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Research Paper

Natural aging of expanded shale, clay, and slate (ESCS) amendment with heavy metals in stormwater increases its antibacterial properties: Implications on biofilter design

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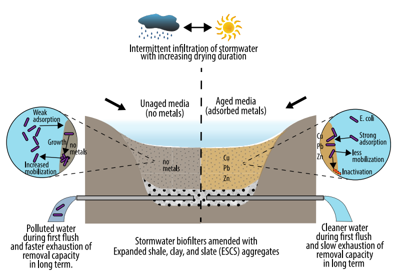
HIGHLIGHTS

- Expanded shale, clay, and slate (ESCS) media adsorb metals irreversibly.
- Heavy metals bind with -OH groups on ESCS and lower surface charge.
- Adsorbed metals increased *E. coli* attachment sites and lower mobilization.
- Increased attachment sites could lower the exhaustion rate of filter media.
- Adsorbed metals on ESCS, not leached metals, inactivated *E. coli* in biofilters.

GRAPHICAL ABSTRACT



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ABSTRACT

Aging is often expected to decrease the pathogen removal capacity of media because of exhaustion of attachment sites by adsorption of co-contaminants and dissolved organics. In contrast, the adsorption of metals naturally present in stormwater during aging could have a positive impact on pathogen removal. To examine the effect of adsorbed metals on pathogen removal, biofilter media amended with expanded clay, shale, and slate (ESCS) aggregates, a lightweight aggregate, were exposed to metals by intermittently injecting natural stormwater spiked with Cu, Pb, and Zn, and the capacity of aged and unaged media to remove *Escherichia coli* (*E. coli*), a pathogen indicator, were compared. Metal adsorption on ESCS media decreased their net negative surface charge and altered the surface properties as confirmed by zeta potential measurement and Fourier-Transform Infrared Spectroscopy (FTIR) analysis. These changes increased the *E. coli* adsorption capacity of aged media compared with unaged media and decreased overall remobilization of attached *E. coli* during intermittent infiltration of stormwater. A live-dead analysis confirmed that the adsorbed metals inactivated attached *E. coli*, thereby replenishing the adsorption capacity. Overall, the results confirmed that natural aging of biofilter media with adsorbed metals could indeed have a net positive effect on *E. coli* removal in biofilters and therefore should be included in the conceptual model predicting long-term removal of pathogens from stormwater containing mixed pollutants.

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1. Introduction

Pathogens or pathogen indicators in urban stormwater is one of the leading causes of surface water and groundwater impairment (Alam et al., 2021; Galfi et al., 2016; McBride et al., 2013). To remove these biological pollutants, stormwater treatment systems such as biofilters have been used (Bratieres et al., 2008; Li et al., 2012), where biofilter media are enhanced with amendments (Ghavanloughajar et al., 2021; Mohanty et al., 2014; Valenca et al., 2021b). However, their removal capacity varies widely (Ghavanloughajar et al., 2021; Valenca et al., 2021b), and the cause of the variability is often attributed to the aging of the filter media (Chandrasena et al., 2016; Li et al., 2012). Aging occurs when biofilter media age naturally due to exposure to stormwater constituents and conditions including drying that can alter their surface properties. Understanding how aging occurs or affects the removal of pathogens can help predict the long-term performance of biofilters during their design lifetime.

Biofilter media can age naturally due to physical, chemical, and biological processes. Physical aging occurs due to temperature fluctuation resulting in drying of media, which has been shown to decrease the overall pathogen removal capacity (Chandrasena et al., 2014b; Fowdar et al., 2021; Li et al., 2012; Nabiul Afrooz and Boehm, 2017). In contrast, another study observed an improved removal capacity of biochar-amended biofilters after aging under dry-wet cycles due to replenished attachment sites (Mohanty and Boehm, 2015). Biological aging occurs due to the growth of biofilms (Nabiul Afrooz and Boehm, 2017), which could also decrease pathogen removal (Chandrasena et al., 2014a). *E. coli* removal could also improve over time due to the growth of protozoa, a natural predator of bacteria (Zhang et al., 2011). Chemical aging occurs when chemical constituents such as natural organic matter (NOM) in stormwater adsorb on media, alter their surface properties, and reduce bacterial removal (Ghavanloughajar et al., 2021; Mohanty and Boehm, 2015). However, previous studies rarely account for other co-contaminants such as dissolved metals on the aging of biofilter media.

Metals are ubiquitous in urban stormwater (Lau et al., 2009; Stein and Tiefenthaler, 2005), and they can adsorb on amendments (Tirpak et al., 2021). The amount of metal adsorbed on amendments can increase with aging, which could affect pathogen removal due to the metal toxicity (Li et al., 2016). For this reason, in some studies, copper and silver nanoparticles were added to adsorbents to improve the pathogen removal (Hrenovic et al., 2012; Li et al., 2014a; Milán et al., 2001; Tuan et al., 2011). The metal nanoparticles could increase the adsorption of pathogens and inactivate them (Kennedy et al., 2008; Li et al., 2014b; Xu et al., 2019). However, these adsorbent media have limited practical relevance due to their cost and limitation on scaling up the production (Li et al., 2014a). They could also leach metals (Li et al., 2014a), which could serve as a secondary contaminant. In contrast, exposure to heavy metal contaminated stormwater could increase the concentration of heavy metals on the media naturally (Al-Ameri et al., 2018; Hermawan et al., 2021). The same process could contribute to the positive effect of aging on pathogen removal. However, it is not clear whether naturally adsorbed metals in biofilters can sufficiently alter the surface properties to have any effect on pathogen removal. As the biofilters are designed to last more than 15–20 years, the total exposure of metals after a few years could be sufficient to alter the surface properties of amendments. However, after installation, biofilters media are rarely monitored for any changes in their surface properties or their performance to remove pathogen beyond the first year (Tirpak et al., 2021). Laboratory studies typically examined the exhaustion in attachment capacity of amendments with aging (Li et al., 2012). However, the positive effect of aging such as increased removal of the pathogen by adsorbed metal has not been tested.

This study aims to test the antibacterial properties to biofilter media aged with heavy metals in stormwater. We hypothesized that the aging of biofilter media with heavy metals in stormwater could improve their bacterial removal capacity. To test the hypothesis, biofilters amended with expanded shale, clay, and slate (ESCS) aggregates, a novel lightweight amendment, were aged by injecting natural stormwater contaminated with metals, and the capacities of aged and unaged biofilters to remove *E. coli* were compared. To isolate the impact of chemical aging because of metal adsorption, other processes of aging were not simulated in this study. The results will help improve the understanding of how the aging of biofilter media with co-contaminants could affect their long-term pathogen removal capacity.

2. Materials and methods

2.1. Stormwater preparation

Natural stormwater was used to simulate the aging of biofilter media. The stormwater was collected from Ballona Creek, Los Angeles, and autoclaved before use to remove any microorganisms to accurately estimate *E. coli* removal by biofilter media. The stormwater naturally contained very low concentrations of Pb (below detection limit, 0.01 μg L^{-1}), Cu (1.8 μg L^{-1}), and Zn (8.3 μg L^{-1}) (Table S1), which was not sufficient to simulate the long-term loading of metals within the experimental time scale. Therefore, stock solutions containing $CuCl_2$, $ZnCl_2$, and $Pb(NO_3)_2$ (Fisher Scientific) were spiked into the stormwater to raise the influent concentration to 500 μg L^{-1} for all metals. The concentration was an order of magnitude higher than typically found in stormwater (Tirpak et al., 2021) to simulate total metal loading in a few years in the field setting. Based on the International Stormwater Best Management Practices (BMP) database, which aggregates data globally from different locations, these three metals were selected because they are typically found at higher concentrations than many other metals in stormwater, and they exhibit a wide range of affinity to amendments (Tirpak et al., 2021). Ni and Cr are also present at high concentrations but they were not used in this study. While Ni exhibits similar adsorption characteristics as Zn (Tirpak et al., 2021), Cr exists as anions (metalloids). Thus, the adsorption of metalloids could have an opposite effect on a surface charge similar to other anions such as phosphate (Appenzeller et al., 2002). However, metalloids are present at a much lower concentration than metal cations (Tirpak et al., 2021), and could be outcompeted by other negatively charged species such as phosphate (Chowdhury and Yanful, 2010).

To test the antibacterial effect of biofilter media with adsorbed metals, a kanamycin-resistant strain of *Escherichia coli K12*, a pathogen indicator was used (Ghavanloughajar et al., 2020; Mohanty et al., 2014). *E. coli* were grown in a Luria-Bertani broth solution to a stationary phase, extracted by centrifugation, washed twice using a phosphate buffer saline (PBS) solution to remove the broth. A small volume of stock *E. coli* concentrated was spiked into the natural stormwater, and their concentration was measured by spread plate technique. The mean concentration of *E. coli* in influent was measured to be $2.8 \pm 0.3 \times 10^5$ CFU mL^{-1}. The concentration is high enough to test the hypothesis and within the range found in natural stormwater (Grebel et al., 2013).

2.2. Model biofilter design

In this study, model biofilters without vegetation were used to estimate the *E. coli* removal capacity of biofilter media without interferences from the confounding factors. Plants' effect on pathogen removal is limited, and any changes in pathogen removal in vegetated biofilters have been attributed to plants' ability to alter the hydraulic retention time (Peng et al., 2016) and moisture content in the filter media (Li et al., 2012). Nevertheless, this reductionist approach without using plants has been used in numerous previous studies to examine the mechanisms of pollutant removal in the biofilters (Hatt et al., 2008; Mohanty et al., 2014; Sun et al., 2020).

Natural biofilter media typically consist of sand or sandy soil with added amendments, if needed, up to 30% by volume (Tirpak et al., 2021). Many adsorbents such as biochar, metallic iron, and zeolite were tested to increase the metal adsorption (Tirpak et al., 2021). Here, Expanded Shale, Clay, and Slate (ESCS) aggregates (<2 mm) was used as biofilter media, which have been shown to remove a wide range of stormwater pollutants (Dordio and Carvalho, 2013; Kalhori et al., 2013; Malakootian et al., 2009; Nkansah et al., 2012). The lightweight media, which can be produced at a wide grain size distribution, has the advantage over others to be used in green roofs or other stormwater infrastructure based on both infiltration and treatment needs. ESCS can replace sand, the most common amendment used for hydraulic control because the aggregate size can be chosen based on design need. They have additional advantages over sand because of lower freight and handling costs with much higher pollutant removal capacity. Thus, ECSC aggregates can be used in rain gardens below the root zone to allow root growth, in a filter strip near the parking lot to permit rapid drainage of water and remove pollutants, and as filtering fills above the collection pipe in the infiltration basin. Different aggregate sizes can be used in permeable pavement. The model biofilters were designed by packing a mixture of quartz sand (ASTM 20-30, Humboldt Mfg Co.) and ESCS aggregate at a ratio of 7:3 in PVC columns (2.54 cm ID x 30 cm length). Compost or soil was not used in the mixture to distinguish the role of ESCS on pathogen adsorption or removal. First, a drainage layer was created using pea gravel up to 6 cm height with a 100 μm nylon membrane on top to prevent biofilter media to get into the drainage layer. Then, sand and ESCS mixture was added in incremental layers to a total filter media depth of 15 cm. A 2-cm layer of pea gravel was added on top to prevent the resuspension or disturbance of the media particles during the stormwater application. Total six columns were packed. Of which, three columns were aged with metals and designated as aged biofilters, whereas the other three columns were exposed to stormwater without metals and designated as unaged biofilters (Fig. S1).

To ensure consistent packing of all biofilters, the bulk density of the packed media was estimated by dividing the weight of the media with the inner volume of the column and comparing between biofilters (Supplementary Material Table S2). The pore volume (PV) was measured by subtracting the weight of the dry media biofilters from the weight of the saturated media biofilters (Borthakur et al., 2021). The residual PV was calculated by draining the saturated biofilters under gravity and subtracting the weight of the dry media biofilters from the weight of the drained biofilters. The hydraulic conductivity of the media was measured using a falling head method (Ghavanloughajar et al., 2020).

2.3. Aging of ESCS media with heavy metals

The experiments were conducted in 5 phases: conditioning, aging, flushing, leaching, and *E. coli* injection. Experiments in the first four phases were designed to age biofilters by injecting stormwater with or without metals, whereas the last phase was designed to compare the *E. coli* removal capacities of aged and unaged biofilters (Table 1). All six biofilters were first conditioned by injecting stormwater at a constant flow rate of 1 mL min^{-1} (11.8 cm h^{-1}) for 24 h to equilibrate the filter media with the stormwater until the effluent pH and ionic strength did not change (Borthakur et al., 2021). To age biofilter media with metals, 500 PV of stormwater spiked with metals was applied on the top of the biofilters at 11.8 cm h^{-1} for 10 days, and the effluent samples were collected at the bottom to measure for heavy metal concentration. The aging phase was estimated to expose 7.2 mg of each heavy metal to biofilter media. This amount is equivalent to 2.1 years of aging of biofilter receiving stormwater with similar metal constituents as Ballona Creek (19.9 ± 29.0 μg L^{-1} Cu, 4.4 ± 12.7 μg L^{-1} Pb, and 83.3 ± 241.2 μg L^{-1} Zn (Stein and Tiefenthaler, 2005) from an acre of catchment area in Los Angeles with an annual rainfall of 379.2 mm yr^{-1}. It should be noted that aging in field conditions can be more complex, and the speciation of adsorbed metals and metal oxides formed during aging could vary widely based on site conditions and time passed after the adsorption (Li et al., 2019; Meng et al., 2018). Therefore, the experimental design tested the effect of adsorbed metals in the short term without accounting for the long-term changes that may occur in natural conditions.

Table 1
Different stages followed during the biofilter experiment.

Stage	Objective	Description	PV injected
Conditioning	Equilibrate the filter media with the stormwater until the effluent pH and ionic strength was consistent	Stormwater injected into all biofilters at mL min^{-1} for 24 h	58
Aging	Contaminate biofilter media with heavy metals	Stormwater injected into control biofilters at 1 mL min^{-1}. Stormwater spiked with 500 μg L^{-1} Pb, Cu and Zn injected into the heavy metal adsorbed biofilters at 1 mL min^{-1}.	500
Flushing	Flush out heavy metals out of the pore water of the heavy metal adsorbed biofilters	Stormwater injected into both sets of biofilters at 1 mL min^{-1}	50
Leaching	Determine the leaching potential of adsorbed heavy metals from the biofilter media	After leaving the columns undisturbed for 24 h, stormwater was reinjected into both sets of biofilters to flush out heavy metals that leached out of the biofilter media during the drying period	8
E. coli injection	Determine the *E. coli* removal capacity of the biofilters	Stormwater spiked with 2.8 ± 0.3 ×10^5 CFU mL^{-1} *E. coli* at 1 mL min^{-1}	64[a]

[a] 64 pore volume includes 8 PV stormwater per injection × 2 injections per drying duration × 4 drying durations

After injection of metal-contaminated stormwater, the contaminated biofilters were then flushed with 50 PV of the natural stormwater without spiked metals, particularly to remove any unabsorbed heavy metals from the pore water. To estimate the leachability of the adsorbed metals, the flow was stopped for 24 h to allow time for loosely bound metals to desorb back into the pore water. Then 8 PV uncontaminated stormwater was injected to estimate the percentage of the adsorbed metals leached into pore water. To prepare the unaged biofilters or biofilters without adsorbed metals, the experiment was repeated by injecting natural stormwater without adding heavy metals into other triplicate biofilters. Effluent samples in all phases were analyzed for heavy metals by using Inductively Coupled Plasma- Optical Emission Spectroscopy (ICP-OES). The samples were centrifuged at 5000 RPM for 15 min to remove any particles, and the supernatant was acidified with nitric acid to lower pH below 1 before analysis.

2.4. Effect of aging on E. coli removal in biofilters

Aged and unaged biofilters were subjected to intermittent infiltration of stormwater contaminated with *E. coli*. Heavy metals were not added to influent when the experiments were carried out for testing of *E. coli* removal capacity of aged and unaged media because dissolved metals can inhibit or inactivate the *E. coli* in stormwater and may overestimate their removal by ESCS media. In each injection, 8 PV of stormwater with 2.8 ± 0.3 ×10^5 CFU mL^{-1} of *E. coli* was applied on the top of the biofilters at 11.8 cm h^{-1} for 3.3 h, and the effluent samples were collected from the bottom at two fractions following the procedure described elsewhere (Ghavanloughajar et al., 2021). The “first flush” sample fraction consisted of the first 5–10 mL of effluent sample collected, which contained the residual water from the previous infiltration event. The second fraction consisted of the stormwater injected during the infiltration events (179.9 ± 7.6 mL). Thus, the *E. coli* concentration in the first flush indicates the fate of *E. coli* in the pore water during the antecedent drying period, whereas the concentration in the second sample presents the overall bacterial removal capacity of the biofilter during the infiltration event. After each infiltration, the biofilters were drained by gravity and left at room temperature (22 °C) for a specific drying duration until the next infiltration event. The process was repeated twice to simulate removal at a specific drying duration. To simulate the effect of varying drying duration on *E. coli* removal or potential growth or inactivation of *E. coli* in biofilters, drying durations of 1, 2, 4, and 7 days were simulated, where two injection events were carried out corresponding to each drying duration. The *E. coli* concentration in the effluent samples was measured using a spread plate technique described elsewhere (Mohanty et al., 2013).

2.5. Heavy metal and E. coli removal by ESCS media

To examine the binding sites on ESCS media for the adsorption of heavy metals, ESCS media with and without adsorbed metals were characterized using Fourier Transform Infrared Spectroscopy (FTIR). To evaluate any change in surface charge of the ESCS media after heavy metal adsorption, the zeta potentials of ESCS media with and without adsorbed metals were measured at the pH (8.5) of stormwater (Method details in Supplementary Material).

E. coli can be removed by aged ESCS media through various mechanisms: adsorption to aged ESCS surface, inactivation of *E. coli* by dissolved metals leached from aged ESCS, and inactivation of *E. coli* by the adsorbed metals on aged ESCS media. To distinguish the role of each process, batch sorption studies were used. To estimate removal by ESCS media without metals, 4 g of ESCS media without metals (termed as unaged media) was mixed with 40 mL of stormwater with spiked *E. coli*, and the change in concentration in water samples was measured. To estimate the removal by aged ESCS, 4 g of ESCS was mixed in triplicated 50-mL centrifuge tubes for 48 h using a wrist action shaker with 40 mL synthetic stormwater (10 mM NaCl) spiked with 300 mg L^{-1} of Pb, Cu, and Zn and adjusted to the same pH and electrical conductivity as the natural stormwater (Ghavanloughajar et al., 2020). This simulated exposure of 12 mg of each heavy metal to 4 g of ESCS media, which corresponded to about 20 days of heavy metal injection in the biofilter experiments. The tubes were centrifuged at 5000 RPM for 15 min, and the supernatants were analyzed for metal concentration to estimate the dissolved and adsorbed metal concentrations. The contaminated media settled in the tube were washed thrice with synthetic stormwater to remove heavy metals in the pore water. The washed media were designated as aged ESCS and were tested to examine their *E. coli* removal capacity by exposing them to stormwater containing *E. coli* for 7 h. In this case, *E. coli* in stormwater may be inactivated by the dissolved metals leached from the aged ESCS or inactivated by the adsorbed metals on the aged ESCS media. To distinguish the contribution of each process, the aged ESCS was mixed with 40 mL synthetic stormwater using an orbital shaker for 48 h to leach any loosely bound metals and centrifuged at 5000 RPM for 15 min. The settled ESCS media contained strongly adsorbed metals that were retained after the leaching test and were exposed to *E. coli* contaminated stormwater to estimate the contribution of the strongly adsorbed metals on *E. coli* removal. The supernatant containing the leached metals was exposed to *E. coli* to quantify the contribution of leached metals on the inactivation of *E. coli*.

Live-dead analysis was performed using fluorescence microscopy to qualitatively examine the fate of the adsorbed *E. coli* on ESCS media with and without aging with metals (Supplementary Material). After exposing *E. coli* to ESCS media with and without adsorbed metals for 7 h, *E. coli* sorbed on both media were exposed to specific dye and observed under a fluorescence microscope to distinguish live cells from the dead cells (Mandakhalikar et al., 2018; Polasko et al., 2021).

2.6. Data analysis

The amount of heavy metals adsorbed on the ESCS media during the injection of the stormwater spiked with metals was estimated using Eq. 1:

$$\% \quad adsorbed = \frac{\sum V_e C_e}{\sum V_i C_i} \times 100 \tag{1}$$

Where V_e and V_i are the volume of the effluent and influent samples, respectively, and C_e and C_i are the heavy metal concentration in the effluent and influent samples, respectively, during the injection and flushing stages. The amount of heavy metals leached during the leaching phase was determined using Eq. 2:

$$\% \quad leached = \frac{\sum V_l C_l}{\sum V_i C_i - \sum V_e C_e} \times 100 \tag{2}$$

Where V_l is the volume of the effluent sample and C_l is the heavy metal concentration in the effluent samples during the leaching phase.

3. Results

3.1. Metal adsorption altered the surface properties of ESCS media

The results reveal how the adsorbed metals change the surface properties of ESCS media. FTIR analysis shows adsorbed metals affect the characteristics of the peak 3500 cm^{-1} (Fig. 1a), which corresponds to the hydroxyl groups ($R-OH$) in the media (Merlic et al., 2001). The peak completely disappeared in the Pb adsorbed ESCS media, while twin peaks were observed in the Cu adsorbed ESCS sample. Adsorption of Zn also showed a sharp peak in that region. Zeta potential measurements showed that the adsorbed metals decreased the net negative surface charge of the ESCS media (Fig. 1b). The zeta potential ESCS media without adsorbed metals at 8.5 pH was − 31 ± 3.3 mV, but it decreased to − 7.7 ± 2.6 mV after the exposure to 300 mg L^{-1} Cu, Pb, and Zn in the batch studies. Typically, ESCS media has a net negative surface charge similar to any other clay minerals. Adsorption of metal cations could lower the negative surface charge by neutralizing the surface charges on clay minerals (Yukselen-Aksoy and Kaya, 2011). These results confirmed that the interaction of metals on ESCS media changed their surface properties, but they did not confirm the mechanisms of metal interaction. It was assumed that the mechanism of metal adsorption on ESCS media is similar to metal adsorption mechanisms on the raw material used to produce ESCS such as clay minerals, which has been extensively studied (Altın et al., 1998; Bradl, 2004). Some other studies on ESCS also focused on ESCS adsorption (Kalhori et al., 2013; Malakootian et al., 2009). Therefore, the scope of the current study is to confirm metal adsorption so that the main hypothesis to understand the effect of adsorbed metal can be tested. We assumed that the added metals (~ 7.2 mg), which was less than 0.0001% of the weight of filter media, would not change the surface area or porosity of the filter media.

3.2. ESCS has a high capacity to remove heavy metals

The goal of this study is to examine if the adsorbed metal has any effect on *E. coli* removal. Thus, metal adsorption is a precondition to test the hypothesis related to the aging effect on E. coli sorption. The results confirmed that the ESCS media had a high removal capacity for Pb and Zn (Fig. S2), meaning they can be tested for their impact on *E. coli* removal. After injecting 500 PV of contaminated stormwater in the aging stage, the concentration of Pb and Zn remained below the detection limit ($C/C_0 = 0.1\%$), whereas the concentration of Cu in the effluent was 31.2% of the influent concentration (C_0). Mass balance analysis (Fig. 2) showed that biofilters adsorbed 95.5 ± 3.9% of the injected Zn, 86.5 ± 4.4% of injected Pb, and 80.5 ± 0.3% of injected Cu. The leaching potential of the adsorbed metals was found to be minimal.

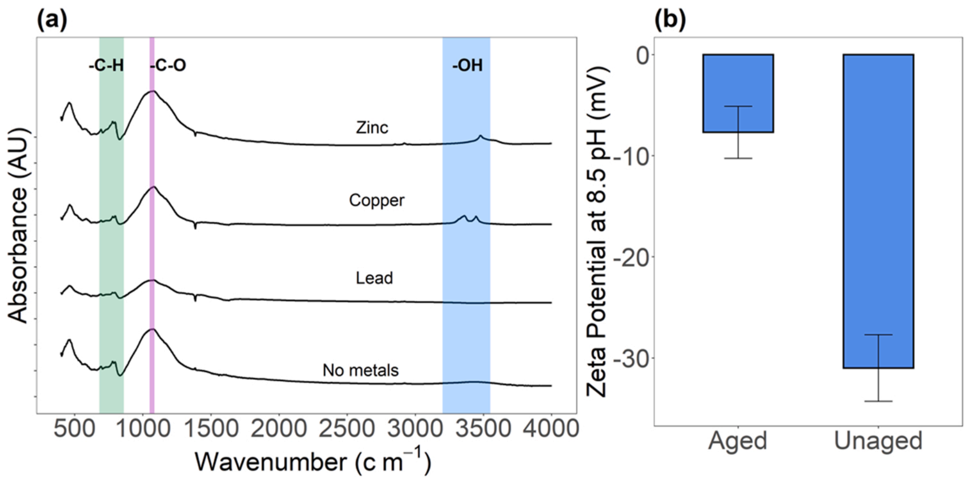

Fig. 1. (a) FTIR analysis of unaged ESCS media (Control) and aged ESCS media with adsorbed metals. The green band refers to the wavenumber range for aromatic C-H bending, the pink band refers to the wavenumber range for C-O stretching bond of primary alcohols and the blue band refers to the wavenumber range for -OH stretch for alcohols/phenols. (b) Zeta potential of unaged ESCS (without metals) and aged ESCS media (with adsorbed metals) at a pH of 8.5 were statistically different ($p < 0.01$), where the error bars represent a standard deviation over the mean from triplicate samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

During the leaching phase, only 0.17 ± 0.02% of adsorbed Cu and 0.02 ± 0.01% of adsorbed Pb were leached. The effluent concentration of Zn was below the influent concentration in natural stormwater, which resulted in additional retention of 0.09 ± 0.005% Zn during the injection of natural stormwater without added metals. Metal adsorption to ESCS media was tested before in the batch studies (Malakootian et al., 2009). The current column study confirmed that the removal is consistent with the batch study even though the hydraulic retention time in the study is an order of magnitude lower than the contact time used in the previous batch study.

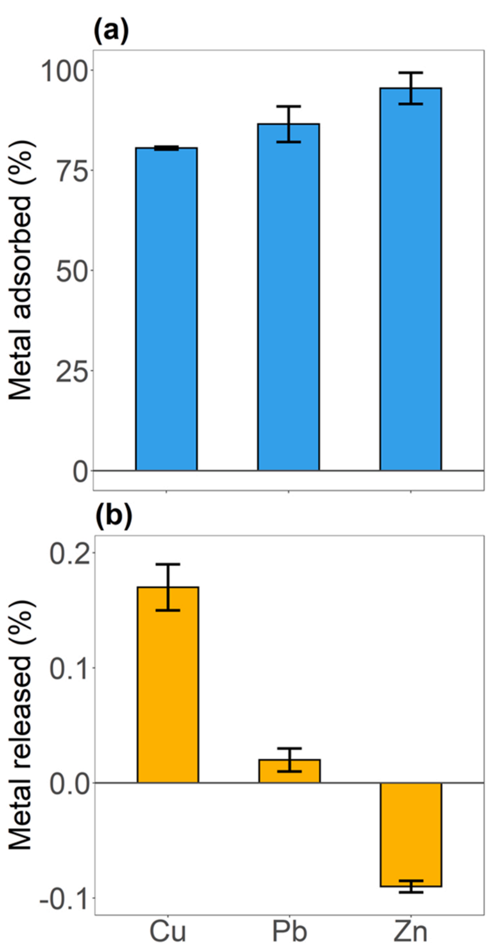

Fig. 2. Mass balance analysis showing (a) percentage of injected metals adsorbed in biofilters after the aging and flushing stages and (b) the percentage of the adsorbed metals leached during the leaching stage. The error bars denote standard deviation over the mean values from triplicate biofilters. Negative % leaching for Zn indicates net adsorption, not leaching, because the Zn concentration in effluent was lower than the concentration in influent stormwater.

3.3. Aging of ESCS with heavy metals improved *E. coli* removal

Aged ESCS media removed more *E. coli* than unaged ECSC media (Fig. 3). *E. coli* concentration in the second sample, which represents biofilter removal capacity during each rainfall event, increased with an increase in the drying duration in successive rainfall events, indicating that *E. coli* removal capacity of the biofilters had decreased with successive infiltration events. However, the rate of loss in removal capacity in the unaged biofilters was higher than that of the aged biofilters. During infiltration events after the 4- and 7-days drying period, the effluent *E. coli* concentration in unaged biofilters without metals was significantly ($p < 0.05$) higher than the effluent concentration in aged biofilters (Fig. S3). The effluent *E. coli* concentration in the unaged biofilters increased exponentially with the increase in drying duration ($R^2 = 0.99$): $C/Co = 0.36 \times e^{0.14 \times Drying\ days}$. However, the effluent *E. coli* concentration in aged biofilters increased logarithmically which appears to flatten out after 7 days of drying ($R^2 = 0.94$): $C/C0 = 0.14 \times \ln(Drying\ days) + 0.40$. The *E. coli* removal capacity of the aged biofilter was consistently higher than unaged biofilters, indicating faster exhaustion of unaged biofilters compared to aged biofilters.

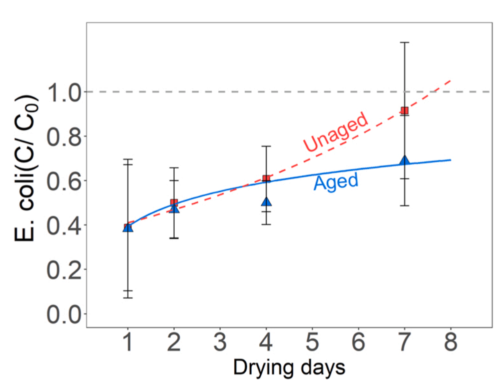

Fig. 3. Change in mean effluent *E. coli* concentration in the effluent with an increase in drying duration for unaged biofilters and aged biofilters (with adsorbed metals). The error bars represent standard deviation over the mean value obtained from 18 samples from triplicate biofilters and duplicate experiments at a specific drying duration. The lines denote the best fits for the mean concentration in the effluents.

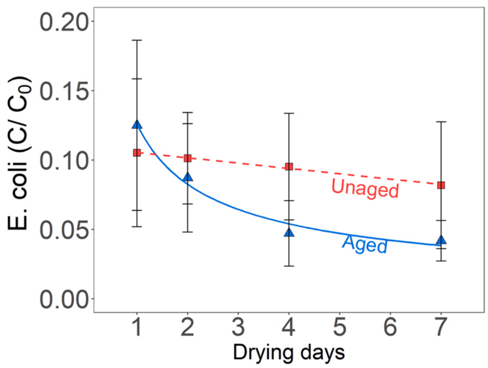

Fig. 4. *E. coli* concentration in the first flush samples with an increase in drying duration in unaged (without adsorbed metals) and aged biofilters (with adsorbed metals). The error bars denote the standard deviation over the mean value obtained from triplicate biofilters and duplicate experiments at specific drying duration (total 18 samples per data point). The lines denote the best fits for the mean effluent concentration from biofilters.

The remobilization of adsorbed *E. coli* from the biofilters during the first flush was lower in the aged biofilters than that in the unaged biofilters (Fig. 4). The *E. coli* concentration in the first flush samples in aged biofilters decreased more rapidly than the unaged biofilters, indicating that the adsorbed metals reduced the leaching of *E. coli* from the biofilters during intermittent infiltration of stormwater. The *E. coli* concentration in the unaged biofilters decreased linearly with the increase in drying duration ($R^2 = 0.99$): $C/Co = -0.0.004 \times Drying\ days + 0.109$. In contrast, the *E. coli* concentration in the aged biofilters decreased much faster by a power function ($R^2 = 0.98$): $C/Co = 0.125 \times (Drying\ days)^{-0.602}$. Due to this, the *E. coli* concentration in the effluent samples from the aged biofilters was significantly lower than the unaged biofilters after 4 and 7 days of drying (Fig. S4).

3.4. Adsorbed metals, not desorbed metals, caused the inactivation of E. coli

Batch experiments validated the result of the column experiments: aged ESCS removed more *E. coli* than unaged ESCS (Fig. 5). When *E. coli* was exposed to aged ESCS, all the injected *E. coli* were removed within 3 h due to a combination of adsorption and inactivation. In contrast, *E. coli* concentration remained high in the entire 7 h of incubation study with unaged ESCS. Removing the readily leachable metals from the ESCS media increased the time to remove all *E. coli* to 5 h. The results from the batch experiments also revealed that removal due to leached metals was negligible compared to the removal by adsorbed metals on ESCS. Exposing the *E. coli* to the metal concentration that was leached from the aged ESCS due to the leaching test (68 μg L^{-1} Cu, 142.8 μg L^{-1} Pb, and 61.5 μg L^{-1} Zn) did not reduce the *E. coli* concentration after 7 h of exposure.

The batch study also confirmed that removal of *E. coli* occurred via adsorption and inactivation, and adsorbed metals, not the leached metals, increased the inactivation of *E. coli*. Live-dead analysis confirmed that heavy metals adsorbed to the ESCS media or aged ESCS inactivated adsorbed *E. coli* (Fig. 5). A high number of green fluorescent cells were observed on unaged ESCS media (Fig. 5b), indicating that most of the adsorbed cells were alive. In contrast, a high number of red fluorescent cells was observed in samples contacted with aged ESCS media (Fig. 5c), indicating heavy metals on ESCS media increased the inactivation of the *E. coli* after adsorption.

4. Discussion

4.1. Reasons for high metal adsorption capacity of ESCS

Heavy metals are typically found in high concentrations in urban stormwater, where their removal can be challenging, particularly when hydraulic retention time (HRT) in biofilters is low. Results in the study showed that ESCS aggregates could adsorb significant amounts of heavy metals without leaching them later. Despite the short HRT of 5–10 mins used in this study, concentrations of each metal in the effluent samples were low, indicating the ESCS aggregates can quickly sorb heavy metals (Fig. 1). The result is similar to a previous study on copper-coated zeolite media, which also removed significant amounts of *E. coli* with a contact time of 4.5 min (Li et al., 2014a). However, copper-coated zeolite is cost-prohibitive to apply on a large scale. The average HRT in field-scale biofilters can range between 20 min to as high as 13 days (Hatt et al., 2009; Zhang et al., 2021a). Since an increase in HRT can increase the removal of pollutants from stormwater (Fang et al., 2021; Zhang et al., 2021b), the metal removal capacity of the ESCS amended biofilters could be much higher in the field setting with longer HRT. Compared to other amendments such as peat, compost, fly ash, or zeolite, ESCS exhibited a comparable or higher metal removal capacity (Lim et al., 2015; Tirpak et al., 2021). Thus, ESCS should be increasingly used as an alternative amendment for biofilters to remove metals from stormwater.

Although two studies tested ESCS media for stormwater treatment, they did not examine the adsorption mechanisms of heavy metals (Malakootian et al., 2009; Shojaeimehr et al., 2014). The quick removal of metals by ESCS was attributed to the strong affinity of metal to specific sites and the porous structure of the ESCS media (Kalhori et al., 2013). Based on FTIR analysis, the heavy metals primarily sorb to the ESCS media by interacting with the hydroxyl ($R-OH$) groups on ESCS (Fig. 2a), where the oxygen atom could act as a strong Lewis base and form complexes with the metal ions (Lim and Lee, 2015). Since the oxygen atom (-O^-) in the hydroxyl group is negatively charged, the adsorption of positively charged heavy metals could reduce the negative surface charge of the ESCS media. The zeta potential measurement of the ESCS media after heavy metal adsorption confirmed a net decrease in the negative surface charge of ESCS media (Fig. 2b). Overall, these results indicate that metals bind strongly on specific sites on ESCS media, which limits the leaching of metals into clean stormwater, and the adsorbed metals reverse the net negative surface charge, which increases the removal of *E. coli*.

4.2. Antibacterial effect of adsorbed heavy metals in biofilters

Pathogen removal in stormwater biofilters is typically challenging because of the possibility of growth of previously removed pathogens and their release during intermittent flow (Mohanty et al., 2014, 2013). Amendments such as biochar or iron filings could improve removal, but the exhaustion of adsorption sites on those media because of bacterial growth limits their utility in long term, indicating aging of these media could lower pathogen removal (Chandrasena et al., 2014a; Valenca et al., 2021b). In this study, aging with metal-contaminated stormwater benefited bacterial removal capacity of ESCS aggregate. The aged biofilters were able to maintain their *E. coli* removal capacity whereas the unaged biofilters exhausted quickly. The results were attributed to the inactivation of adsorbed *E. coli* in the aged biofilters. The ability of the heavy metals to limit *E. coli* growth or enhance inactivation increased with an increase in exposure time during the drying period between rainfall events. This result indicates that the growth of attached *E. coli* in between rainfall events is limited, and most adsorbed *E. coli* could be

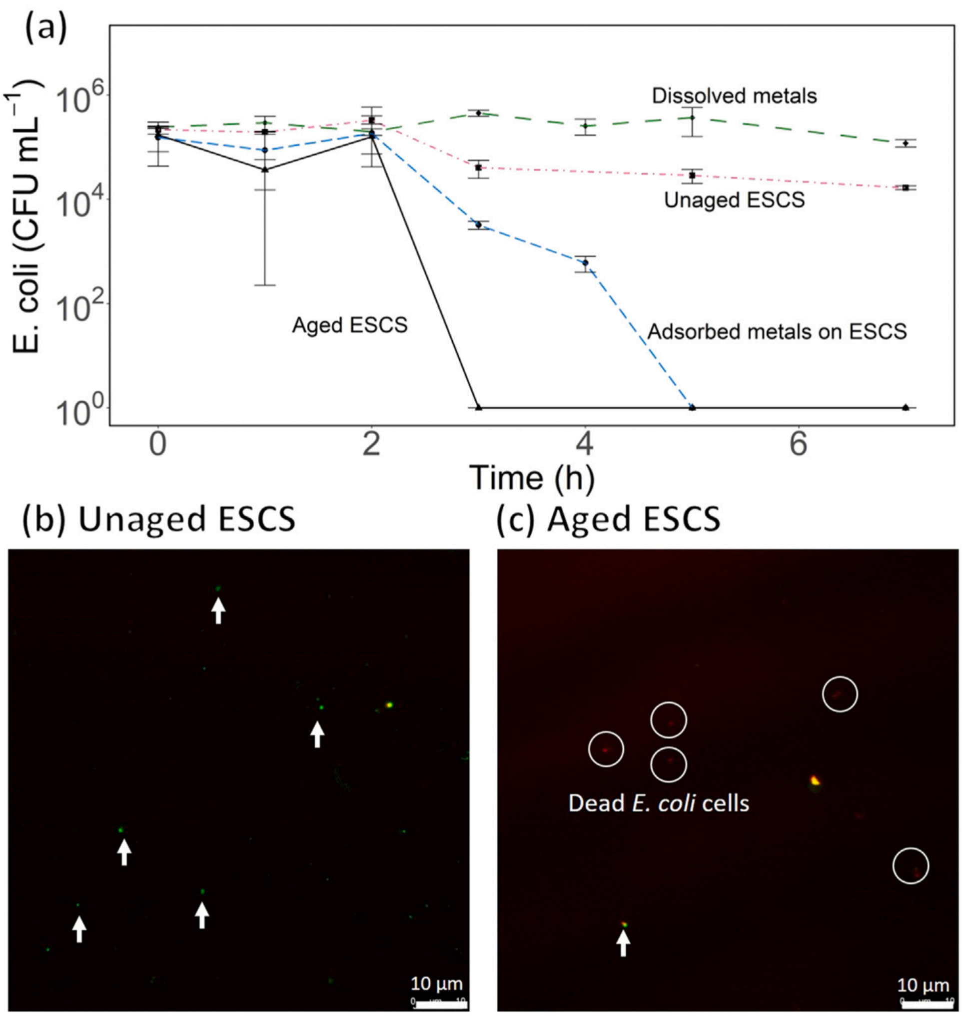

Fig. 5. (a) Change in *E. coli* concentration in batch studies after exposure to unaged ESCS (control), Dissolved metals (Metals leached from ESCS), Adsorbed metals on ESCS (ESCS with strongly adsorbed metals), and aged ESCS (ESCS media after metal adsorption). The error bars denote standard deviation over the mean value obtained from triplicate experiments. (b) Fluorescence microscopy images for *E. coli* adsorbed on unaged ESCS (no adsorbed metals). (c) Fluorescence microscopy images for *E. coli* extracted from aged ESCS (adsorbed metals). The white arrows point toward the green live cells whereas white circles enclose the red dead cells.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

eventually inactivated during a long period between the rainfall events. Although the negative effect of aging is often highlighted in the literature (Valenca et al., 2021a), the positive effect of aging, particularly due to adsorption of metals, on pathogen removal has not been demonstrated before. We expect that other amendments that have high metal adsorption capacity as ESCS should also exhibit the positive effect of adsorbed metals on the pathogen removal (Tirpak et al., 2021) as long as the adsorbed metal is sufficiently high to alter surface charge and interaction with bacteria. Future studies should confirm the finding in field studies or using other amendments. We used zeta potential measurement to prove the changes in surface charge with aging, FTIR analysis to confirm possible changes in surface properties of clay minerals after metal adsorption, and live-dead analysis to confirm the inactivation of bacteria. Thus, surface properties of media aged in field conditions should be measured to confirm the findings and their relevance in field settings.

The remobilization of attached pathogens during intermittent infiltration of stormwater is a concern (Chandrasena et al., 2012; Li et al., 2012; Mohanty et al., 2013) because it would make the biofilter a source of pathogens. The results show that the adsorption of heavy metal on ESCS media also reduced the remobilization of adsorbed *E. coli* during intermittent infiltration of stormwater. Although unaged biofilters reduced the *E. coli* remobilization as well, the aged biofilters reduced the mobilization to a greater extent. This reduction in the remobilization of *E. coli* increased with exposure time drying periods between the rainfalls. An increase in exposure time during drying did not improve the first flush leaching of *E. coli* in other studies with biochar (Valenca et al., 2021b) and iron filings (Ghavanloughajar et al., 2021), but those studies did not use amendments aged with heavy metals. Most media are inert to pathogens, where pathogens are adsorbed on the surface without resulting in their inactivation (Peng et al., 2016; Tirpak et al., 2021). To facilitate inactivation, silver or copper nanoparticles or other metals were mixed with media (Li et al., 2016, 2014a, 2014b; Matsumura et al., 2003; Zeng et al., 2015), but these media are cost-prohibitive for large scale applications and thus have low practical significance. In contrast, ESCS media, which is produced at an industrial scale, can develop antibacterial coating by adsorbing metals naturally present in stormwater. Thus, with an increase in the age of biofilters, the ESCS media can become better at removing pathogens due to an increase in the concentration of metals on the media surface.

4.3. E. coli removal processes on metal-coated ESCS media

Metals in the biofilter media could mainly remove *E. coli* by two different processes: inactivation and/or adsorption (Hrenovic et al., 2012; Li et al., 2014a; Nan et al., 2008). Biofilter media could leach heavy metal into pore water, which could inactivate *E. coli* due to metal toxicity. However, media could also adsorb *E. coli* and inactivate them due to interaction with the adsorbed metals (Nan et al., 2008). Batch experiments showed that leached heavy metals did not remove *E. coli* from the stormwater (Fig. 5a), indicating the contribution of leached metals on *E. coli* removal in the biofilters was negligible. In contrast, adsorbed *E. coli* were inactivated as confirmed by live-dead analysis (Fig. 5c). The heavy metals could inactivate *E. coli* by damaging the cell walls and accumulating in the *E. coli* cells (Raffi et al., 2010; Siddiqi et al., 2018; Tian et al., 2012). Overall, the results confirmed that the inactivation of *E. coli* was caused by adsorbed metals, not the leached metals from the contaminated ESCS. This study compared inactivation processes by adsorbed and dissolved metals, which can be present in biofilters pre-contaminated with metals. Future studies should examine the mechanism by probing the oxidative stress (Engel et al., 2018; Yang et al., 2021), and observing the changes in the cell wall characteristics (Glasauer et al., 2001).

These results have practical significance in predicting the performance lifetime of biofilters. Biofilters are constructed with a service life of at least 20 years. However, the media could become exhausted long before the design lifetime (Blecken et al., 2009; Mohanty and Boehm, 2015; Okaikue-Woodi et al., 2020; Zhang et al., 2014), particularly for bacterial pollutants due to their growth between rainfall events (Chandrasena et al., 2014a, 2012). For the same reason, unaged ESCS in the study became exhausted and lost the bacterial removal capacity after a few rainfall events. The exhaustion rate was much slower in aged ESCS with adsorbed metals, even with increasing in drying duration. Thus, the aging of ESCS could help the performance life of the biofilters amended with ESCS. However, the experiment should be repeated in the field settings to include additional complexities such as competitive adsorption by organic matter, nutrients, or ions (Charbonnet et al., 2020; Mohanty and Boehm, 2015; Okaikue-Woodi et al., 2020).

5. Conclusions

The study shows that aging with heavy metals, which occur naturally, could have a net positive effect on the long-term removal of *E. coli* in biofilters. Results of batch experiments and column studies reveal that biofilters amended with ESCS aggregates could adsorb significant amounts of metals such as Pb, Cu, and Zn from stormwater and decrease net negative surface charge on ESCS media, and these changes increased *E. coli* removal. Spectroscopic analysis and surface charge measurement confirmed that ESCS media irreversibly bind heavy metals from contaminated stormwater and their surface charge becomes less negative, thereby attracting more *E. coli* from stormwater. The metal coating developed with aging could help maintain the long-term *E. coli* removal capacity of the biofilter by increasing adsorption or inactivation of *E. coli* or limiting their growth. The live-dead analysis confirmed that the adsorbed metals inactivated *E. coli* by damaging the cell walls. Any metals that could be leached from the metal-contaminated ESCS were found to be insufficient to inactivate *E. coli*, indicating adsorption was a precondition to inactivation of *E. coli* This enhanced adsorption and inactivation of *E. coli* cells enabled the aged ESCS media to exhaust at a slower rate than the rate in the unaged ESCS media. Thus, filter media aging with metals should be considered in predicting the long-term pathogen removal in biofilters.

CRediT authorship contribution statement

For "Aging of expanded shale, clay, and slate (ESCS) amendment with heavy metals in stormwater increases its antibacterial properties: Implications on biofilter design". **Annesh Borthakur:** Conceptualization, Methodology, Data Analysis, Writing. **Kristida L. Chhour:** Methodology and data collection. **Hannah L. Gayle:** Methodology, Data collection. **Samantha R. Prehn:** Methodology, Data collection. **Michael K. Stenstrom:** Writing – review & editing. **Sanjay Mohanty:** Acquisition of the financial support for the project, Conceptualization, Editing, Revision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.128309.

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