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Effects of solid manure particle fractionation on transport, retention, and release of *Escherichia coli*

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ABSTRACT

Understanding the effect of manure particle fractionation on transport, retention, and release of bacteria plays a critical role in manure management and environmental policies that address soil and water bacterial pollution. Compared to soil particle size, there is less understanding of the importance of solid manure particle size and fractionation on bacterial fate and transport in soils. Four different cow manure particle sizes (0.25, 0.5, 1, and 2 mm) were used to investigate *Escherichia coli* fate in a saturated loamy sand soil. Leaching experiments were performed for up to 20 pore volumes. Preferential transport of chloride mitigated as manure particle size increased. The larger manure fractions (1 and 2 mm) showed greater heterogeneity in bacteria transport and release; smaller manure fractions (0.25 and 0.5 mm) had a greater bacteria retention with retarded release. Bacteria release was associated with transport and re-entrainment of manure particles through soil columns. The results highlighted the contribution of fine and transported particles as of primary importance for retention near the surface and transporting bacteria in soil. Similar retention shapes (*i.e.*, exponential) for different fractions illustrated the similarity of manure source, where greater retention was observed at 0–3 cm depth for the smallest (0.25 mm) and largest (2 mm) manure fractions. The findings also highlighted the dependency of bacteria transport, retention, and release on manure physical fractionation, which should be considered in managing soil and manure practices in the field.
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1. Introduction

The application of solid manure for fertilization is a common agricultural practice mainly due to the high nutrient and water content that reduces the need to purchase chemical fertilizers. A large volume of manure is produced in agriculture and animal production systems (for example, a rough dry mass of 7.3 kg per day for a full-grown milking cow; Bowman and Bowman, 2009), and therefore, its fate should be key part of the waste management program in agricultural settings (Bowman and Bowman, 2009; Loyon et al., 2016; Font-Palma, 2019).

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Improved manure management is important because inadequate management poses a risk for point source or non-point (diffuse) bacterial pollution, which can compromise soil, air, and water quality (Herrero et al., 2018; Liu et al., 2018). Previous events in Canada (*e.g.,* an event in May, 2000 in Walkerton, Canada when 2300 people required medical attention, 7 of whom died), Finland, France, Germany and the USA show that bacterial pollution is still possible even under the best manure management practices and can strongly influence social health (Kukkula et al., 1997; Krapac et al., 2002; Unc and Goss, 2004; Gallay et al., 2006; Goss and Richards, 2007).

Previous studies, extending from nano and micro- to watershed-scales, have systematically explored the mechanisms of bacteria, or colloid transport in general and reported the importance of soil properties particularly the particle size, water content, flow conditions (*e.g.,* Auset et al., 2005; Bradford et al., 2003, 2006a, 2013, 2015; Bradford and Torkzaban, 2008; Cho et al., 2016; Jamieson et al., 2002; Sepehrnia et al., 2019; Wu et al., 2019), and manure type (Unc and Goss, 2004; Bradford et al., 2006a,b; Guber et al., 2006, 2005a,b, 2007; Hodgson et al., 2009; Soupir et al., 2010; Sepehrnia et al., 2018; Li et al., 2020). However, the physical characterization of effects caused by solid manure has received less attention (Pachepsky et al., 2006, 2008, 2009; Guber et al., 2011, 2013).

Solid components in manure allow bacteria to attach to particulate materials (bacteria-associated particles), causing increased survival time and prolonging the potential of contamination when the attached organisms are released into water (Bradford et al., 2006c; Oliver et al., 2007; Soupir et al., 2010; Soupir and Mostaghimi, 2011). Pachepsky et al. (2009) reported that particle size distribution in manure runoff and leachate suspensions remained remarkably stable after 15 min of runoff initiation, in which the manure particles had a median diameter of 3.8 μm with 90% of the particles between 0.6 and 17.8 μm. Guber et al. (2007) found that manure colloids can decrease bacteria attachment to clay, silt, and organic coated sand particles when compared to bacteria attachment in the absence of manure particulates. Bacteria transport is associated with particles and their fate can be greatly modified by the presence of manure solid materials (Cardoso et al., 2012; Shelton et al., 2003; Stocker et al., 2015). However, a mechanistic understanding of how the microbes are released from different manure fractions and subsequently affected in soil is scarce. Sepehrnia et al. (2017, 2018) compared three manure-treated soils (sandy, silty, and silty clay loam) with three different manures (cow; *Bos taurus*, sheep; *Ovis aries*, and poultry; *Gallus gallus domesticus*) and concluded that the ratio of particulates to suspended or soluble materials was a determining factor in bacteria transport and retention, especially when the ratio increased (*i.e.,* higher particulate vs. soluble materials). It remains unknown which fractions of solid manure play greater roles in bacteria fate in terms of transport and retention if a bulk of solid manure is applied to fields.

In this study, we considered common land manure application management practices in which reducing the particle size makes the application possible. We conducted a series of column experiments to compare *Escherichia coli* (*E. coli*) transport and retention from four different fractions of cow manure (0.25, 0.5, 1, and 2 mm). The air-interface contribution (Bradford and Torkzaban, 2008) was minimized by saturating columns to explicitly explore the effects of manure particle size. The release, the most important aspect of bacteria transport, was also assessed through the long leaching process for each manure fraction and manure treated-soil column (Blaustein, 2014; Blaustein et al., 2015). Attachment and detachment of bacteria were assessed in batch experiments with no hydrodynamic dispersion and the effects from pores (Bradford et al., 2015; Sasidharan et al., 2016). The latter could highlight the heterogeneities of bacteria retention as a function of manure particle size. The main objectives of this study were thus to: 1) assess the effect of manure fraction size on bacteria transport and retention; and 2) evaluate the effect of manure heterogeneity on bacteria release from manure and re-entrainment of transported manure particles and consequently bacteria through the soil. The goal was to use the results to improve uncertainties of systematic prediction, conduct mathematical modeling of solid manures, and provide precise insight into better manure management in terms of bacteria release from solid manure as stressed by Pachepsky et al. (2009) and Blaustein et al. (2015).

2. Material and methods

2.1. Soil columns and manure preparation

Soil sample was collected from 0−10 cm depth of an agricultural field located in Shahrekord, Iran (32.3526° N, 50.8261° E). The soil is classified as an Inceptisol (Mosleh et al., 2017; Soil Survey Division Staff, 1993). The soil materials were air-dried and sieved through a 2-mm sieve. Fifteen PVC columns (5 cm diameter and 12 cm height,) were filled with 10 cm soil of equal mass, leaving a 2 cm headspace to uniformly distribute manure on the top of the soil columns. The chemical properties of soil sample were measured using standard methods. Soil pH was measured by a pH-meter in saturated paste (Rhoades, 1996), electrical conductivity (EC) by an EC-meter in 1:5 soil: water suspension (Sims, 1996), total organic carbon by wet-digestion method (Walkly and Black, 1934), soluble sodium by a flame photometer, and soluble calcium and magnesium by EDTA titration method in 1:5 soil:water suspension (Page et al., 1992). Further soil physical and hydraulic properties were measured by methods used in previous studies including percentage of primary particles by Bouyoucos (1936), particle and bulk density by Black and Hartage (1986), saturated hydraulic conductivity (K_s) using constant hydraulic head (Klute and Dirksen, 1986), saturated water content (θ_s), pore water velocity ($v = q/\theta_s$) (q is steady-state flux density), and porosity by Kirkham (2005).

Cow (*Bos taurus*) manure was collected fresh as excreted (less than 5 min after deposition and therefore was quite wet). The manure was air-dried for 72 h at room temperature, then passed through 0.25, 0.5, 1, and 2 mm sieves. The fractions were therefore < 0.25 mm, < 0.5 mm, < 1 mm, and < 2 mm. The manure was separated for each fraction and kept at 4 °C for the leaching experiments. The manure application rate was 30 Mg ha^{-1} (dry basis) which was based on water content after air-drying (Sepehrnia et al., 2021).

2.2. Control, manure, and manure-treated soil leaching

The leaching was performed based on pore volume (PV = $\theta_v \times V_t$; θ_v and V_t are volumetric water content and total column volume, respectively) with the soil columns initially saturated by submerging them in tap water for 24 h (Sepehrnia et al., 2014, 2017). The columns were then leached with up to 20 PVs with a volume of 6.6 ml tap water (equal to 0.1 PV) for the first and 66 ml (1 PV) for the following nineteen PVs continuously poured onto the top of the columns. This procedure established the column saturation using variable water head as upper boundary condition and gravity drainage (*i.e.,* seepage face) as the lower boundary condition (Šimůnek and van Genuchten, 2008). The effluents were sampled similarly to the leaching increments so 29 samples were collected into sterile containers for each column. The controls including soil (without manure) and manure fractions (without soil) were also similarly leached in triplicate. The latter can be found in Sepehrnia et al. (2021). Furthermore, the changes of chloride (Cl^-), as a conservative chemical tracer, were evaluated in the effluent samples of the manure treated soil columns. The results are presented in Fig. 1, normalized by measuring the concentration of Cl^- in the counterparts in the manure fractions effluents.

2.3. Bacteria release from manure in the absence of a soil pore system

Batch experiments were conducted to determine the release behavior of bacteria from different manure fractions in the absence of soil pores while the entire system is in motion (Sasidharan et al., 2016). Six samples of bulk manure for each fraction size were poured into centrifuge tubes (50 mL), in triplicate, and 6.6 mL of tap water was added. The aliquots were centrifuged at 100 ×g for 5 min at room temperature. The supernatant was filtered through a 5 µm filter (Merck Millipore, Germany) and the bacteria were recovered according to live cell culture (as described in Section *2.4*) for both supernatants and the residual solid materials.

2.4. Recovery of transported and retained bacteria cells

Transported and retained bacteria from soil columns were separately recovered using live cell count method according to previous studies by Sepehrnia et al. (2017, 2018) and Guber et al. (2006, 2007). For the transported bacteria in effluents, a ten-fold serial dilution (1:10) was used to have a suitable countable number of bacteria colonies on the culture media plates. The samples were typically diluted 1,000 or 10,000 fold. A small amount (0.1 ml) of final diluted sample was plated on Eosin Methylene Blue (EMB) agar and incubated at 37 °C for 18 to 24 h. EMB agar contains sucrose and lactose, utilized as fermentable carbohydrates substrates, which encourages the growth of gram-negative bacteria, especially fecal and non-fecal coliforms. Lactose-fermenting gram-negative bacteria acidify the medium, which reduces the pH, and the dye produces a dark purple complex usually associated with a green metallic sheen. EMB agar media assists in the visual distinction of *Escherichia coli*, colonies are 2–3 mm in diameter grow with a greenish metallic sheen in reflected light, dark or even black center in transmitted light (MacFadden, 1985).

For the retained bacteria, which included the viable bacteria in solution and attached to the soil (Guber et al., 2006, 2007), the columns were fully drained and disassembled at the end of the experiment and the soil was extruded and sliced into 0–3, 3–6, and 6–10 cm depths. One g of the sliced soil was homogenized and added to 9 ml sterilized distilled water (in triplicate and from each depth), then mixed and centrifuged at 300 rpm (Guber et al., 2006). The suspensions were diluted as explained above (*i.e.,* 1,000 or 10,000) and 0.1 ml of the subsamples were placed on EMB medium and incubated for 18 to 24 h at 37 °C. The viable bacteria grown on the medium culture plates were then counted. The concentration of bacteria were reported based on the colony forming units (CFUs) of fecal coliforms per mL effluent (CFU mL^{-1}) and g soil (CFU g^{-1}) for effluents and residual solids, respectively (Swanson et al., 1992; Guber et al., 2006).

3. Results and discussion

3.1. Soil and manure properties

The soil had sandy loam texture (83.4% sand, 6.7% silt, and 9.9% clay) and the bulk and particle densities were 1.60 (±0.10) and 2.70 (±0.03) g cm^{-3}, respectively. The organic matter content of the soil and manure were 0.22% and 69%, respectively. The chemical properties of cow manure including pH, EC, and soluble ions were similar to those presented by Mosaddeghi et al. (2009) and Sepehrnia et al. (2014, 2018) as given in Table 1. Additional physical properties of the soil used in the columns and flow conditions are in Table 2.

3.2. Chloride transport

The Cl^- breakthrough curves (BTCs) related to the manure-treated soils are in Fig. 1. Three regions were observed in the tracer BTCs indicating early (I), peak transport (II), and tailing (III) phases. The greatest changes occurred in the first 4 PVs of leaching. The BTCs related to the finest (0.25 mm) and largest (2 mm) fractions had the highest and lowest peaks in Cl^- transport, respectively. All peaks were observed around 2 PVs indicating the columns were reasonably homogeneous with respect to the effects of manure in bacteria transport. The slight increase in Cl^- effluent concentration

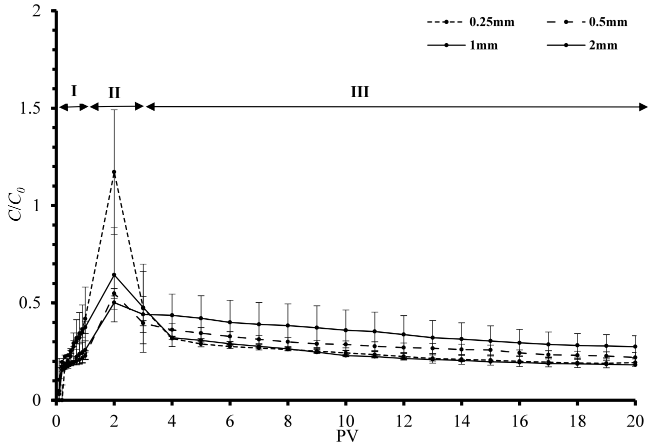


Fig. 1. Normalized conservative tracer (Cl^-) transport through manure-treated soil columns with different manure size fractions.

Table 1
Chemical properties of the soil and manure. EC: electrical conductivity, Na^+: soluble sodium, Ca^{++}: soluble calcium, Mg^{++}: soluble magnesium, OM: Organic matter.

Property	pH	EC	Na^+	Ca^{++}	Mg^{++}
Material	dS m^{-1}		meq L^{-1}		
soil	8.05	0.65	10.12	4.0	5.0
manure	8.55	3.75	24.34	12.50	28.50

Table 2
Physical properties of the soil used in the columns. θ_m: gravimetric water content, θ_s: saturated water content during steady-state, q: steady-state flux density, v: apparent pore-water velocity (q/θ_s), V_t: volume of columns, PV: pore volume. The numbers in parentheses are standard deviation.

Property	θ_m	θ_s	q	v	V_t	PV
	(%)		(cm min^{-1})		(cm^3)	
Sandy loam	25.50 (±0.02)	40.50 (±0.03)	1.57 (±0.25)	4.12 (±0.34)	196.30 (0.00)	75.00 (±6.20)

compared to the influent (*i.e.*, $C > C_0$) for the 0.25 mm size fraction likely indicates a low intensive preferential flow signal (Sirivithayapakorn and Keller, 2003). The Cl^- transport trend for the 0.25 and 2 mm fractions was displaced for the rest of leaching (4 PVs to 20 PVs), causing 2 mm fraction to show greater tailing (Fig. 1). This result demonstrates that the larger fractions influenced Cl^- transport through pores, probably by changing water partitioning and incorporation of a wider soil pore size in comparison to fine manure fractions (Unc, 2002). However, this does not necessarily mean bacteria transport obeys such trends as reported by Wang et al. (2013) and Sepehrnia et al. (2018) and described below.

3.3. Bacteria transport, retention, and release

The effect of the manure fractions on transport and retention was observed in the bacteria BTCs of manure-treated soil columns (Fig. 2a). The bacteria BTCs are normalized using bacteria concentrations obtained from manure leaching (without soil), presented in Sepehrnia et al. (2017).

Four different phases are discriminated in the curves shown in Fig. 2a. Bacteria transport was initially very irregular, and the highest concentrations were observed in the first phase (I). Bacteria transport can be categorized into two groups for large (1 and 2 mm) and small (0.25 and 0.5 mm) manure fractions in this phase, indicating that the larger the manure size, the greater the transport. The 2 mm-treated soil showed concentrations greater than influent ($C > C_0$) which demonstrates preferential transport of bacteria (Sepehrnia et al., 2017, 2018). The tracer (Cl^-) showed a slight preferential transport, but only for the 0.25 fraction, indicating that bacteria transport does not necessarily follow the tracer trend (Wang et al., 2013). This most probably highlights that the finer manure fractions could mitigate preferential bacteria transport by pore blocking. This could also be ascertained from the tracer (Cl^-) BTCs, where all maximum peaks were observed around 2 PVs with different magnitudes (Fig. 1). This indicates that the larger manure fractions (1 and 2 mm) contained greater particle size distributions and heterogeneous medium, which could simultaneously participate in blocking an extended soil pore network and facilitating bacteria transport compared to finer ones that might only be efficient for a narrower soil pore network. Therefore, values of $C > C_0$ are more likely bacteria-associated particles that

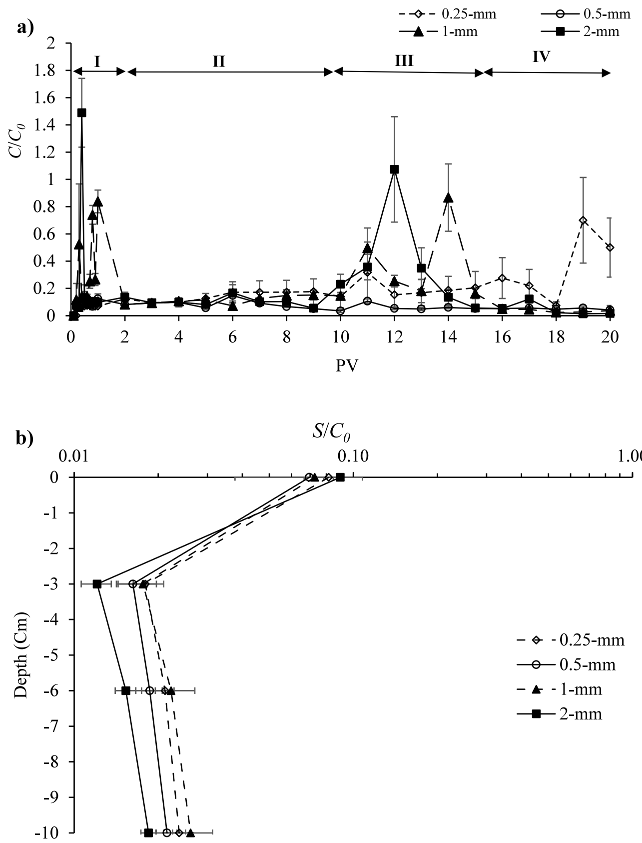


Fig. 2. Normalized *Escherichia coli* breakthrough curves (a) and retention profile (b) for manure-treated soil columns with different manure size fractions.

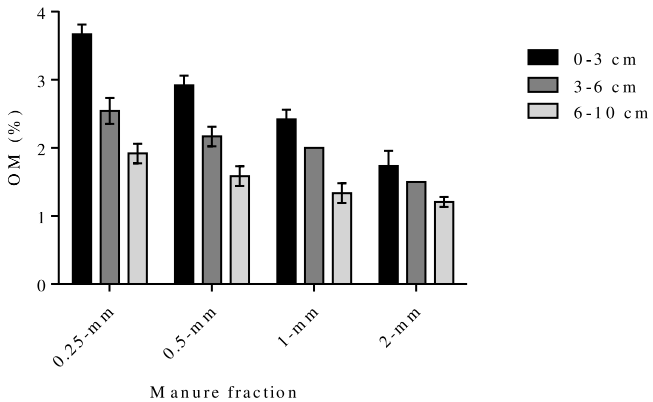
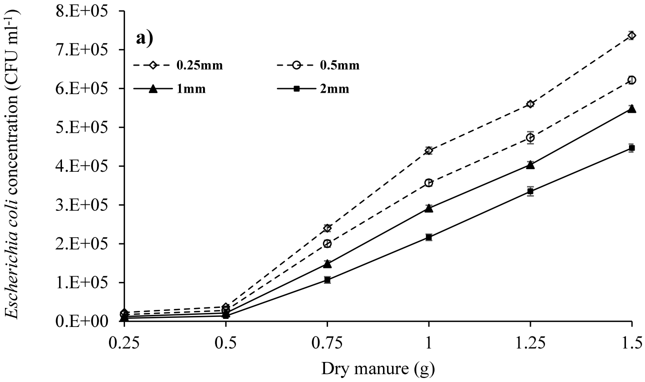


Fig. 3. The percentage of transported organic matter through different depths of manure-treated soil columns.

can be related to the size exclusion where pore blocking occurred due to manure rather than soil pore contrast effect. This resulted in bacteria tending to disperse less and move in faster streamlines because they were not filtered ((Keller and Sirivithayapakorn, 2004)).

Following leaching, the BTCs showed a tailing phase (II, 2 to 10 PVs) when we believe most retention occurred for bacteria, so all fractions had similar trends (Fig. 2b). This phase of the BTCs is clearly the consequence of soil function in which free bacteria or those associated with particles could reach effluent. In the third phase (III), the BTCs entered a release mode in which the physically trapped (*i.e.*, pore effect), strained (*i.e.*, grain–grain contact effect), and/or weakly attached bacteria (through physiochemical processes) were most probably released into soil solution (Bradford and Torkzaban, 2008).



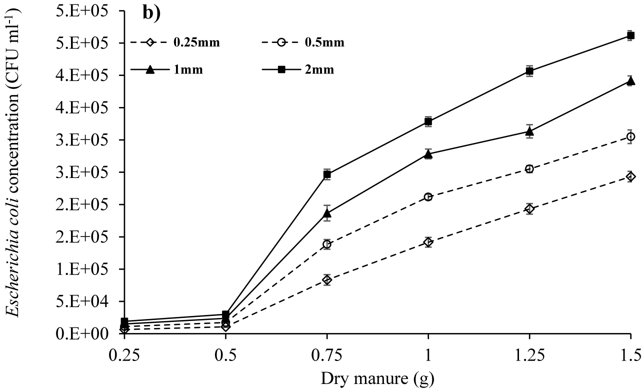


Fig. 4. *Escherichia coli* concentration in liquid (a) and solid (b) phases for the specific mass of manures measured in batch experiments.

A fourth phase (IV) can be attributed to bacteria release, which was accompanied with less tailing tendency for the 0.25 mm fraction than the other fractions. The larger fractions (1 and 2 mm) again showed greater tendency for release when compared to the third and fourth phases. In other words, the heterogeneity in release increased as manure particle size increased. This demonstrates a release mode that has most likely originated from the constrained bacteria carried with manure particles (*i.e.*, bacteria-associated particles) at different depths and released due to change in water regime around trapped particles during leaching (Liu et al., 2019).

Soupir et al. (2010) examined cowpats using portable box-plots and concluded that most fecal bacteria are attached to and transported with manure colloids (8 to 62 μm) in sediment-laden flow regardless of the soil texture. Therefore, the pattern of organic matter in soil columns were investigated after leaching, as illustrated in Fig. 3. The total organic matter content of the applied manure (69%) was considerably higher than that of the soil (0.22%, Table 1). Therefore, the measured organic matter through the columns after leaching could be reasonably attributed to the leached organic matter supplied from the manure fractions. Organic matter regularly decreased as depth increased in all manure fractions (Fig. 3). The highest organic matter content was observed at the surface (0–3 cm) where manure was applied. Surprisingly, the difference between organic matter content of various depths decreased as manure fraction size increased. This demonstrates that the finer particles in the larger fractions are transported through the profile and trapped in pores, while, the movement of such particles were limited at the inlet (0–3 cm) and blocked pores for the 0.25 mm fraction, which supposedly have less heterogeneity in particle size distribution. This is the most obvious reason for the lower transport of bacteria for 0.25 and 0.5 mm fractions versus greater transport and release in the 1 and 2 mm fractions (Fig. 2a).

Mode of retention was exponential and, interestingly, similar for all fractions added to the soil (Fig. 4). This illustrates that the manure size only affected the bacteria retention rate, not the shape mode and the manure source (*i.e.*, cow) was also a determining factor for retention shape (Sepehrnia et al., 2017). The 0.25 and 2 mm fractions had the higher rates in bacteria retention at the surface (0–3 cm).

3.4. Manure fraction heterogeneity harbors bacteria

Figs. 4a and b show the bacteria concentrations as a function of manure bulk in the batch experiment. The manure fractions can release (Fig. 4a) and retain (Fig. 4b) bacteria significantly differently in the absence of soil pores while the entire system is in motion. The smallest fraction (0.25 mm) released proportionally more bacteria into the solution than the other fractions (Fig. 4a). However, the number of bacteria retained in the solid materials of manure fractions was reversed as 2 mm >1 mm> 0.5 mm> 0.25 mm (Fig. 4b). The trend either in release or in retention increased as manure bulk increased (Figs. 4a and 4b), indicating dependency of pollution rate on the amount of manure. However, this dependency decreased as manure size increased, so the ratio of bacteria in solution to that remaining in manure decreased as 3.2 ($\pm$0.49), 1.70 ($\pm$0.21), 1.04 ($\pm$0.25), and 0.63 ($\pm$0.23) for 0.25 mm, 0.5 mm, 1 mm, and 2 mm fractions, respectively. This finding shows that the larger manure particles with higher particulate content acted as harbors for bacteria and impede bacteria release to the solution phase. These findings explain the bacteria BTCs of the manure-treated soil (Fig. 1a), and the greater bacteria retention at the soil surface for 0.25 mm and 2 mm fractions (Fig. 1b), which correspond to changes in retained organic matter at different depths of the manure-treated soil columns (Fig. 3).

The findings demonstrate that manure fractions can greatly affect transport, retention, and release of bacteria from solid manure through soil and the dominant bacteria filtration process can be attributed to the reversible physical trapping that depends on manure fractions. Manure, with regard to chemical, physical, and biological properties, however, can change the physical and electrochemical properties of soil solution, pores, and microbial population that can subsequently affect interactions between bacteria and soil particles in different ways that increase or decrease filtration, modify the kinetics of the physico-chemical interactions between charged surfaces, and alter the competition for retention sites between suspended soluble and particulate compounds (Soupir et al., 2010). This should be further investigated for manures with different solid material contents.

4. Conclusion

A four-phase versus a three-phase trend was observed for the bacteria transport compared to a non-reactive tracer. The largest manure fraction (2 mm) mitigated preferential transport of the tracer but accelerated bacteria transport. This observation reversed for the finest fraction (0.25 mm). Contradictions in tracer and bacteria transport were due to the greater heterogeneity of materials in the 2 mm fraction and greater efficiency of the finest fraction (0.25 mm) at blocking pores near the inflow. Therefore, manure application consist of smaller aggregates that can inhibit accelerated transport of bacteria to reach subsurface waters.

In comparison to previous studies regarding the physical configuration of soil, our results illustrated that the bacteria retention also depends on the manure fraction from which bacteria cells are released. Bacteria retention was clearly influenced by manure size, while similar retention shapes (exponential mode) reflected similar manure source.

Our findings thus demonstrate that manure management strategies and decision making in field manure application should include the importance of manure particle size in bacterial pollution of soil and water. The transported components of a specific solid manure such as straw, fine and coarse materials can cause heterogeneities in bacteria release and transport and may be subjected to preferential or macropore flows. This is a crucial point in areas where the manures are traditionally distributed into the fields without any physical pre-treatment.

CRediT authorship contribution statement

Sayyed-Hassan Tabatabaei: Conceived and designed the experiments, Reviewing & editing. **Nasrollah Sepehrnia:** Conceptualization, Methodology, Conceived and designed the experiments, Analyzed and interpreted the data, Contributed reagents, Materials, Analysis tools or data, Wrote the paper. **Hamdollah Norouzi:** Performed the experiments. **Hossein Shirani:** Reviewing & editing. **Fereidoun Rezanezhad:** Reviewing & editing.

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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