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Attenuation of Migration of Bacteria with Porosity and Depth in Homogeneous Sand Media.

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Abstract

The depth of strata layer and nature of soil strata below the absorption boundary of soakaways/ pit latrines/ septic systems play significant roles in protection of groundwater supply source. An experimental work was carried out to examine the effect of porosity and depth on the attenuation of migration of bacteria in homogeneous sand media using Escherichia coli and Staphylococcus Aureus. Column filtrations were implemented at five depths: 10, 20, 30, 40 and 50cm for each sample of porosities: 0.28, 0.36, 0.37, 0.40 and 0.42. Volumetric approach was used to determine the porosity, plate count method and vertical downward flow were used for bacterial count and column experiment respectively. The coefficient of attenuation as a function of time was calculated. The results showed that attenuation capacity of media increases with increase in depth and decrease in porosity. We are able to observe a variation in attenuation capacity with both bacteria due to difference in their morphological characteristics. This investigation revealed that we can predict threshold depth and porosity for significant and meaningful attenuation of migration with minimum depths of 14cm and 21cm and maximum porosities of 0.34 and 0.36 for E. coli and Staphylococcus respectively. This will be applicable in better design of hydrogeological barrier against pathogenic contamination of groundwater supply source and their associated diseases.

Keywords: Bacteria, Groundwater, Filtration, Hydrogeological barrier, Attenuation, Migration.

1.0 Introduction

Groundwater is the water that infiltrates the soil from rainfall and penetrates to the underlying strata. The water bearing strata is called aquifers and consists of unconsolidated material like sandstones and limestone. The underground water supplies are usually considered safe provided they are properly located, constructed and operated according to the world health organization guidelines for drinking water [1]. However, microbial pollutants released from industries, domestic and agricultural activities to the ground can work their way down into groundwater. The movement and dispersion within the aquifer can spreads the contaminants over a wide area, which can then intercept with groundwater wells or find their way back to surface water making the water supplies unsafe. Thus, groundwater that was traditionally assumed to be pure and safe for drinking without treatment have now been found not to be so due to increased human activities [2].

The contamination of subsurface water by pathogenic bacteria and viruses has caused large outbreaks of water borne diseases [3]. In USA, poor microbiological quality of groundwater systems has caused many disease outbreaks. A review by Macler and Merkle [4] reported the initial results of a major public health study of the occurrence of pathogens in U. S groundwater supplies involving the sampling of 244 public water-supply wells prior to any treatment. About 50% of wells initially considered more vulnerable to contamination and 40% of wells considered less vulnerable were positive for one or more faecal indicators (total coliform bacteria, E. coli, enterococci, viruses infecting coliform bacteria, and human viruses). Craun and Calderon [5] reported that between 1971 and 1994, 58% of U.S. waterborne disease outbreaks (356 in number) were caused by contaminated groundwater systems. That 70% of these outbreaks were considered to be due to contamination of the groundwater source as opposed to the distribution system.

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The situation in Nigeria is disturbing as recent reports suggested that 80% of the hospital patients on admission in Nigeria had water related problem [6]. Ibe and Okplenye [7] sampled water from four different boreholes within Ulli, Anambra state, Nigeria and subjected them to bacteriological analysis to assess their portability; the finding showed that the water from all the boreholes did not meet the world health organization standards for drinking water and should be treated, boiled and filtered before drinking. In spite of these coupled with several reports both in developed and developing countries including Nigeria where majority of people lived in rural and sub-urban area; private groundwater supplies such as shallow wells and boreholes which all have their origin linked with groundwater serve as main sources of water for drinking, agriculture and domestic use. There is a need to find a better approach to source-water contamination by microorganisms, to reduce the likelihood of bacterial infections in drinking water supplies. This approach will favour long travelling times of bacteria from contaminant sources such as septic tank, soakaway and pitlatrines through elimination/ attenuation of the microbial contaminants with the use of sorted sand soil as hydrogeological barrier. Long travelling times favour the natural attenuation/ elimination of micro-organisms, but most private water supplies have a very limited catchment area which shortens the travelled distance of bacteria from the soakaway, septic tank and pit-latrines to the water source, a problem which this work intend to suggest a solution.

Soil is the first barrier to pathogen contamination of aquifer, but due to variability in the structural and compositional distribution of strata it is therefore vulnerable. Using a sorted sand media of prescribed porosity and depth would help in increasing the distance of travel/time of travel of bacteria which favour attenuation and elimination of micro-organisms into the groundwater. This will not only reduce the cost of water treatment such as use of ultraviolet ray for disinfection against micro-organisms but also reduce the outbreak of diseases associated with microbial contamination and the cost of their treatment. This will save individual, state and national government huge amount of money in containment of waterborne diseases.

This above can be achieved by attenuating the influx of bacteria from soak away into the groundwater sources by filling the bottom of the soak away/pit latrines with sand media of predetermined parameters (porosity and depth). These parameters are the basic quantities that their threshold needed to be determined to know the type of porous media (porosity) and the depth which the attenuation and elimination of bacteria would be significant in protecting groundwater source from pathogenic contamination and their associated diseases.

There are several research studies on the transport of microorganism in porous media relating to chemical and microbiological aspect of this problem but little work had been done in respect to the physical parameters on the migration of bacteria in the subsurface. Smith et al [8] investigate bacteria migration through undisturbed natural deposits using laboratory based column studies. They noted a significant decline on bacteria concentrations in the column effluent once the material had subsequently been homogenized and repacked, thus illustrating the importance of bacterial migration through preferential flow paths. Harvey et al., [9] studied the effects of surface characteristics upon retardation and soil attenuation of bacteria with microsphere. Barton [10] examined an experiment on measurements of bacterial penetration in sand columns with uniform particle size and results showed that effective random motility decreases with decreasing particle diameter. Barton and Ford [11] studied the impact of particle diameter on the effective transport coefficient using silica sand (Homogeneous) of sizes between 80μm and 800μm. They observed that effective random motility of bacteria population decreases with decreases particle diameter. Kouznetson et al [12] also compare and simulate the transport and fate of microorganism from waste water for surface and subsurface irrigation methods and observed that surface irrigation appeared to be efficient in decreasing the number of pathogenic in irrigated water. Antonina et al [13] examined the effect of media characteristics (type of soil, hydraulic load, and depth and water application) on the removal of bacterial and viral indicators in vertical flow constructed wetland and intermittent sand filter. They observed that filter with a depth of 65cm presented significant ($P<0.05$) higher removal of bacteria than those of 25cm.

Of all these studies, there has not been any that was tailored toward protecting groundwater supplies source, nor study extensively on the effect of porosity on the attenuation of migration of bacteria in sand media. In this work we examined the effectiveness of sand as a protective layer against bacteria migration into the groundwater supply sources. The objective is to determine the porosity and depth of the sand media for significant bacteria migration attenuation.

2.0 Theoretical Background

Coefficient of Attenuation is the measure of reduction of migration of bacteria (motility). It is controlled by dispersion, retardation (attachment/detachment) and filtration processes. The coefficient of attenuation can be approximated by exponential function [3].

$$C = Co \exp(-\lambda_t t) \quad (1)$$

Factors known to influence the attenuation and retention of bacteria in porous media are; Straining: porous media, bacteria cell size and shape, Adsorption: physical, chemical and microbiological. Physical porous media, flow velocity & temperature;Chemical Ionic strength and pH; Microbiological chemotaxis, hydrophobicity, electrostatic charges and bacteria concentration.

3.0 Materials and Method

Samples collection and determination of porosity

Sand samples were collected from the river bed in Osun state, one from Osun-river along Iwo – Gbongan road and the other from Waterworks in Iwo. Sizeable sand quantities were brought to the laboratory in polythene bags, washed with deionised water in order to remove fine organic materials that were in the process of decaying as a result of the work of soil micro organism [14]. The samples were then boiled in 1M hydrochloric acid for 2 hrs and latter treated with 1M of NaOH to remove metallic oxide coating on the sand and equilibrate the pH respectively. The resulting sand samples were washed again in deionised water two to three times; sundried and stony pebbles were removed. Sieve analysis was conducted using electric shaker with different sieve sizes (0.150mm, 0.212mm, 0.300mm, 0.425mm and 0.600mm) which gave different soil textures and labeled A, B, C, D, and E respectively. The porosity of each of the samples were determined using volumetric approach (equation 2).

$$\Phi = \frac{(a+b)-c}{a} \qquad (2)$$

Where 'a' is volume of sand (bulk volume) in mL, 'b' is volume of water (mL) and 'c' is volume of mixture of water and sand (mL)

Bacteria collection and Preparation

The fecal samples were collected in sterile sample bottles from septic tank and were subjected to microbiological analysis for isolation and characterization. Isolation was done using the pour plate method [15] on Eosin Methlene Blue Agar. The isolates with characteristic dark centre greenish metallic sheen was subcultured repeatedly by streaking on the new agar plates until pure culture were obtained. The isolates were characterized morphologically and biochemically, and the results were compared with Bergey's manual of determinative bacteriology [16] to deduce the bacteria identity.

The pure culture of the bacteria was inoculated with nutrient broth that have been prepared by sterilization at 121^{o}C for 15 minutes and cooled. This was then incubated with identified bacterial for 48 hrs at 37^{o}C. The inoculated broth was centrifuged by dispensing them into sterilized vial and spinned at 4000 rpm to harvest the bacteria cells. The cells were washed two to three times, after centrifugation and suspended in normal saline to avoid osmotic burst. This was then kept aseptically in the refrigerator at 4^{o}C. Exactly 1ml of the suspension was serial diluted and plated to determine the colony forming unit per milliliter (CFU/ml).

Column preparation and operation

Glass columns (1m long, 2.79cm diameter) pyrex were washed and disinfected with 70% ethanol and sterilized in hot air oven at 120^{o}C for 2 hrs. The column cylinder which was covered with muslin cloth at outlet was blocked so as to prevent water passage. The saturated sand was then poured into the water column up to height 'h' referred to the depth equal 10cm. The column was repeatedly tapped during packaging to prevent any entrapment of air bubble [17]. The screen cover was removed and water was allowed to pool down, until the dripping water from the column has a frequency of one drop per 10 seconds. Then, 2ml of bacterial suspension of known concentration was dropped onto the sand bed in the column with the aids of 5ml sterile syringe and this was followed by intermittent supply of 250 ml of distilled water. The effluents of the column was collected and analyzed for the bacteria load. This was done five times to have five rainfall simulations as it may occur under natural condition. Five effluents were collected differently in sterile beakers and covered immediately and subjected to microbial analysis using pour plate technique for the bacteria count. The time for various flows were taken and recorded. The effluents concentrations of bacteria were normalized to the respective influent concentration [18]. This process was repeated for four different depths (20, 30, 40 and 50cm). The coefficients of attenuation were determined using equation (1).

4.0 Results and Discussion

The column experiment was conducted to determine the fractional (relative) recovery of bacterial strains for the five samples of different porosity and each at five depths. This is to unveil the influence of depth and porosity on the migration of bacteria and to determine the maximum porosity and minimum depth at which the attenuation of migration would be significant (i.e threshold depth and porosity below and above which the attenuation and retention would be negligible respectively). The time taken for each flush at various depths for different sample was taken to determine the coefficients of attenuation using equation (1) [3]. Variation in the depth and porosity of the sand media produces changes in the time of flow for same quantity of loading which indicates a change of average pore velocity (Darcy) which is a function of porosity; thus porosity is an indispensable parameter when designing hydrogeological barrier against microbial contaminants. Table 1 presents the values of average relative concentration of bacterial recovered at various depths for different porous sample for Escherichia coli while Table 2 presents the same for staphylococcus aureus. These were used to determine the coefficients of attenuation which in this context is the summation of filtration and adsorption processes.

Our observation revealed that as the depth of the media increases, relative concentration of bacteria recovered decreases which implied more bacterial are retarded /attenuated with increasing time, therefore increasing the residence time of bacteria before elution, thus a reduction in migration (Table 1 and 2). Table 3 presents the determined values of coefficient of attenuation (λ_t) at various depths for porosity ranges between 0.28 and 0.42 considered in the work, while Table 4 presents coefficients of attenuation at various porosities. The trend in Table 3 revealed that as depth increases, the coefficient of attenuation increases; the zero magnitude at 40 cm and 50 cm for E. coli does not meant zero attenuation. We observed through point extrapolation that these are indication that there exist a minimum depth at which attenuation is significant below which the attenuation and retention would be insignificant. Table 4 revealed that as porosity increases, the coefficients of attenuation decreases for both organisms.

Table 1: Computed average value of normalized concentration (C/C_o) for five drains at different porosity for the five depths considered (Escherichia coli).

Porosity (ϕ)	**C/Co @ 10.00cm**	**C/Co @ 20.00cm**	**C/Co @ 30.00cm**	**C/Co @ 40.00cm**	**C/Co @ 50.00cm**
0.28	0.1228	0.1186	0.0885	0.0744	0.0514
0.36	0.2904	0.1846	0.1789	0.1577	0.0846
0.37	0.1734	0.1699	0.1445	0.1538	0.0694
0.40	0.2986	0.2296	0.1986	0.0902	0.0845
0.42	0.3514	0.2972	0.1910	0.1164	0.0949

Table 2: Computed average values of normalized concentration(C/C_o) for drains at various depths for five samples (Staphylococcus Aureus).

Porosity (ϕ)	**C/Co @ 10.00cm**	**C/Co @ 20.00cm**	**C/Co @ 30.00cm**	**C/Co @ 40.00cm**	**C/Co @ 50.00cm**
0.28	0.1307	0.0742	0.0597	0.0484	0.0307
0.36	0.1450	0.0883	0.0800	0.0733	0.0467
0.37	0.2675	0.1100	0.0775	0.0588	0.0463
0.40	0.2485	0.1809	0.1559	0.1162	0.0838
0.42	0.2356	0.1976	0.1485	0.0896	0.0724

Table 3: Determined values of coefficient of attenuation (λ_t) at various depths).

Depth (cm)	**10.00**	**20.00**	**30.00**	**40.00**	**50.00**
Attenuation coefficient(λ)/cm					
Escherichia coli	-0.15	-0.05	-0.02	0.00	0.00
Staphylococcus aureus	-0.14	-0.07	-0.02	-0.01	-0.01

Table 4 : Determined values of coefficient of attenuation (λ_t) at various porosities.

Depth (cm)	**0.28**	**0.36**	**0.37**	**0.40**	**0.42**
Attenuation coefficient(λ)/cm					
Escherichia coli	-0.01	-0.05	-0.09	-0.28	-0.53
Staphylococcus aureus	-0.01	-0.05	-0.22	-0.21	-0.47

With increase quest for the protection of groundwater supplies source against bacterial contamination and their associated diseases, few research works have been aimed at verifying the effect of porosity and depth on the attenuation and retention of bacteria migration through saturated porous media. More so in developing countries, like Nigeria where the distance between groundwater supply source and the soakaways, pit latrines and septic systems is very small (<25m), and thus a need for the use of hydrogeological barrier in shielding the influx or reducing the rate of inflow of this contaminants into the water wells. It is prominent to know the minimum depth and maximum porosity of the sand media that will be applicable in design of a functional barrier layer.

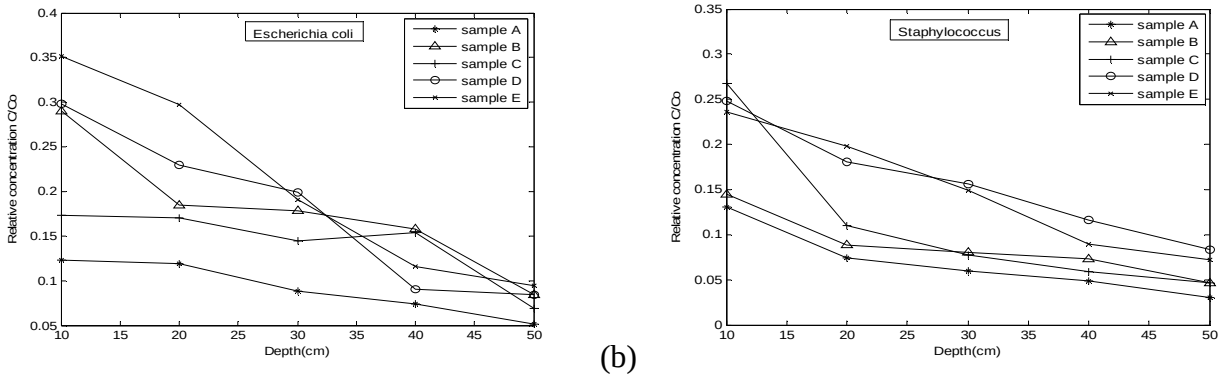
Effect of media depth

The depths of the media considered in the research work are 10, 20, 30, 40 and 50 cm for different porous samples. From figure 1(a & b), it was approximately revealed that the higher the depth the higher the coefficients of attenuation for both

organisms (E coli and staphylococcus respectively). With sample A given the lowest relative recovery of bacteria with depth for both organism and sample E the least. Our results agreed with the findings of Antonina et al. [13] that filter with a depth of 65 cm presented significant ($P < 0.05$) higher removal of bacteria than those of 25 cm, and work of [19] that the efficiency of a rapid filter is directly related to the media depth; but contrary to the work of Jose et al.[20] and Reddy et al. [21] that there is no significant effect of soil depth on the fecal coliform removal from the fact that straining occurs over a relatively short time, but they did not put into consideration that straining is not the only mechanism responsible for the retention/attenuation of migration of bacteria in porous media. The plot of coefficient of attenuation versus depth was used to determine the threshold depth for both bacteria strains. We observed minimum depth of 14 cm and 21 cm for Escherichia coli and Staphylococcus aureus respectively (Figure 3(a)). The variation in the threshold depth for bacterial attenuation in sand media is as a result of their shape coupled with other microbiological characteristics.

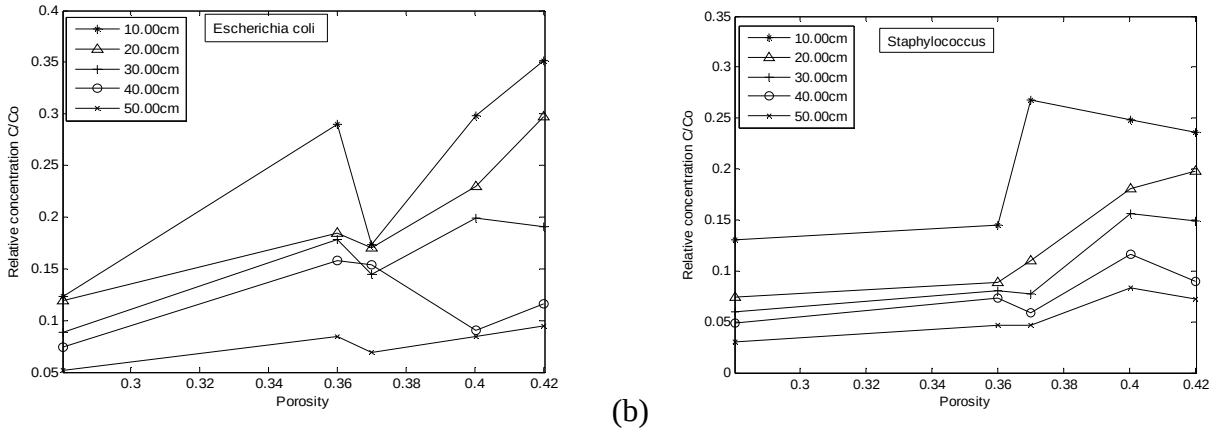
Effects of Porosity

Porosity is a property of porous material that depicts the macro properties of the material. It is a basic physical parameter that characterizes the effectiveness of the sand media as a filter. The result of average normalized concentration of bacteria and porosities for the depths considered revealed that attenuation of migration of bacteria depend on porosity for all depths as shown in figure 2 (a & b). The attenuation capacity of media with lowest porosity for sand porous media is greater than for other due to larger contact time between the grain surface and bacteria strains that led to multiple adsorptions to grain surfaces. As porosity increases, the permeability increases, this reduces the contact time between the particle and the grain surfaces thus reducing the rate of adsorption which directly lead to the reduction the attenuation capacity of the media for all the depths. It was observed via extrapolation of data (Table 4) in this work that sand of porosity 0.34 and 0.36 are the maximum porosity for E coli and Staphylococcus aureus respectively (Figure 3(b)) for significant attenuation and retention. The use of fine to medium size media enable us to unveil this relationship of porosity with bacteria migration attenuation/retention which in contrary to most of the findings which observed that there is no relationship between porosity and bacteria motility.



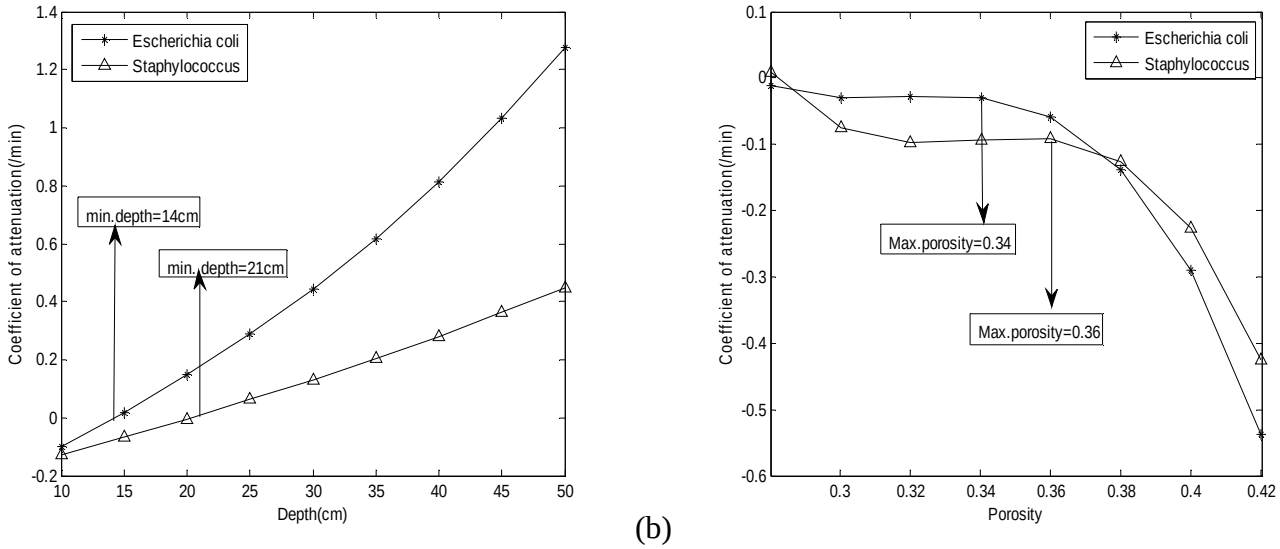
(a) (b)

Figure 1 (a) and (b) show the plot of average relative concentration of bacteria recovery at different depths for the various sample of different porosities for Escherichia coli and Staphylococcus aureus respectively.



(a) (b)

Figure 2 (a) and (b) show the plot of average relative concentration of bacteria recovery versus porosity for different depths for Escherichia coli and Staphylococcus aureus respectively.



(a) (b)

Figure 3(a) show the plot of coefficient of attenuation versus depth to determine the minimum depth at which the media start attenuating the migration for both bacteria while figure 3(b) the graph of coefficient of attenuation versus porosity to determined the threshold porosity above which the porosity has no effect on attenuation of bacteria in sand media.

Conclusion

The attenuation of migration of bacterial in porous media depends grossly on the depth and the porosity of the media which directly determined the efficiency of such media (sand) as hydrogeological barrier and filter for protection of groundwater supplies source. This can be achieved by filling the bottom of earthen septic tank, soakaway and pitlatrines by sand media of homogenized and predetermined porosity and significant depth. This will increase the retention time of travel (travelled distance) and increases the attenuation capacity. From this work, it can be concluded that the combination of low porosity and high depth proves to be critical.

- The findings suggest a minimum depth of 14 cm for E coli and 21 cm for Staphylococcus for significant attenuation.
- It was also observed that the maximum porosity is 0.34 for E coli and 0.36 for staphylococcus for significant attenuation and retention.

From the above results, porosity and depth of the media are significant physical parameters to be considered when designing sand media as a protective layer against groundwater contamination. The results of our finding can also be applicable in the design of water treatment sand filter. In design a layered media for water filtration, the minimum depth of a single layer should not be less than 14 cm and porosity not greater than 0.36 for effective filtration and purification.

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