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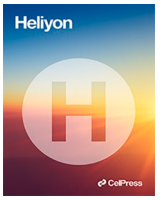

Research article

Plasmid size determines adsorption to clay and breakthrough in a saturated sand column

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ABSTRACT

Horizontal gene transfer (HGT) is a major factor in the spread of antibiotic resistant genes (ARG). Transformation, one mode of HGT, involves the acquisition and expression of extracellular DNA (eDNA). eDNA in soils is degraded rapidly by extracellular nucleases. However, if bound to a clay particle, eDNA can persist for long periods of time without losing its transformation ability. To better understand the mechanism of eDNA persistence in soil, this experiment assessed the effects of 1) clay mineralogy, 2) mixed salt solution, 3) plasmid size on DNA adsorption to clay and 4) breakthrough behavior of three differently sized plasmids in an environmentally relevant solution. Batch test methods were used to determine adsorption trends of three differently sized DNA plasmids, pUC19, pBR322, and pTYB21, to several pure clay minerals, goethite (α-FeOOH), illite, and kaolinite, and one environmental soil sample. Results show not all sorbents have equal adsorption capacity based on surface area with adsorption capacities decreasing from goethite > illite = kaolinite > bulk soil, and low ionic strength solutions will likely not significantly alter sorption trends. Additionally, plasmid DNA size (i.e., length) was shown to be a significant predictor of adsorption efficiency and that size affects DNA breakthrough, with breakthroughs occurring later with larger plasmids. Given that DNA persistence is linked to its adsorption to soil constituents and breakthrough, eDNA size is likely an important contributor to the spread of ARG within natural microbial communities.

1. Introduction

Horizontal gene transfer is a major factor in microbial evolution and adaptation [1]. Though conjugation has historically been understood as the primary driver of HGT in the environment, transformation has recently received more attention due to its role in the spread of ARG [2]. Over 80 different species of microbes are capable of natural transformation [3] and some can be transformed by eDNA fragments as short as 20 base pairs. Natural transformation occurs in soil environments and can involve the transfer of entire genes [4–6].

For natural transformation to occur, several conditions must be met. One such condition is that the DNA must persist long enough to contact a receptive microbial host [7]. Most free-floating DNA in the environment, however, will be rapidly degraded by nucleases,

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which are nearly ubiquitous in soil [8]. To avoid enzymatic degradation, DNA must adsorb to soil constituents, such as clay minerals [9,10]. Once adsorbed, DNA may be physically protected from enzymatic activity while maintaining its transformation ability [11,12].

DNA adsorption to iron oxyhydroxide (e.g., goethite) and phyllosilicate clays (e.g., kaolinite and illite) is controlled by the interactions (i.e., hydrogen bonding, electrostatic forces, and ligand exchange) between outwardly facing phosphate moieties of the DNA and surface hydroxyl groups of the minerals [13]. As such, the mechanism of DNA adsorption is largely dependent on pH, solute concentrations, and clay mineralogy [14–22]. Above pH 5, the phosphate moieties are negatively charged and require polyvalent cations (e.g., Ca^{2+} and Mg^{2+}) to bridge them to the permanent negative charges on the mineral, a process known as cation bridging. Below pH 5, the phosphate groups of DNA are protonated and interact directly with the mineral's adsorption sites. Because DNA does not require cation bridging, DNA adsorption is considerably higher below pH 5 [23]. However, in the presence of inorganic anions (e.g., SO_4^{2-} and $H_2PO_4^-$), DNA adsorption is reduced, due to competition for anions blocking adsorption sites on the clay [20,24]. Effects of homoionic solutions on DNA adsorption have been well characterized for plasmid DNA [8,25]. To the authors' knowledge, however, no study has yet to examine plasmid DNA adsorption in an environmentally relevant solution, which contain a complex mixture of monovalent and polyvalent cations and anions.

The size of the DNA molecule also influences its adsorption efficiency [15,26]. Smaller DNA fragments tend to adsorb preferentially over larger DNA molecules, due to slower diffusion rates with increasing molecular size. Additionally, steric hindrance reduces the degree that multiple phosphate moieties can interact with the clay surface as the size of the DNA molecule increases. Due to its compact structure, plasmid DNA adsorbs more readily than linear DNA for a given base pair size [15]. However, there is a paucity of research comparing the impact of plasmid DNA on abiotic partitioning reactions. Numerous studies have estimated the capacity of soil materials to adsorb DNA, typically with linear, model eDNA such as calf-thymus, herring sperm, and/or salmon sperm DNA [23,27,28]. Interestingly, reported maximum adsorption capacities vary greatly, with one study never finding a maximum adsorption capacity [27]. Extrinsic factors partially explain this observed variation. For example, Gardner and Gunsch [29] showed that pretreating soil samples (e.g., making homoionic) greatly increased DNA adsorption.

Nevertheless, this variation in reported adsorption capacity could also be partly due to the intrinsic properties of clay, such as surface area (SA) of clay particles. DNA only interacts with the external surfaces of clay particles, as DNA molecules are too large to penetrate interlayer sites [11,19]. Thus, the total amount of available adsorption sites on the surface, not clay mass, determines the potential maximum adsorption capacity [30]. Unfortunately, few studies have considered the effect of clay SA on DNA adsorption, with most studies failing to report the SA of the clays used. This makes it difficult to compare DNA adsorption capacity across studies, because the SA of a clay in one study might be different than a similar clay in a different study. Therefore, to limit variability among sorbent materials, SA was normalized across batch samples in this study.

Finally, plasmid DNA has the potential to spread ARG through natural transformation, and due to their smaller size, are more mobile than bacteria [31]. Though the migration and filtration of microbes in porous media have been extensively studied [32,33], few column studies have examined DNA transport and those were mostly tracer studies for groundwater flow [34,35]. To our knowledge, only three studies have examined plasmid DNA filtration in column experiments [31,36,37] and only one of those evaluated at the effects of size on plasmid DNA transport [37]. Additionally, no study has yet to examine plasmid DNA transport in an environmentally relevant background solution. Because of the importance of DNA transport for microbial transformation in the environment [7], more research on the effects of plasmid DNA size on transport is greatly needed.

The objectives of this study were to determine the effects of 1) clay mineralogy, 2) mixed salt solution, 3) plasmid size on DNA adsorption to clay and 4) breakthroughs of three differently sized plasmids in an environmentally relevant solution. In this experiment, batch test methods were used to determine adsorption trends of three differently sized DNA plasmids (i.e., pUC19, pBR322, and pTYB21) to several pure clay minerals (i.e., goethite (α-FeOOH), illite, and kaolinite) and one environmental soil sample. Batch tests were conducted with deionized water (DIW) and an artificial groundwater (AGW) surrogate to assess if plasmid DNA adsorption changes in an environmentally relevant solution. Additionally, saturated sand columns were used to assess the breakthroughs of these three plasmids.

2. Materials and methods

2.1. Sorbent materials

Kaolinite (KGa-1) and illite (IMt-2) were obtained from the Clay Minerals Society Source Repository (Washington County, GA). The Fe oxyhydroxide, goethite (30–63 % Fe), was purchased from Sigma-Aldrich (St. Louis, MO). The bulk soil material (Table S1), identified as a sandy clay loam (textural classification based on the USDA classification scheme), was collected from the Department of Energy's Savannah River Site (SRS; Aiken, South Carolina). The clay fraction of the test soil is dominated by kaolinite and Fe-

Table 1
BET surface area of clay minerals and bulk soil.

Minerals	BET surface area (m^2/g)
Goethite	12.9
Kaolinite	11.8
Illite	20.5
Bulk soil	12.6

oxyhydroxides, such as goethite, with very limited organic matter. The soil material was air dried and homogenized before use. Prior to conducting the batch experiments, the minerals and soil were suspended in UltraPure DNase/RNase-Free Distilled Water as a stock suspension (20 mg/mL) and the pH was lowered to pH 5, which reflects the typical pH of soils at SRS. BET surface areas were determined using N_2 as a probe molecule [38] (Table 1).

2.2. Plasmid DNA

Original solutions of ampicillin-resistant plasmids pUC19 (2686 bp), pBR322 (4361 bp), and pTYB21 (7514 bp) were purchased from New England BioLabs (Ipswich, MA). JM109 *E. coli* (Promega, Madison, WI) were transformed with the plasmids and grown in Luria-Bertani (LB) broth dosed with 100 μg/mL ampicillin. Plasmids were extracted with ZymoPure Plasmid Maxiprep Kit (Zymo, Irvine, CA) by following manufacturer's instructions, and suspended in UltraPure DNase/RNase-Free Distilled Water (DIW). Concentrations and purity of plasmid stock solutions were determined with a NanoDrop One Microvolume UV–Vis Spectrophotometer (Thermo Scientific, Waltham, MA) and DNA integrity was assessed by gel electrophoresis.

2.3. DNA adsorption experiments

Batch experiments were conducted in duplicate to study the sorption of plasmids to goethite, kaolinite, illite, and the bulk soil. Sorbent amounts were normalized to have total surface area of 10 cm^2. Thus, 77.5 μg of goethite, 84.7 μg of kaolinite, 48.8 μg of illite, and 79.4 μg of bulk soil were suspended in DIW or AGW (Strom and Kaback, 1992; Table S2). After plasmids were added, solutions were raised to a final volume 0.5 mL with either DIW or AGW. Plasmid concentrations for batch tests ranged from 0.83 nM to 33.5 nM. A batch test with a plasmid concentration of 1.7 nM and no sorbent was used as a control. The suspensions were equilibrated for 2 h on an orbital shaker at room temperature, and then centrifuged at 10,000×*g* for 30 min. Supernatants were drawn off with a pipette and DNA concentrations left in solution were analyzed with an Invitrogen Qubit 3.0 Fluorometer (Thermo Fisher, Waltham, MA).

2.4. Column design

Clear polyvinyl chloride (PVC) tubing with a diameter of 2.5 cm and a length of 7.5 cm was used for the column. Plastic meshes were placed at the inlet and outlet to retain column material. For all experiments, columns were packed with approximately 65 g of Ottawa sand. Therefore, based off the specific gravity of the material (2.65 g cm^{-3}), the column had approximately 11.5 mL of pore volume. The sand was cleaned before each experiment by soaking the material in trace metal grade hydrochloric acid overnight and then rinsing it several times with Milli-Q water. During the experiment, a peristaltic pump was used to maintain constant flow rate of 0.2 mL min^{-1}.

2.5. Column experiment

Before each experiment, the column was fully saturated with AGW. Each plasmid DNA was diluted to a concentration of 33.5 nM in an AGW solution spiked with tritiated water (^3H_2O), which was used as a conservative tracer. For each experiment, the DNA-^3H_2O samples were leached into the column at rate of 0.2 mL min^{-1}. After a volume of 1 mL entered the column, the inlet solution was immediately switched to a DNA-^3H_2O free AGW solution. To confirm that 1 mL was injected into the column, DNA-^3H_2O samples were weighed before and after each experiment. A fraction collector was used to collect effluent samples in LoBind 2 mL microcentrifuge tubes for DNA and tritium analysis. After each experiment, DNA concentrations in effluent samples were analyzed with an Invitrogen Qubit 3.0 Fluorometer (Thermo Fisher, Waltham, MA). All column experiments were run in duplicate. Effluent tritium was analyzed by liquid scintillation counting (LSC). Effluent fractions were mixed with 10 mL of Ultima Gold™ scintillation cocktail and counted for 60 min on a Beckman Coulter LS6500. Breakthrough estimates were based on relative counts per minute for known dilutions of the inlet treatment solution. A detection limit of ~1 % of the inlet tritium concentration was typically achieved using this technique.

2.6. Data analysis

The resulting batch data was analyzed using the common Freundlich and Langmuir isotherms (Eq. (1) and Eq. (2)). Concentrations were analyzed using the Freundlich isotherm equation:

$$S = K_F C^n \quad \text{Eq. 1}$$

where S is the amount DNA sorbed per unit mass of clay minerals, K_F is the Freundlich partitioning coefficient, and *n* is the unitless exponential Freundlich term, and C is the equilibrium concentration of DNA in solution. Concentrations were also analyzed using the Langmuir isotherm equation:

$$X = \frac{X_{max} K_L C}{(1 + K_L C)} \quad \text{Eq. 2}$$

where X is the amount of DNA sorbed per unit mass of clay minerals, X_{max} is the maximum amount of DNA that may be sorbed, K_L is the Langmuir partitioning coefficient related to the sorption energy, and C is the equilibrium DNA concentration in solution. Each isotherm

was linearized to determine Langmuir and Freundlich constants and coefficients of determination (R^2). Analysis of variance (ANOVA) and paired t-tests were used to determine statistical differences in adsorption maximums. Analysis was conducted using Microsoft Excel (ver. 16.0) and R (3.4).

3. Results

3.1. Adsorption isotherms

Adsorption isotherms of DNA plasmids pUC19, pBR322, and pTYB21 onto goethite, illite, kaolinite, and bulk soil in DIW were determined (Fig. 1). For this experiment, the adsorption sites were considered saturated when DNA adsorption failed to increase with increasing DNA equilibrium concentration. Adsorption data was generally well described by both Langmuir and Freundlich isotherms ($R^2 > 0.9$). Only pTYB21 in kaolinite, illite, and bulk soil was poorly described by the Freundlich isotherms. For all plasmids, adsorption occurred on all sorbent samples and increased past initial levels. For all sorbent types, goethite showed the highest adsorbed amount, followed by illite, then kaolinite, and finally the bulk soil. Additionally, pUC19, the smallest of the three plasmids tested, was found to have the highest adsorption to all sorbent types, followed by next largest plasmid, pBR322, and then pTYB21. For most samples, DNA sorption began to decrease around 5 nM, and only pUC19 in goethite did not plateau after 5 nM. Adsorption isotherm constants and additional information for DNA adsorption in DIW can be found in Table 2.

Additionally, results showed that adsorption increased in the presence of AGW (Fig. 2). Again, the clay sorption sites were considered fully saturated after DNA partitioning remained consistent with increasing DNA equilibrium concentrations. Adsorption data for pUC19 was generally well described by both Langmuir and Freundlich isotherms ($R^2 > 0.9$), except for illite. pBR322 in goethite, illite, and bulk soil was poorly described by Freundlich isotherms. Additionally, pTYB21 in bulk soil was described by the Langmuir isotherm. Goethite was found to have the highest adsorption capacities for all plasmids, followed by illite, then kaolinite, and finally bulk soil. pUC19 was also found to adsorb the most, followed by pBR322, and finally pTYB21, consistent with increasing plasmid size. In adsorption isotherms for pBR322 and pTYB21, DNA adsorption in sorbents except for goethite peaked at around 5 nM, with lower relative adsorption at higher concentrations. In the case of pTYB21, the last point in the illite adsorption isotherm dropped below the last point of the kaolinite adsorption isotherm. Adsorption isotherm constants and additional information for DNA adsorption in AGW can be found in Table 3.

The four tested sorbent types were found to have significantly different adsorption capacities for pUC19 ($p = 0.001$), pBR322 ($p = 0.005$), and pTYB21 ($p = 0.007$) in DIW as determined by one-way ANOVA. When analyzed individually with a paired *t*-test, however,

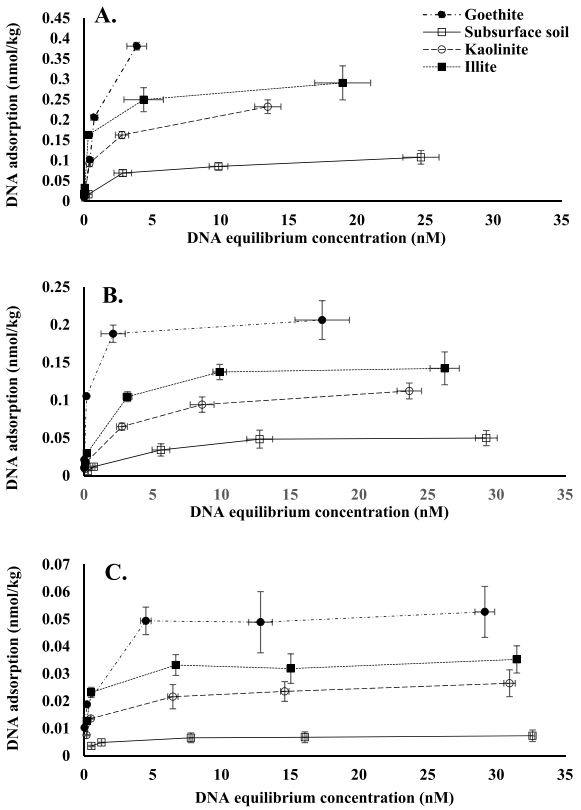

Fig. 1. Adsorption isotherm of (A) pUC19, (B) pBR322, and (C) pTYB21 on goethite, illite, kaolinite, and bulk soil in deionized water (±1 SD).

Table 2
Isotherm properties from adsorption batch tests in deionized water. Langmuir adsorption constants (K_L) and Freundlich constants (K_F) were determined using linearized forms of the isotherm equations.

Soil matrix	DNA	Langmuir			Freundlich		
		X_{max} (nmol/kg)	K_L	R^2	K_F	n	R^2
Goethite	pUC19	0.388	0.383	0.925	0.190	0.620	0.967
	pBR322	0.225	0.381	0.993	0.081	0.421	0.835
	pTYB21	0.053	0.125	0.932	0.026	0.254	0.925
Kaolinite	pUC19	0.192	0.339	0.956	0.744	0.455	0.896
	pBR322	0.050	0.347	0.965	0.027	0.273	0.917
	pTYB21	0.016	0.199	0.893	0.011	0.114	0.615
Illite	pUC19	0.291	0.437	0.978	0.130	0.434	0.836
	pBR322	0.070	0.469	0.885	0.034	0.265	0.837
	pTYB21	0.353	0.324	0.917	0.021	0.174	0.781
Bulk soil	pUC19	0.790	0.884	0.909	0.017	0.424	0.935
	pBR322	0.024	0.735	0.961	0.004	0.258	0.815
	pTYB21	0.007	0.494	0.802	0.004	0.160	0.671

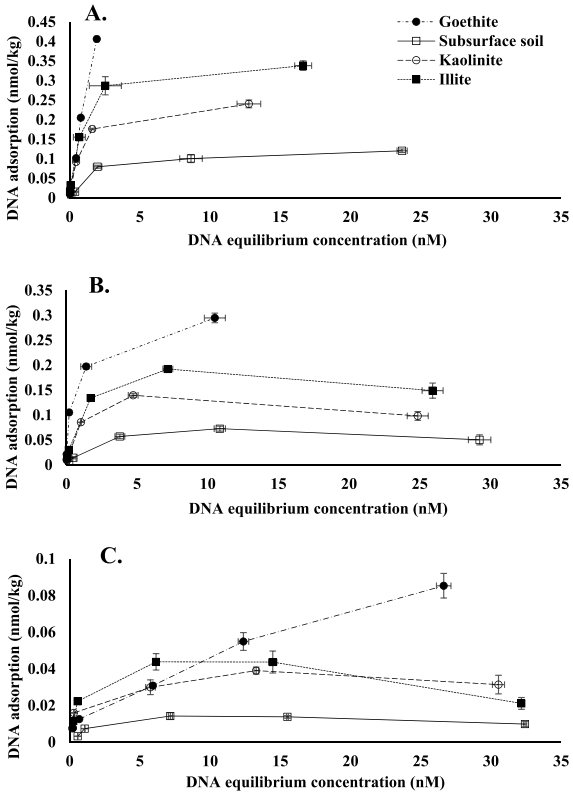

Fig. 2. Adsorption isotherm of (A.) pUC19, (B.) pBR322, and (C.) pTYB21 on goethite, illite, kaolinite, and bulk soil in artificial groundwater (±1 SD).

illite and kaolinite were found not to have significantly different adsorption capacities for pUC19 ($p = 0.112$), pBR322 (0.073), or pTYB21 ($p = 0.215$) in DIW.

When comparing adsorption capacities in AGW, the four sorbent types were found to have significantly different in capacities in pUC19 ($p < 0.001$), pBR322 ($p = 0.001$), and pTYB21 ($p = 0.005$). Kaolinite and illite were found to have significantly different adsorption capacities for pUC19 ($p = 0.028$) and pBR322 ($p = 0.043$) but not pTYB21 ($p = 0.279$) when compared individually. Additionally, when adsorption capacities between DIW and AGW were compared, paired t-tests revealed that no combination of sorbent and plasmid was found to be significantly different ($p > 0.05$).

Finally, one-way ANOVA showed that DNA size significantly affected the adsorption capacities of goethite ($p = 0.007$), bulk soil ($p = 0.007$), kaolinite ($p < 0.001$), and illite ($p = 0.006$) in DIW. Additionally, adsorption capacities in goethite ($p = 0.001$), bulk soil ($p = 0.042$), kaolinite ($p = 0.003$), and illite ($p = 0.002$) were found to be significantly different due to DNA size in AGW.

Table 3
Isotherm properties from adsorption batch tests in artificial groundwater. Langmuir adsorption constants (K_L) and Freundlich constants (K_F) were determined using linearized forms of the isotherm equations.

Soil matrix	DNA	Langmuir			Freundlich		
		X_{max} (nmol/kg)	K_L (1/nM)	R^2	K_F (1/nM)	n	R^2
Goethite	pUC19	0.406	0.590	0.948	0.228	0.715	0.980
	pBR322	0.295	0.587	0.985	0.186	0.501	0.901
	pTYB21	0.085	1.184	0.938	0.015	0.498	0.967
Kaolinite	pUC19	0.241	0.721	0.958	0.031	0.519	0.906
	pBR322	0.140	0.559	0.965	0.046	0.423	0.804
	pTYB21	0.039	0.649	0.897	0.017	0.264	0.809
Illite	pUC19	0.339	0.539	0.993	0.093	0.542	0.916
	pBR322	0.192	0.492	0.969	0.065	0.408	0.862
	pTYB21	0.044	0.490	0.776	0.021	0.169	0.383
Bulk soil	pUC19	0.121	0.986	0.920	0.130	0.489	0.902
	pBR322	0.073	1.345	0.948	0.020	0.419	0.809
	pTYB21	0.010	1.902	0.890	0.006	0.276	0.614

3.2. Breakthrough assessment

Plasmid DNA size was associated with decreased filtration through a saturated sand column (Fig. 3). Effluent concentrations for ^3H_2O and the three plasmids are represented by measured concentration (C) over initial concentration (C_0). Breakthrough for ^3H_2O was observed at 1.5 pore volumes and peak concentration was reached at 2 pore volumes. Percent recovery of ^3H_2O was >0.9. Breakthrough for all plasmids occurred after ^3H_2O detection, with only trace pUC19 detection occurring before the ^3H_2O peak concentration. Breakthrough for the DNA plasmids were observed at 1.5, 3.5, and 4.7 pore volumes for pUC19, pBR322, and pTYB21, respectively. Peak concentrations for pUC19, pBR322, and pTYB21 were observed at 4.4, 4.7, and 5.9 pore volumes, respectively. Both pUC19 and pBR322 concentrations leveled off at around at around 0.03 C/C_0. Whereas pTYB21 only showed a trend to that level. Mass recovery for plasmids were as followed: 0.875 for pUC19, 0.672 for pBR322, and 0.728 for pTYB21.

4. Discussion

After normalizing SA for all minerals, sorbent type was found to be a significant predictor of adsorption capacity in our study, with adsorption capacities decreasing from goethite > illite = kaolinite > bulk soil. Cai et al. [16] reported that kaolinite had a higher adsorption capacity than goethite at pH 5, despite kaolinite having a lower SA than goethite. However, the batch experiments conducted by Cai et al. [16] were done in with low concentrations of tris(hydroxymethyl)aminomethane (Tris) buffer, which has been shown to increase DNA adsorption capacities of kaolinite [39]. Additionally, our observations conform to the findings of Schmidt and Martínez [13], who observed that hydroxyl groups on colloidal goethite particles create strong, monodentate bonds to DNA phosphate moieties.

As suspected, illite and kaolinite had similar adsorption capacities. Both minerals are phyllosilicates with limited layer charge from isomorphic substitution. Unlike kaolinite, which is a 1:1 clay, illite is a 2:1 clay. Typically, 2:1 clays (i.e., montmorillonite) are found to have a completely different DNA adsorption capacity than 1:1 clays [15,17,19,29,30]. Nevertheless, the few layers of illite typically have smaller interstitial space than montmorillonite, and the external SA of illite is likely equal to the total SA [30]. Therefore, illite and kaolinite, with their similar physiochemical natures, likely have a similar number of surface sites available for DNA adsorption when SA is equal, and thus, similar adsorption capacities.

The bulk soil was found to have a relatively low adsorption capacity. Though SRS soil is composed of goethite, kaolinite, and illite, the soil sample is mostly sand (68 %), which might be hindering adsorption. Analysis of the soil sample revealed very little organic carbon, which is known to greatly increase DNA adsorption in untreated soil [40]. Finally, the soil sample was not made homoionic or treated in any way beyond initial homogenization. As such, the lack of organic material or perhaps the presence of native cations/anions could have decreased adsorption capacity.

Batch experiments conducted with DIW or AGW did not have significantly different maximum adsorption capacities, probably due to the low ionic strength of the AGW solution, which was too dilute to impact DNA sorption. Most studies examining the role of divalent cations typically use high, non-environmentally relevant solute concentrations [14–16,29]. Compared to those concentrations, the ionic concentrations in AGW are low. Also, the anions present in the solution are likely to counteract the benefits to adsorption from the divalent cations [24]. Interestingly, unlike in the DIW, adsorption capacity decreased at the highest concentration range. A similar phenomenon was observed by Poly et al. [30], who also reported decreased adsorption at the higher end.

Our results showed that the size of DNA plasmid affects its adsorption efficiency, with decreasing DNA adsorption with increasing size. These results are unsurprising, as most batch test studies have shown that DNA adsorption depends on size [15,26]. In the context of the spread of ARG, this seems to suggest that smaller plasmids would have higher transformation capabilities compared to larger molecules, because smaller plasmids will persist longer in the soil.

Finally, plasmid DNA filtration and the effects of size on plasmid transport were examined in an environmentally relevant ionic background solution and flow rate. Three column tests were conducted, using three differently sized plasmids. By comparing

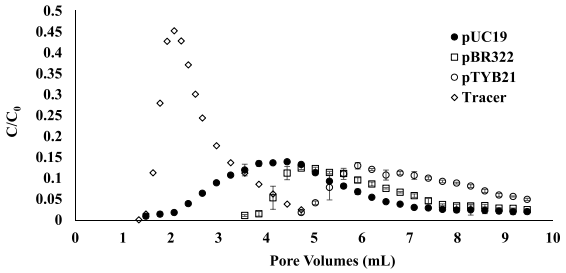

Fig. 3. Breakthrough curves of DNA plasmids pUC19, pBR322, and pTYB21(±1 SD) alongside $^{3}H_2O$. One pore volume is equivalent to 11.5 mL. C, measured concentration. C_0, initial concentration.

breakthroughs with $^{3}H_2O$, which does not interact with the column media, our observations reveal that plasmid DNA breakthrough is hindered by column material. Additionally, results show that plasmids DNA size effects transport, with peak breakthroughs occurring in the following pattern: pUC19, pBR322, and pTYB21. Finally, irreversible plasmid DNA adsorption was observed. These results are similar to transport behavior observed by Chen et al. [37], who also reported that size effects plasmid DNA transport. Results indicate that free-floating plasmid DNA may transport across long distances in porous media under environmentally relevant conditions, though some irreversible binding may occur. However, though smaller plasmid DNA might migrate further than larger plasmid DNA, they will also be adsorbed more readily to soil constituents than larger plasmid DNA [15,26], which will impact the transformation potential.

5. Conclusions

Though understudied, extracellular plasmid DNA has the potential to spread ARG to indigenous microbes through natural transformation. For transformation to occur, several conditions must be met [7]. As part of an ongoing project to assess the potential of natural transformation leading to the spread of ARG, this study examined two of two factors of those conditions: plasmid DNA adsorption and breakthroughs.

We found that not all sorbents have equal adsorption capacity based on surface area, and low ionic strength solutions will likely not significantly alter sorption trends. We also found that plasmid DNA size is shown to be a significant predictor of adsorption efficiency and that size effects DNA transport, with breakthroughs occurring later with larger plasmids.

The spread of ARG in the soil environment through transformation depends on the gene of interest reaching microbial hosts. Because DNA persistence is linked to its adsorption to soil constituents and transport, size should be considered when considering the potential of plasmid to lead to the spread of ARG.

Data availability

Data are available upon request.

CRediT authorship contribution statement

Jarad Cochran: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Liyun Zhang:** Investigation. **Benjamin B. Parrott:** Resources, Conceptualization. **John C. Seaman:** Writing – review & editing, Methodology, Conceptualization.

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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29679.

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