not consider specific antimicrobial contaminant degradation by microbial processes, hydrolysis, or
interactions of antimicrobials with dissolved organic matter. Continued use and refinement of the LETA model by the drinking water research and operations community is greatly encouraged, and the model program code is provided in Appendix A.

Conclusions

- The LETA slow sand filter computer model was developed to investigate the behavior of ionizable antimicrobial contaminants in slow sand filtration, using sorption and photolysis coefficients and treatment system operational parameters.

- Results of the LETA model simulation confirmed high adsorption rates observed in previous soil studies for the tetracycline, quinolone, and macrolide classes of antimicrobials. The 60-day filtration simulation suggested greater than 4-log removal from 1 μg/L influent concentrations in the top 40 cm of the sand column.

- Conservative estimates of schmutzdecke sorption were comparable to land-applied waste sludge, and accumulating contaminants may inhibit filter microbial diversity.

- A 60-day pilot experiment, injecting 1 μg/L tylosin to a pilot slow sand filter, showed up to 0.25 mg/kg of the macrolide antimicrobial remaining in the schmutzdecke layer normally removed during filter maintenance. This value was one order of magnitude below the average conservative predicted sorbed concentration.

- Antimicrobial contaminant biodegradation and sorption/desorption data relevant to drinking water source environmental conditions is limited. Considering the conservative estimates of the LETA model and the extracted antimicrobial concentration from a pilot-scale filter, continued research investigating antimicrobial compound mobility in schmutzdecke biomass is warranted for adequate environmental risk assessment.
CHAPTER 4

ANTIMICROBIAL CONTAMINANT REMOVAL BY MULTI-STAGE DRINKING WATER FILTRATION

Stephen J. Rooklidge, Jennifer A. Field, Peter O. Nelson, M. Robin Collins

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Introduction

Recent investigations of pharmaceutical contamination in surface water revealed that antimicrobial compounds are present at trace concentrations (Kolpin et al., 2002; Golet et al., 2002; Calamari et al., 2003). Exposure to antimicrobials in the environment may enhance selection of resistant bacteria strains in drinking water sources. The resulting human consumption of antimicrobials below therapeutic levels may reduce the effectiveness of drug regimens prescribed to treat infectious diseases (Levy, 1998). Aquatic systems in rural areas may be contaminated by antimicrobials from wastewater treatment plant effluent or diffuse pollution introduced by land application of animal manure and processed biosolids (Rooklidge, 2004). Water-soluble antimicrobials are used in livestock and poultry production to promote growth and avoid easily spread bacterial infections, and waste streams from these operations are potential routes of environmental contamination (Campagnolo et al., 2003; Yang and Carlson, 2003).

Rural water treatment systems that rely on contaminated sources may not include oxidation or adsorption processes commonly used in larger municipal water treatment facilities, which have been shown to be effective methods for pharmaceutical contaminant removal (Huber et al., 2003). Regions with available land experienced a resurgence of slow sand filtration during the last century, and a review of recent research and operational parameters is provided by Logsdon et al. (2002). Roughing filter pretreatment of source water (multi-stage filtration) removes suspended solids prior to
slow sand filtration and prolongs the filtration cycle during seasonal periods of poor water quality (Galvis et al., 1993). Roughing filters are generally designed in a manner similar to slow sand filters, with the exceptions of larger media grain size and faster filtration rates. First Nation communities, rural areas of North America, and many developing nations are the primary benefactors of multi-stage filtration technology due to the passive nature of the treatment process, which relies on sorption, biodegradation, and predation within the filter column (Weber-Shirk and Dick, 1997). Although an effective treatment method for removal of pathogenic microorganisms and suspended particulate matter, multi-stage filtration was not specifically designed to remove modern organic contaminants.

An investigation of herbicide contaminant behavior in slow sand filters indicated that although extensive microbial degradation occurred within the first eight days of filtration, sorption within the filter column was not significant (Woudneh et al., 1997). Four polar pharmaceuticals were initially reported to be biodegraded during slow sand filtration (Sacher et al., 2000); however, subsequent studies indicated significant removal of the compounds was inconclusive (Ternes et al., 2002). Antimicrobial pharmaceutical removal has yet to be investigated in many types of water treatment processes. Conventional drinking water treatment processes incorporating carbon adsorption and oxidation were shown to be effective methods for sulfonamide removal (Adams et al., 2002). However, these treatment techniques may have limited feasibility in developing nations and rural areas due to cost and maintenance considerations.
This study examined roughing and slow sand filter removal efficiency for five compounds from four classes of antimicrobials identified as contaminants in a recent U.S. nationwide surface water investigation (Kolpin et al., 2002). The representative antimicrobials used for this study included veterinary medications and drugs prescribed for human use. Sulfamethazine (SMZ) is used in veterinary applications for control of livestock and poultry coccidiosis, and sulfamethoxazole (SMX) is commonly combined with trimethoprim (TRI) to treat human pneumonia and urinary tract infections (Fullerton, 1998). Lincomycin (LIN) is prescribed to treat human infections caused by gram-positive organisms and has veterinary applications for growth promotion in swine and poultry. Tylosin (TYL) is used to promote growth in cattle and treat poultry respiratory infections (VPB, 1997).

An antimicrobial challenge experiment was conducted on a pilot roughing and slow sand filter system built at the municipal water treatment facility of the City of Salem, Oregon. The municipality uses slow sand filtration to treat source water from the North Santiam River. The study was performed to calculate the antimicrobial removal efficiency of roughing filter pretreatment and slow sand unit processes subjected to 0.2 mg/L influent concentrations of each antimicrobial for greater than twenty detention periods. The roughing filter was dosed with antimicrobials for 4 days and aqueous samples were collected at influent and effluent sample ports. The pilot slow sand filter system was dosed with antimicrobials for 14 days and water samples were collected from the influent, effluent, and sample ports at 10-cm intervals down the sand column every day
for 7 days and on the final day. Aqueous samples were analyzed for the injected antimicrobials using high performance liquid chromatography/tandem mass spectrometry (HPLC MS/MS).

Laboratory sorption studies were conducted on filter biomass formed at the sand/water interface (schmutzdecke) of the municipal system to generate isotherms and determine sorption coefficients ($K_d$, $K_{oc}$, $K_{om}$) for the studied antimicrobials. Batch sorption data were compared to values estimated by empirical equations previously derived to examine hydrophobic chemical partitioning behavior in natural sediments. This research was designed to investigate the removal of antimicrobials in multi-stage filtration, provide information for rural communities impacted by antimicrobial surface water pollution, and aid environmental and human health risk assessments.

Methods

The pilot horizontal roughing filter had an influent flow rate of 0.5 m/h provided by a constant-head reservoir filled from the municipal facility’s source water intake structure. The filter was manufactured from exterior-grade plywood supported by a beam and pier-block foundation, and sealed with epoxy at wood connections and fastener points to form a watertight box. Dimensions of the box construction containing the media was 0.6 meters high, 1.22 meters wide, and 2.44 meters long, and the filter had an initial layer of calcite limestone in the first filter cell (Figure 16) with decreasing sizes of basalt river
rock (Table 8) making up the remaining filter media at a final cell length ratio of 0.45:2.55:2:1. The calcite amendment was found to enhance influent clay turbidity removal and increase corrosion control characteristics of multi-stage filter effluent (Rooklidge and Ketchum, 2002a). Crushed and cleaned basaltic river rock and sand media were provided by Morse Bros. (Stayton, OR), and high-calcium limestone was donated by Ash Grove Cement Co. (Portland, OR).

![Diagram of Pilot roughing filter longitudinal section diagram.](image)

**Figure 16** Pilot roughing filter longitudinal section diagram.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Size (mm)</th>
<th>Porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>30-20</td>
<td>0.53</td>
</tr>
<tr>
<td>Second</td>
<td>30-20</td>
<td>0.51</td>
</tr>
<tr>
<td>Third</td>
<td>20-12</td>
<td>0.49</td>
</tr>
<tr>
<td>Fourth</td>
<td>12-9</td>
<td>0.47</td>
</tr>
</tbody>
</table>

The pilot slow sand filter was a 3-m tall polyethylene tank with a 1.2-m internal diameter (Poly Processing Co., French Camp, CA) fed source water at a rate of 0.15 m/hr (Figure 17). The 1-m deep basaltic sand media had a uniformity coefficient of 2.26 and effective
size of 0.30 mm. To mature the schmutzdecke for the antimicrobial challenge study, the pilot slow sand filter effluent was run to waste for three weeks. The slow sand filter was regarded as mature when effluent turbidity fell below 1 nephelometric turbidity unit (ntu), the supernatant level reached 25-cm above the schmutzdecke, effluent total coliform was < 2 cfu/100 mL, and *E. coli* was not detectable. Water quality characteristics were determined for the filtration period using methods referenced in Table 9.
All antimicrobials were analytical grade and provided by MP Biomedicals, Inc. (Aurora, OH). The daily injected mass of all five analytes was dissolved in 200 mL methanol and diluted in 4 L distilled water brought to neutral pH with NaOH. Analytes were injected into the filter influent pipe by peristaltic pump from 4-L clean amber glass bottles to provide a 0.2 mg/L final influent concentration of each antimicrobial. The total antimicrobial concentration fed to the pilot system (1 mg/L) was not considered detrimental to slow sand filter biological activity and treatment performance because concentrations did not exceed inhibitory limits of the aerobic, primarily gram negative, bacteria of schmutzdecke microbial populations (Ingerslev et al., 2001).

Aqueous samples for chromatographic analysis were collected during the challenge study in 40-mL clean amber glass vials, amended with 3 mM sodium azide (Weber-Shirk and Dick, 1997), and stored at 4 °C until analyzed. Duplicate samples of 1.5 mL from each

Table 9 Summary of water quality analytical methods.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowrate</td>
<td>Grad. cylinder/stopwatch</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>Potentiometric</td>
<td>Standard Methods, 4500</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>H₂SO₄ Titration</td>
<td>Standard Methods, 2320</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Conductivity Meter</td>
<td>Standard Methods, 2510</td>
</tr>
<tr>
<td>Calcium</td>
<td>Inductively Coupled Plasma</td>
<td>Standard Methods, 3120</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Inductively Coupled Plasma</td>
<td>Standard Methods, 3120</td>
</tr>
<tr>
<td>Hardness</td>
<td>By Calculation</td>
<td>Standard Methods, 2340</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Nephelometric Meter</td>
<td>Standard Methods, 2130</td>
</tr>
<tr>
<td>Temperature</td>
<td>Thermometer</td>
<td>Standard Methods, 2550</td>
</tr>
<tr>
<td>Total Solids</td>
<td>Dried to 105°C</td>
<td>Standard Methods, 2540</td>
</tr>
<tr>
<td>Total Volatile Solids</td>
<td>Dried to 550°C</td>
<td>Standard Methods, 2540</td>
</tr>
<tr>
<td>Total Organic Carbon</td>
<td>Dried to 105°C</td>
<td>Standard Methods, 5309</td>
</tr>
<tr>
<td>Total Coliform/E. coli</td>
<td>Enzyme Substrate (Idexx)</td>
<td>Standard Methods, 9223</td>
</tr>
</tbody>
</table>
vial were centrifuged at $10^5 \times g$ for 20 minutes and 1 mL of the supernatant was transferred to HPLC autosampler vials. Sulfadimethoxine (SDM) was added to each HPLC vial as a 20 µg/L internal standard.

A Luna C8(2) 3 µ 4.6 mm x 150 mm column was used (Phenomenex, Torrance, CA) with a Waters 2690 HPLC system (Milford, MA) and Quattro Micro mass spectrometer (Manchester, UK). The sample injection volume was 350 µL at a mobile phase flow rate of 0.35 mL/min, and the mobile phase consisted of 2-propanol (A) and distilled water with 0.6% formic acid for a pH of 2.5 (B). The mobile phase gradient was 10% mobile phase A increased to 100% by 13 minutes, kept at 100% for 2 minutes, decreased to 10% by 18 minutes, with another 7 minutes of equilibration time. Quantification was performed using MassLynx 4.0 software, which multiplied the peak area of analyte by the concentration/area ratio of the internal standard.

Limits of detection (LOD) and quantitation (LOQ) for all analytes were quantified by calculating the signal to noise ratio (S/N) from triplicate, 6-point calibration curves made with centrifuged river water. The LOD for all compounds at a S/N of 3:1, LOQ at a S/N of 10:1, and multiple reaction monitoring MS parameters are listed in Table 10. Blanks and water for the calibration curves were made by spiking centrifuged river water collected on each day of the study. Antimicrobial concentrations were calculated from 6-point linear calibration curves ranging from 0.1-500 µg/L. Recovery from standards made in river water ranged from 93-110% and $R^2$ for all calibration curves were > 0.98.
Table 10  HPLC MS/MS parameters for analytes and internal standard.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mass Transition (m/z)</th>
<th>Cone Voltage (V)</th>
<th>Collision Energy (eV)</th>
<th>Retention Time (min)</th>
<th>LOD (μg/L)</th>
<th>LOQ (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRI</td>
<td>290.92 &gt; 261.03</td>
<td>38</td>
<td>29</td>
<td>8.25</td>
<td>0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>SMX</td>
<td>253.79 &gt; 155.81</td>
<td>20</td>
<td>11</td>
<td>11.76</td>
<td>0.05</td>
<td>0.17</td>
</tr>
<tr>
<td>SMZ</td>
<td>278.84 &gt; 91.58</td>
<td>26</td>
<td>23</td>
<td>9.53</td>
<td>0.10</td>
<td>0.33</td>
</tr>
<tr>
<td>SDM</td>
<td>310.83 &gt; 107.71</td>
<td>30</td>
<td>30</td>
<td>12.33</td>
<td>0.05</td>
<td>0.12</td>
</tr>
<tr>
<td>LIN</td>
<td>407.03 &gt; 359.13</td>
<td>37</td>
<td>18</td>
<td>8.11</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>TYL</td>
<td>916.34 &gt; 772.32</td>
<td>50</td>
<td>30</td>
<td>11.51</td>
<td>0.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Schmutzdecke was collected from the municipal filter by pressing clean glass petri dishes into the drained sand column and removing the top 1-cm of material. The mass fraction of organic carbon in the schmutzdecke was 0.4%, and the fraction of organic matter was 1.54% after maturation in the municipal slow sand filter system. Schmutzdecke sorption studies of each antimicrobial were performed by placing 5 g of air-dried solid in 50-mL centrifuge tubes with 30 mL of river water containing 3 mM sodium azide. Samples were shaken at 200 rpm for 24 hours, centrifuged, and the aqueous antimicrobial concentrations were immediately analyzed by HPLC MS/MS. Calibration curves were generated as before, using centrifuged river water from a schmutzdecke control vial containing sodium azide that had not been spiked with analyte.

The sorption study river water collected in March, 2004 had a pH of 7.2, and was not adjusted during the sorption experiments. The final pH of shaken schmutzdecke samples ranged from 7.1 to 7.6. Sorption coefficients derived for this pH range were regarded as representative of the studied source water because the Santiam River fluctuates annually.
between pH 6.8-7.6 (Wujcik, 2004). Analysis of the blank river water indicated none of the antimicrobials were present in detectable concentrations.

The sorption coefficient \( K_d \) was calculated for all analytes using equation 17, where \( C_s \) is the mg of sorbed antimicrobial per kg of solid and \( C_e \) is the aqueous analyte concentration (mg/L) after 24 hours of equilibration time. Isotherms were created by analyzing well-mixed samples at five increasing analyte concentrations (100-500 \( \mu \)g/L). Linearity was evaluated by the coefficient of determination \( (R^2) \) calculated from linear regression of data by Excel 2002 (Microsoft, Redmond, WA).

\[
K_d = \frac{C_s}{C_e}
\]  

(17)

The schmutzdecke sorption coefficients normalized to the fraction of organic carbon \( (K_{oc} = K_df_{oc}) \) and organic matter \( (K_{om} = K_df_{om}) \) were calculated and compared to estimates using the 1-octanol-water partition coefficient \( (K_{ow}) \) of each antimicrobial and empirical equations from literature (Karickhoff et al., 1979; Briggs, 1981). The \( K_{ow} \) was estimated using the KowWin computer program at neutral pH (Meylan and Howard, 1995).

Results

Sorption of all five antimicrobials reached equilibrium within 24 hours (Appendix C). \( K_d \) values determined by regression of data exhibited significant linearity and were well-
represented by equation 17 (Figure 18). Schmutzdecke antimicrobial sorption followed the order TYL > TRI > LIN > SMX > SMZ, and the results for TYL were comparable to coefficients found for soil (Rabølle and Spliid, 2000) and manure (Loke et al., 2002). Sulfonamide sorption to schmutzdecke was the same low order of magnitude as found for sandy and clay loam soils, and indicated a potential for high mobility in filtration systems (Boxall et al., 2002).

![Figure 18](image.png)

**Figure 18** Antimicrobial sorption isotherms for schmutzdecke with regression lines forced through zero.

Normalization of sorption coefficients to the sorbent fraction of organic carbon or organic matter has been used for many years to predict partitioning behavior of hydrophobic contaminants in natural sediments (Brown and Flagg, 1981). Calculation of contaminant $K_{oc}$ from the laboratory-derived coefficient $K_{ow}$ allows environmental partitioning behavior to be evaluated without extensive field investigation. However, linear
relationships between antimicrobial $K_{oc}$ and $K_{om}$ have not been consistently observed (Tolls, 2001; Loke et al., 2002). Empirical equations for hydrophobic chemical behavior in soils and sediments are important tools for environmental risk assessment, and measured schmutzdecke $K_{oc}$ and $K_{om}$ were compared to values calculated by equation 18 for organic carbon (Karickhoff et al., 1979) and equation 19 for organic matter (Briggs, 1981).

\[
\log K_{oc} = 1.00 \log K_{ow} - 0.21 \tag{18}
\]

\[
\log K_{om} = 0.52 \log K_{ow} + 0.64 \tag{19}
\]

Comparison of measured and calculated parameters indicated octanol-water partition coefficients and empirical equations of hydrophobic compounds are not suitable descriptors of antimicrobial sorption to schmutzdecke (Table 11). The order of $K_{ow}$, $K_{oc}$, and $K_{om}$ did not correlate with the observed sorption order and it was evident the sorption behavior could not be explained by purely hydrophobic partitioning. The calculated values of $\log K_{oc}$ and $\log K_{om}$ from empirical equations 18 and 19 did not significantly approximate the measured values. As a result, field studies were considered necessary to investigate sorption and removal behavior in drinking water filtration.
Table 11  Measured and calculated schmutzdecke sorption parameters for five antimicrobials.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Measured Sorption Parameters</th>
<th>Calculated Sorption Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log$K_d$ ($R^2$)</td>
<td>Log$K_{oc}$</td>
</tr>
<tr>
<td>TRI</td>
<td>1.61 (0.98)</td>
<td>4.01</td>
</tr>
<tr>
<td>SMX</td>
<td>0.71 (0.92)</td>
<td>3.11</td>
</tr>
<tr>
<td>SMZ</td>
<td>0.56 (0.91)</td>
<td>2.95</td>
</tr>
<tr>
<td>LIN</td>
<td>0.84 (0.97)</td>
<td>3.24</td>
</tr>
<tr>
<td>TYL</td>
<td>1.88 (0.83)</td>
<td>4.28</td>
</tr>
</tbody>
</table>

The mean and 95% confidence intervals of influent water quality parameters from grab samples collected during the pilot study filtration period are shown in Table 12. The antimicrobial removal efficiencies of roughing filtration exhibited the same behavior as the schmutzdecke sorption order (Table 13). This behavior was not surprising, since the schmutzdecke was primarily composed of sand filter media processed from the same material as the basaltic gravel media of the roughing filter. A significant trend of increasing removal efficiency was not observed for any of the antimicrobials during the study period. Roughing filtration is a physical removal system for particulate matter that impacts slow sand filtration, and biological growth within the gravel media is not considered a relevant mechanism for contaminant removal because of fast filtration rates and low detention periods. The removal of strongly sorbed antimicrobials, such as TYL, indicated an accumulation of sorbed contaminants in roughing filter waste may occur between media wash cycles that could be reintroduced to the environment when the waste is spread on agricultural soils.
Table 12  Mean and 95% confidence intervals of Santiam River water quality parameters from grab samples collected during the antimicrobial challenge study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.26 ± 0.29</td>
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<tr>
<td>Alkalinity (mg/L as CaCO₃)</td>
<td>18.7 ± 1.3</td>
</tr>
<tr>
<td>Conductivity (µS/cm)</td>
<td>30 ± 2.1</td>
</tr>
<tr>
<td>Calcium (mg/L)</td>
<td>2.71 ± 0.05</td>
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<tr>
<td>Magnesium (mg/L)</td>
<td>0.72 ± 0.01</td>
</tr>
<tr>
<td>Hardness (mg/L as CaCO₃)</td>
<td>10.4 ± 1.6</td>
</tr>
<tr>
<td>Turbidity (ntu)</td>
<td>1.79 ± 0.61</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>7.0 ± 1.7</td>
</tr>
<tr>
<td>Total Solids (mg/L)</td>
<td>32.3 ± 1.6</td>
</tr>
<tr>
<td>Total Volatile Solids (mg/L)</td>
<td>3.3 ± 0.97</td>
</tr>
<tr>
<td>Total Organic Carbon (mg/L)</td>
<td>0.69 ± 0.03</td>
</tr>
</tbody>
</table>

Table 13  Roughing filter (RF) antimicrobial removal efficiency calculated for each day during the 4-day challenge experiment.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>1 d</th>
<th>2 d</th>
<th>3 d</th>
<th>4 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRI</td>
<td>25.9</td>
<td>23.7</td>
<td>20.6</td>
<td>27.3</td>
</tr>
<tr>
<td>SMX</td>
<td>3.0</td>
<td>9.7</td>
<td>20.7</td>
<td>8.9</td>
</tr>
<tr>
<td>SMZ</td>
<td>12.4</td>
<td>4.5</td>
<td>2.9</td>
<td>0.3</td>
</tr>
<tr>
<td>LIN</td>
<td>19.3</td>
<td>12.3</td>
<td>1.4</td>
<td>9.9</td>
</tr>
<tr>
<td>TLY</td>
<td>68.5</td>
<td>68.5</td>
<td>63.0</td>
<td>57.7</td>
</tr>
</tbody>
</table>

No significant trends of increasing removal were observed for any of the antimicrobials during the slow sand filter challenge study, and increased antimicrobial removal efficiency would be expected if the antimicrobials were degraded by microbial populations within the filter media (Woudneh et al., 1997). Schmutzdecke maturation appeared not to be affected during the study because filter headloss increased from 25 to 50 cm within 14 days. Slow sand filter removal efficiencies of the studied antimicrobials
(Table 14) indicated average removal efficiencies decreased in a manner comparable to the batch sorption order of schmutzdecke (TYL>TRI>LIN>SMX>SMZ). TYL and TRI were almost 100% removed during slow sand filtration. LIN exhibited less than 25% removal, and slow sand filtration removed less than 4% of the influent sulfonamide concentrations by the last day of the study period.

Table 14  Slow sand filter (SSF) antimicrobial removal efficiency calculated during the 14-day challenge experiment.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>1 d</th>
<th>2 d</th>
<th>3 d</th>
<th>4 d</th>
<th>5 d</th>
<th>6 d</th>
<th>7 d</th>
<th>14 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRI</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>99.5</td>
</tr>
<tr>
<td>SMX</td>
<td>7.2</td>
<td>4.7</td>
<td>19.8</td>
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<td>15.7</td>
<td>12.7</td>
<td>26.4</td>
<td>3.1</td>
</tr>
<tr>
<td>SMZ</td>
<td>8.9</td>
<td>2.9</td>
<td>6.9</td>
<td>1.1</td>
<td>6.1</td>
<td>12.1</td>
<td>17.1</td>
<td>3.9</td>
</tr>
<tr>
<td>LIN</td>
<td>98.4</td>
<td>74.8</td>
<td>56.5</td>
<td>43.4</td>
<td>45.2</td>
<td>25.4</td>
<td>24.1</td>
<td>24.2</td>
</tr>
<tr>
<td>TYL</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

The low removal efficiency of SMX and SMZ in unit processes of multi-stage filtration suggested the presence of sulfonamides in source water will impact the quality of filter effluent and additional treatment processes are necessary to remediate the contaminants and protect water consumers. Sulfonamides may be considered as indicator contaminants for source waters suspected of veterinary and human pharmaceutical contamination because of their observed persistence in aquatic systems and lack of significant removal by conventional and slow sand treatment processes.
The percent of influent aqueous concentrations at the end of the filtration period for 10-cm intervals within the sand column are shown in Figure 20. TYL concentrations fell below the LOQ within 40 cm of the sand column and these results verified earlier laboratory soil column studies that found almost complete TYL sorption within a 30-cm column (Rabølle and Spliid, 2000).

![Graph showing percent of influent analyte concentrations at 10-cm depth intervals down the sand column (day 14).](image)

Figure 19. Percent of influent analyte concentrations at 10-cm depth intervals down the sand column (day 14).

If the source water biota are already acclimated to the presence of antimicrobials, contaminants sorbed to filter media may be partially degraded over a 60 to 90-day filtration period (Liguoro et al., 2003; Teeter and Meyerhoff, 2003). However, aerobic biodegradation of antimicrobials by planktonic bacteria may not be relevant within the ~12-h slow sand filter detention period (Ingerslev et al., 2001). Antimicrobials with the highest sorption response attach to media in the region of greatest biological activity of a
slow sand filter (Ellis and Aydin, 1995) and the biological effects of continued contaminant exposure to slow sand filter microbial communities have yet to be investigated. Sorption of antimicrobials in the schmutzdecke may enhance development of bacterial resistance and the application of slow sand filter waste products to agricultural land may distribute resistant microbial populations to regions previously unexposed to this selective pressure (Rooklidge, 2004). The impact and significance of resistant organisms to agricultural soil are difficult to assess (Sengeløv et al., 2002; Onan and LaPara, 2003), but waste application could contribute to aquatic organism resistance by the introduction of antimicrobial contaminants mobilized through diffuse pollution pathways. Further research is needed to investigate resistance transfer in heterogeneous microbial communities of biological filtration and antimicrobial contaminant mobilization into environmental systems that may adversely impact humans and aquatic organisms.

Conclusions

- Sorption coefficients of five antimicrobials to mature schmutzdecke grown on basaltic sand filter media were comparable to those previously found for soils.

- Antimicrobial sorption to slow sand filter schmutzdecke did not correlate well with octanol-water partition coefficients and empirical equations derived to investigate hydrophobic contaminant behavior in natural sediments.

- Roughing filtration exhibited low removal efficiencies for antimicrobials with low schmutzdecke sorption coefficients, but antimicrobials with high sorption coefficients, such as tylosin, accumulate in roughing filter waste and may eventually be applied to agricultural soils.
Slow sand filtration exhibited antimicrobial removal efficiencies in the order of tylosin>trimethoprim>lincomycin>sulfamethoxazole>sulfamethazine, and trimethoprim and tylosin were almost 100% removed by this treatment method. Tylosin was primarily sorbed within the top 40 cm of the sand filter column and these results supported earlier findings of laboratory-scale soil column studies.

At the end of a 14-day filtration period, slow sand filtration exhibited less than 25% removal of lincomycin and less than 4% removal of the sulfonamide class of antimicrobials from spiked river water. These results were consistent with the low schmutzdecke sorption coefficients, and multi-stage filtration is regarded as an ineffective treatment method for lincomycin and sulfonamides.

Sulfonamides, because of their greater relative persistence among pharmaceutical antimicrobial compounds, may be a suitable indicator for suspected veterinary and human pharmaceutical contamination of conventional and slow sand drinking water treatment processes.

Considering the antimicrobial contaminant behavior within a biologically-active slow sand filter, continued research investigating bacterial resistance transfer in biological filtration systems and antimicrobial compound mobility in land-applied roughing filter waste and schmutzdecke are warranted for adequate environmental risk assessment.
CHAPTER 5

GENERAL CONCLUSIONS
Slow sand filtration is still a viable method of water treatment most suitable for raw water sources low in turbidity and suspended solids. Pretreatment can expand the treatment method to include source waters with higher turbidity.

Roughing filtration prior to slow sand filtration (multi-stage filtration) has been shown to be an efficient and effective treatment technique for source waters subjected to high sediment loads.

Multi-stage filtration is used in rural regions that may be subjected to antimicrobial contamination from upstream waste discharges or diffuse pollution.

Few organic contaminant studies and no quantitative antimicrobial removal efficiency studies have been conducted on multi-stage filtration systems.

To keep the continued use of multi-stage filtration applicable to systems burdened with trace organic pollutants, research must be conducted that investigates the fundamental removal processes and efficiencies of these filtration techniques.

The proliferation of resistant bacteria and diminishing effectiveness of human therapeutic drugs reach far beyond the geographic origin of antimicrobial compounds, and these consequences present a truly global concern.

The focus on reducing contaminant concentrations in water destined for human consumption may enhance proliferation of bacteria species insensitive to antimicrobials by spreading inadequately treated waste into disparate geographical regions.

To determine true antimicrobial contaminant removal from the cycle of pollution, analytical methods quantifying effluent concentrations, degradation, and mobility in amended soils must be applied to wastewater treatment processes. Engineers and water purveyors need to account for all pollution pathways in the design of treatment plants and rehabilitation of older facilities.

Although terraccumulation and diffuse pathways contribute to antimicrobial residues found in surface and groundwater, recent human and aquatic organism risk assessments rarely include this phenomenon.

Collection of pharmaceutical sales data by geographic region and regulations curbing extensive agricultural use of growth-promoting antimicrobials have been implemented in several countries, but are unlikely to be instituted in the United
States within the near future. Current practices may be altered if local economic benefits of growth-promoting feed additives include impacts along other routes of the diffuse pollution cycle.

- Strategies used by the health community to maintain effectiveness of human drug therapy, such as public education, judicious use of antimicrobials, identification of contaminant pathways, and investigation of resistant bacteria proliferation, are applicable methods for the environmental scientific and engineering communities to examine global antimicrobial contamination before eventual consequences are fully realized.

- The LETA slow sand filter computer model was developed to investigate the behavior of ionizable antimicrobial contaminants in slow sand filtration, using sorption and photolysis coefficients and treatment system operational parameters.

- Results of the LETA model simulation confirmed high adsorption rates observed in previous soil studies for the tetracycline, quinolone, and macrolide classes of antimicrobials. The 60-day filtration simulation suggested greater than 4-log removal from 1 μg/L influent concentrations in the top 40 cm of the sand column.

- Conservative estimates of schmutzdecke sorption were comparable to land-applied waste sludge, and accumulating contaminants may inhibit filter microbial diversity.

- A 60-day pilot experiment, injecting 1 μg/L tylosin to a pilot slow sand filter, showed up to 0.25 mg/kg of the macrolide antimicrobial remaining in the schmutzdecke layer normally removed during filter maintenance. This value was one order of magnitude below the average conservative predicted sorbed concentration.

- Antimicrobial contaminant biodegradation and sorption/desorption data relevant to drinking water source environmental conditions is limited. Considering the conservative estimates of the LETA model and the extracted antimicrobial concentration from a pilot-scale filter, continued research investigating antimicrobial compound mobility in schmutzdecke biomass is warranted.

- Sorption coefficients of five antimicrobials to mature schmutzdecke grown on basaltic sand filter media were comparable to those previously found for soils.

- Antimicrobial sorption to slow sand filter schmutzdecke did not correlate well with octanol-water partition coefficients and empirical equations derived to investigate hydrophobic contaminant behavior in natural sediments.
• Roughing filtration exhibited low removal efficiencies for antimicrobials with low schmutzdecke sorption coefficients, but antimicrobials with high sorption coefficients, such as tylosin, accumulate in roughing filter waste and may eventually be applied to agricultural soils.

• Slow sand filtration exhibited antimicrobial removal efficiencies in the order of tylosin>trimethoprim>lincomycin>sulfamethoxazole>sulfamethazine, and trimethoprim and tylosin were almost 100% removed by this treatment method. Tylosin was primarily sorbed within the top 40 cm of the sand filter column and these results supported earlier findings of laboratory-scale soil column studies.

• At the end of a 14-day filtration period, slow sand filtration exhibited less than 25% removal of lincomycin and less than 4% removal of the sulfonamide class of antimicrobials from spiked river water. These results were consistent with the low schmutzdecke sorption coefficients, and multi-stage filtration is regarded as an ineffective treatment method for lincomycin and sulfonamides.

• Sulfonamides, because of their greater relative persistence among pharmaceutical antimicrobial compounds, may be a suitable indicator for suspected veterinary and human pharmaceutical contamination of conventional and slow sand drinking water treatment processes.

• Considering the antimicrobial contaminant behavior within a biologically-active slow sand filter, continued research investigating bacterial resistance transfer in biological filtration systems and antimicrobial compound mobility in land-applied roughing filter waste and schmutzdecke are warranted for adequate environmental risk assessment.


Baker M. (1948) The Quest for Pure Water: The History of Water Purification From the Earliest Records to the Twentieth Century. AWWA, Denver, CO, USA.


Elanco MSDS (1999) Material Safety Data Sheet—Micotil 300. Elanco Animal Health, Lilly Corporate Center, Indianapolis, IN, USA, 46285


APPENDIX A

LETA SLOW SAND FILTER MODEL
LETA Model Time Step

The convergence of aqueous antimicrobial concentrations at various time steps ($\Delta t$) during the early part of the simulated filtration period is illustrated in Figures 21 and 22. A time step of 0.002 days was considered sufficient for the LETA model to calculate aqueous concentrations within the supernatant and throughout the depth of filter column. This time step was within the practical data overflow limit imposed by the Visual Basic computer program (Microsoft Excel, Redmond, WA).

![Graph](image)

**Figure 21** Convergence of aqueous antimicrobial concentrations ($C_A$) at model time steps between 0.03 and 0.0005 days.
125

\[ y = 6617.5x^3 - 104.96x^2 + 0.3402x + 0.2035 \]

\[ R^2 = 0.9893 \]

Figure 22  Effect of time step on the filtration time necessary to reach maximum supernatant antimicrobial concentration.

LETA Model User Interface

The LETA model code is written in Visual Basic, and the user interface is a Microsoft Excel 2002 spreadsheet shown in Table 15. The model is designed to be used and modified by operations, engineering, and research personnel that may have limited programming experience. The user enters values into each cell, or leaves the cell in its default value, and simply clicks the “Run LETA” dialog box to initiate model calculation. The computation time can take up to two minutes, depending upon the number of days or time steps the user enters in the input page cells. The output is generated at the end of the run, and examples of output tables are presented in Tables 16 and 17. Output data graphs are shown in Figure 23.
Table 15  
Input page for Visual Basic LETA Slow Sand Filter Model.

<table>
<thead>
<tr>
<th>Run LETA</th>
<th>Chemical Properties</th>
<th>Supernatant Grab Sample Data</th>
<th>Sand Column Core Sample Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day</td>
<td>$S_x$ (g/l)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
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</tr>
<tr>
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Table 16  Output data from LETA model calculations.

| A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| 0 | 0.02 | 0.05 | 0.10 | 0.20 | 0.40 | 0.80 | 1.60 | 3.20 | 6.40 | 12.80 | 25.60 | 51.20 | 102.40 | 204.80 | 409.60 | 819.20 | 1638.40 | 3276.80 |
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| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
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| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
### Table 17: Output data from LETA model calculations (page 2)

#### Log Values for Graphs (Default at -0.6)

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#### Supernatant Parameter Values Calculated by LETA

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#### Additional Notes
- Table entries are rounded to two decimal places for simplicity.
- The values presented are as calculated by the LETA model, indicating key parameters such as nutrient concentrations and flow rates.
- Further analysis may be required to interpret these values in the context of the model's application to specific environmental conditions.
Figure 23  Output graphs from LETA model.
LETA Visual Basic Slow Sand Filter Model Code

Private Const SVCOUNT As Integer = 10
Dim U As Double
Sub Main()
    Dim time As Double
    Dim step As Double
    Dim endTime As Double
    Dim i As Integer
    Dim iout As Integer
    Dim loopCount As Integer
    Dim Sv(SVCOUNT) As Double

    ' Initial conditions
    time = Cells(22, 2)
    endTime = Cells(23, 2)
    step = Cells(24, 2)
    iout = 0
    loopCount = endTime / step
    Sv(0) = Cells(4, 5)
    Sv(1) = Cells(4, 6)
    Sv(2) = (Cells(4, 5) - Cells(4, 6)) / Cells(4, 5)
    Sv(3) = Cells(4, 6) / Cells(4, 5)
    Sv(4) = Cells(4, 7) * Cells(19, 2)
    U = Cells(13, 2) / (Cells(17, 2) * Cells(19, 2))
    Sv(6) = 0
    Sv(7) = 0
    Sv(8) = 0
    Sv(9) = 0

    ' Clear old values from spreadsheet cells
    Sheet3.Range("a6:e306").ClearContents
    Sheet3.Range("y6:ad306").ClearContents

    For i = 0 To loopCount
        Call IntRK4(Sv, time, step)
        time = time + step
        ' Send 1 of every 100 calculated values to spreadsheet
        If i Mod 100 = 0 Then
            Sheet3.Cells(iout + 6, 1).Value = time
            Sheet3.Cells(iout + 6, 25).Value = Sv(0) ' Total solids
            Sheet3.Cells(iout + 6, 26).Value = Sv(1) ' Volatile solids
            Sheet3.Cells(iout + 6, 28).Value = Sv(2) ' Fraction of inorganic matter
            Sheet3.Cells(iout + 6, 29).Value = Sv(3) ' Fraction of organic matter
            Sheet3.Cells(iout + 6, 2).Value = Sv(4) ' Supernatant volume
            Sheet3.Cells(iout + 6, 30).Value = U ' Sand column interstitial water velocity
            Sheet3.Cells(iout + 6, 3).Value = Sv(6) ' Supernatant aqueous concentration
            Sheet3.Cells(iout + 6, 4).Value = Sv(7) ' Supernatant solids-sorbed concentration
            Sheet3.Cells(iout + 6, 5).Value = Sv(9) ' Accumulating mass in influent solids
            iout = iout + 1
        End If
    Next i
End Sub
'Runge-Kutta integration sub-routine
Public Sub IntRK4(Sv() As Double, ByVal time As Double, dt As Double)
    'Create temporary storage for intermediate values
    Dim i As Integer
    Dim y(SVCOUNT) As Double
    Dim k1(SVCOUNT) As Double
    Dim k2(SVCOUNT) As Double
    Dim k3(SVCOUNT) As Double
    Dim k4(SVCOUNT) As Double
    For i = 0 To SVCOUNT - 1
        y(i) = Sv(i) 'Remember initial values
    Next i

    'Pass 1
    Call Derive(Sv, k1, time) 'Get derivatives
    For i = 0 To SVCOUNT - 1
        y(i) = Sv(i) + k1(i) * dt / 2 'Update state variables
    Next i

    'Pass 2
    time = time + dt / 2
    Call Derive(y, k2, time) 'Get derivatives
    For i = 0 To SVCOUNT - 1
        y(i) = Sv(i) + k2(i) * dt / 2 'Update state variables
    Next i

    'Pass 3
    Call Derive(y, k3, time) 'Get derivatives
    For i = 0 To SVCOUNT - 1
        y(i) = Sv(i) + k3(i) * dt 'Update state variables
    Next i

    'Pass 4
    time = time + dt / 2
    Call Derive(y, k4, time) 'Get derivatives
    'Final update
    For i = 0 To SVCOUNT - 1
        Sv(i) = Sv(i) + (k1(i) + 2 * k2(i) + 2 * k3(i) + k4(i)) * dt / 6
    Next i
End Sub
'State variable derivatives
Public Sub Derive(y() As Double, k() As Double, time As Double)
    Dim Kf As Double 'Aqueous antimicrobial photolysis coefficient
    Dim Kp As Double 'Organic matter partitioning coefficient
    Dim Kc As Double 'Clay adsorption coefficient
    Dim Ks As Double 'Sand adsorption coefficient
    Dim Tsl As Double 'Initial influent total solids
    Dim TsF As Double 'Final influent total solids
    Dim VsI As Double 'Initial influent volatile solids
    Dim VsF As Double 'Final influent volatile solids
    Dim fomi As Double 'Initial organic solids fraction
    Dim fomI As Double 'Final organic solids fraction
    Dim fimi As Double 'Initial inorganic solids fraction
    Dim fimF As Double 'Final inorganic solids fraction
    Dim HI As Double 'Initial headloss
    Dim HF As Double 'Final headloss
    Dim Qin As Double 'Influent flow
    Dim Cin As Double 'Influent aqueous antimicrobial concentration
    Dim A As Double 'Filter area
    Dim n As Double 'Sand media porosity
    Dim Cm As Double 'Aqueous concentration at 0.01-cm sand layer depth
    Dim day As Integer 'Truncated time to nearest day
    Dim Dell As Integer 'Time between sample data collection (default is daily)
    day = Int(time + 0.0001)

'User input values
    Kp = Cells(8, 2)
    Kc = Cells(9, 2)
    Ks = Cells(10, 2)
    Tsl = Cells(day + 4, 5)
    TsF = Cells(day + 5, 5)
    VsI = Cells(day + 4, 6)
    VsF = Cells(day + 5, 6)
    HI = Cells(day + 4, 7)
    HF = Cells(day + 5, 7)
    A = Cells(19, 2)
    n = Cells(17, 2)
    Qin = Cells(13, 2)
    Cin = Cells(14, 2)
    Dell = 1

'Calculated values
    fimi = (Tsl - VsI) / Tsl
    fimF = (TsF - VsF) / TsF
    fomI = VsI / Tsl
    fomF = VsF / TsF

'Get schmutzdecke aqueous concentration from the spreadsheet
    If Time < Day + 0.2 Then
        Cm = Sheet3.Cells((Day * 5 + 0) + 6, 6)
    ElseIf time >= day + 0.2 And time < day + 0.4 Then
        Cm = Sheet3.Cells((Day * 5 + 1) + 6, 6)
    ElseIf time >= day + 0.4 And time < day + 0.6 Then
        Cm = Sheet3.Cells((Day * 5 + 2) + 6, 6)
Elseif time >= day + 0.6 And time < day + 0.8 Then
   Cm = Sheet3.Cells((day * 5 + 3) + 6, 6)
Elseif time >= day + 0.8 Then
   Cm = Sheet3.Cells((day * 5 + 4) + 6, 6)
End If

'Photolysis reaction for 10 hours of the day, penetrating to 10-cm depth
If time < day + 0.42 And HI < 0.1 Then
   Kf = Cells(7, 2)
Elseif time < day + 0.42 And HI > 0.1 Then
   Kf = Cells(7, 2) * 0.1 / HI
Else
   Kf = 0
End If

'State Variable Equations

'Influent total solids
k(0) = (T5F - Tsl) / DeIT

'Influent volatile solids
k(1) = (VsF - VsI) / DeIT

'Supernatant fraction of inorganic matter
k(2) = (fimF - fiml) / DeIT

'Supernatant fraction of organic matter
k(3) = (fomF - foml) / DeIT

'Supernatant volume
k(4) = A * (HF - HI) / DeIT

'Sand column interstitial water velocity
U = (Qln - (HF - HI) / DeIT) / (n * A)

'Supernatant aqueous antimicrobial concentration
k(6) = (Cin - y(6)) * Qin / (y(4) * (1 + Kp * y(3) * y(0) + Kc * y(2) * y(0))) * (Kf + Kp * (k(3) * y(0) + y(3) * k(0) + y(3) * y(0) * k(4) / y(4)) + Kc * (k(2) * y(0) + y(2) * y(0) * k(4) / y(4)) * y(6) / (1 + Kp * y(3) * y(0) + Kc * y(2) * y(0))

'Supernatant sorbed antimicrobial concentration
k(7) = (Kp * y(3) * y(0) / Cin + Kc * y(2) * y(0) * Cin - y(7)) * Qin / (y(4) + Kp * (k(3) * y(0) + y(3) * k(0) + y(3) * y(0) * k(6) + y(3) * y(0) * y(6) * k(4) / y(4)) + Kc * (k(2) * y(0) * y(6) + y(2) * k(0) * y(6) + y(2) * y(0) * k(6) + y(2) * y(0) * y(6) * k(4) / y(4))

'Accumulating antimicrobial mass sorbed to influent solids
k(8) = y(7) * (Qin - k(4)) * 1000

'Accumulating antimicrobial mass sorbed in schmutzdecke
k(9) = (Qin - k(4)) * (y(6) - Cm) * 1000

End Sub
APPENDIX B

HPLC MS/MS METHOD PARAMETERS
### Table 18  
**Mass spectrometer tune settings (Quattro Micro MassLynx 4.0).**

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<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Ion Energy 2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Multiplier (V)</td>
<td>650</td>
<td>650</td>
<td>650</td>
<td>650</td>
<td>650</td>
<td>650</td>
</tr>
</tbody>
</table>

#### Pressure

<table>
<thead>
<tr>
<th>Gas Pirani Pressure (mbars)</th>
<th>2.21(10^3)</th>
<th>2.21(10^3)</th>
<th>2.21(10^3)</th>
<th>2.21(10^3)</th>
<th>2.21(10^3)</th>
</tr>
</thead>
</table>

### Table 19  
**HPLC MS/MS parameters for analytes and internal standard.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mass Transition (m/z)</th>
<th>Cone Voltage (V)</th>
<th>Collision Energy (eV)</th>
<th>Retention Time (min)</th>
<th>LOD (µg/L)</th>
<th>LOQ (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRI</td>
<td>290.92 &gt; 261.03</td>
<td>38</td>
<td>29</td>
<td>8.25</td>
<td>0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>SMX</td>
<td>253.79 &gt; 155.81</td>
<td>20</td>
<td>11</td>
<td>11.76</td>
<td>0.05</td>
<td>0.17</td>
</tr>
<tr>
<td>SMZ</td>
<td>278.84 &gt; 91.58</td>
<td>26</td>
<td>23</td>
<td>9.53</td>
<td>0.10</td>
<td>0.33</td>
</tr>
<tr>
<td>SDM</td>
<td>310.83 &gt; 107.71</td>
<td>30</td>
<td>30</td>
<td>12.33</td>
<td>0.05</td>
<td>0.12</td>
</tr>
<tr>
<td>LIN</td>
<td>407.03 &gt; 359.13</td>
<td>37</td>
<td>18</td>
<td>8.11</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>TYL</td>
<td>916.34 &gt; 772.32</td>
<td>50</td>
<td>30</td>
<td>11.51</td>
<td>0.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Figure 24  Chromatogram of six antimicrobials (100 μg/L).

Figure 25  Mass spectrum of six antimicrobials.
APPENDIX C

ANTIMICROBIAL SORPTION STUDY
Schmutzdecke Sorption Equilibrium Results

Individual antimicrobials were placed in six 40-mL glass vials, with 30 mL river water and 5 g of air-dried schmutzdecke for a final concentration of 500 µg/L and 3mM NaN₃. The vials were shaken for up to 24 hours, and duplicate samples were collected from each vial at the times indicated in Figure 23. For the purpose of estimating the equilibrium sorption coefficient ($K_d$) of each antimicrobial, equilibrium sorption was assumed to occur within 24 hours.

Figure 26  Sorption of antimicrobials on schmutzdecke over 24 hours.