Efficiency of saprolite for removing *E. coli* from simulated wastewater

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ABSTRACT

Saprolite, weathered bedrock, is being used to dispose of domestic sewage through septic system drainfields, but its ability to remove coliforms is unknown. This study determined if *Escherichia coli* could be removed by a sandy loam saprolite material. Triplicate columns containing saprolite were prepared with lengths of 30, 45, and 60 cm. A 215-mL solution containing 1×10^5 CFU/100 mL of non-toxic *E. coli* was applied to the top of each column for 5 days/week for 13 weeks, and selected outflow samples were analyzed for *E. coli.* Control columns had only tap water applied to them at the same time. Significantly higher ($p \leq 0.10$ compared to controls) *E. coli* concentrations were only detected in samples collected at the end of week 3 for the 30-cm columns and week 4 for the 45-cm columns. *E. coli* concentrations were small and ranged from approximately 2 to 3 MPN/100 mL. No *E. coli* were detected in any outflow from the 60-cm columns. From weeks 5 to 13, *E. coli* concentrations from all columns were either undetectable or not significantly different from the control. The results showed that 60 cm of sandy loam saprolite was sufficient for the removal of *E. coli* from simulated wastewater.

Key words | coliforms, filtration, piedmont soils, septic systems, weathered bedrock

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HIGHLIGHTS

- A laboratory study showed that a sandy loam saprolite (weathered bedrock) material can remove *E. coli* from simulated wastewater.
- Saprolite at least 60 cm thick appears suitable for use with septic systems to treat wastewater on-site.
- Results of the study will be useful for state health regulators in the USA to assess saprolite suitability for on-site wastewater disposal.

INTRODUCTION

On-site wastewater management systems (OSWMS), commonly referred to as septic systems, are the most common means of treating and disposing of sewage in areas not served by a sewer system. Approximately 20% of the households in the USA, and one-half of those in North Carolina (NC), manage their domestic sewage on-site using one of the OSWMS (US Census Bureau 2004, 2011). It is estimated that currently approximately 24,000 new OSWMS are installed each year in NC.

A conventional on-site wastewater management system consists of a septic tank and a drainfield (US EPA 2002). The septic tank provides primary treatment to the sewage by allowing solids to settle and go through anaerobic digestion. After the liquid (referred to as wastewater) containing dissolved and suspended organic materials, as well as biological (e.g., *Escherichia coli* (*E. coli*)) and chemical pollutants, leaves the septic tank, it is dispersed into the soil through a series of trenches in the drainfield. In unsaturated and aerated soil, some pathogenic bacteria are removed through physical filtration, and the anaerobic bacteria typically die off in the aerobic soil environment (Foppen & Schijven 2006).

Both field and laboratory studies have shown that 60 cm of aerated, unsaturated sandy soils performs well as a filter for *E. coli* (Cogger *et al.* 1988; Stall *et al.* 2014). According

doi: 10.2166/wst.2020.514

to NC OSWMS regulations, a minimum of 30 cm of unsaturated suitable soil must be present below the bottom of the trenches for most OSWMS. The trenches for conventional systems are typically 60-cm deep; therefore, a minimum of 90 cm of suitable soil must be available for installing a septic system (NCPHEH 2017). Such a suitable soil depth is becoming more difficult to find in the rapidly growing urban populations of the Piedmont and Mountain regions of NC and other Eastern states of the US.

Saprolite (commonly known as rotten rock) is isovolumetrically-weathered bedrock that has had some of its original minerals dissolved and removed, while new pores have developed between the mineral grains that were in the original rock (Vepraskas *et al.* 1991, 1996). Minerals that remain in the saprolite, such as quartz, muscovite, and feldspars, also are found in the overlying soil. While saprolite does contain fractures and veins that were present in the original rock, these features are frequently plugged with clay or oxide minerals (Williams *et al.* 1994). Water moves through saprolite by flowing around mineral grains, or through small root channels (Williams & Vepraskas 1994; Li *et al.* 1997). The structure of saprolite is described as 'massive-rock controlled', meaning that the planar voids commonly found between the structural units of soil (i.e., soil peds) are absent in saprolite. Saprolite is found under virtually all soils in the Piedmont and Mountain regions of the Southeastern USA, and extends from the bottom of the soil solum to solid bedrock (Daniels *et al.* 1999). The thickness of saprolite materials is variable and can range from <1 m where bedrock is shallow to over 50 m thick where the bedrock is deep (Daniels *et al.* 1999). In NC, saprolite can be used for OSWMS, but the regulations for its use are much more stringent compared to soils with comparable texture (NCPHEH 2017). One reason for these restrictions is the lack of knowledge regarding the efficacy of pathogen removal by saprolite within the unsaturated zone below the trenches.

Because few OSWMS have been installed in saprolite, determining the ability of this material to remove coliforms has to be performed in the laboratory using indicator organisms. *E. coli* is commonly used as an indicator organism because it is a major coliform found in the feces of humans (Medema *et al.* 2003; Gerba & Smith 2005). Viruses are also present in waste materials but are more difficult to detect and use as indicator organisms. For a preferred single indicator organism, Karim *et al.* (2004) recommended *E. coli* be used, because in the sites they examined these coliforms were found in most cases (82%). Coliforms such as *E. coli* can be removed from soil water by: (1) attachment to soil particles, (2) die-off or being consumed by larger soil microorganisms, and (3) straining whereby coliforms are trapped in pore throats that are too small for the organisms to pass through (Foppen & Schijven 2006). The objective of this study was to determine the effectiveness of a saprolite material in removing *E. coli* from simulated wastewater.

METHODS

Saprolite

Saprolite material (C horizon) was collected from a site in northern Guilford County, NC, approximately 16 km north of Greensboro at N 36.21162°, and W −79.784433°. This saprolite was formed from a felsic crystalline rock, similar to a granite gneiss or granite. The overlying soil was mapped as Clifford sandy loam (fine, kaolinitic, mesic Typic Kanhapludults). The site was selected because the saprolite below the soil solum was considered suitable for wastewater disposal by the NC On-Site Water Protection Branch. Approximately 50 kg of saprolite material was excavated from a pit, collected in 5 gallon (approximately 19 L) buckets, and transported to the laboratory. Three undisturbed cores were also collected from the pit in 76 mm diam. × 76 mm tall cylinders.

In the laboratory, the undisturbed cores were oven-dried at 105 °C for 24 hours in order to determine bulk density (mass/total volume) by the core method (Grossman & Reinsch 2002). The bulk saprolite material was air-dried and passed through a 2-mm mesh sieve. A particle size analysis was performed using the hydrometer method (Gee & Or 2002). Three cores were packed with the sieved material to the same bulk density as undisturbed saprolite in the field. The saturated hydraulic conductivity of the repacked saprolite was determined using the constant-head method (Amoozegar & Wilson 1999). The cores were then re-saturated with tap water from the bottom, placed into pressure cylinders containing porous ceramic plates, and desorbed incrementally at soil water pressure heads ranging from 0 to −400 cm to determine a water characteristic curve (Dane & Hopmans 2002). Water retention data were used to estimate the diameters of the pores in the cores using an abbreviated version of the capillary rise equation:

$$D = 0.3/h \quad (1)$$

where D is the diameter of the pore in cm, and h is the water pressure head in cm.

Experiments

Cylinders (40, 55, and 70-cm long) were constructed from 6-inch (15 cm inside diameter) polyvinyl chloride (PVC) pipe (Figure 1). A drainage outlet at the bottom was installed to allow the collection of pore water (or solution) samples. Air-dried, sieved saprolite material was packed into each cylinder over a 2-cm thick layer of coarse sand to the natural bulk density of the saprolite in the field. The filled cylinders contained saprolite columns in lengths of 30, 45, and 60 cm (Figure 2). Three replicate columns were prepared for each of the 30, 45, and 60-cm treatment lengths that were to only receive the tap water–*E. coli* suspensions. One column of each size was used as a control and received tap water (no *E. coli*). The columns were arranged in a randomized pattern on the laboratory benchtop.

Initially, the saprolite columns were saturated with tap water from the bottom, and then allowed to drain to wet all absorbent surfaces. After reaching equilibrium (no drainage water after 2 d), 215 mL (equivalent to 0.3 gal/(ft^2 d); (12 L/m^2 d) of tap water was applied to the top of each saprolite column daily for 5 d to establish a flow regime and to verify that clogging did not occur.

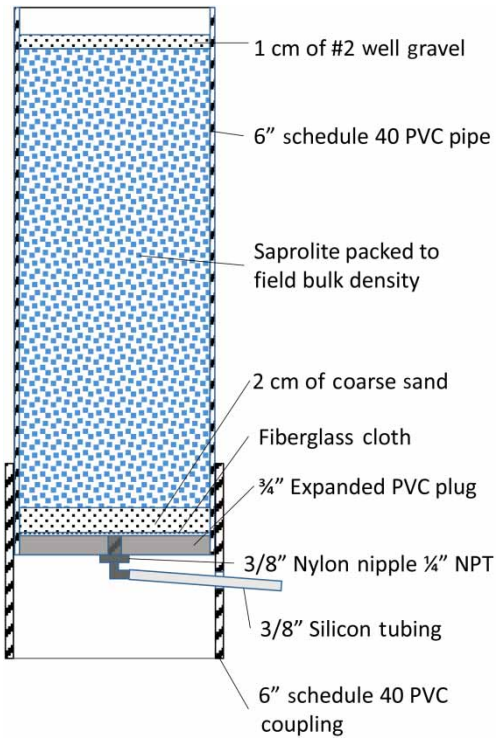

Figure 1 | Cross-section of a saprolite-filled cylinder showing how it was constructed (1″ = 2.54 cm; NPT: American National Standard Pipe Thread).

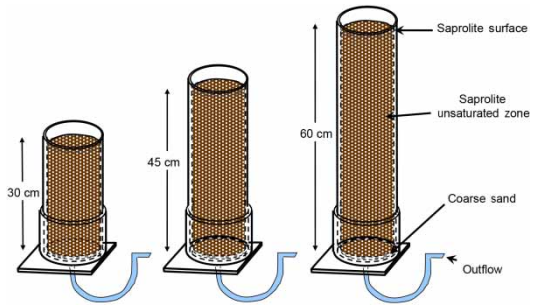

Figure 2 | A replicate set of saprolite-filled cylinders with saprolite in lengths of 30, 45, and 60 cm.

Non-pathogenic *E. coli* (*E. coli* K-12, Carolina Biological, NC) were inoculated into nutrient broth (Difco Laboratories, Detroit, MI, USA) and grown overnight at 37 °C without agitation. Cells were harvested by centrifugation at 10,000×*g* for 15 min. The bacteria pellets were re-suspended in autoclave-sterilized tap water (215 mL each), to a concentration of 1×10^5 CFU/100 mL, to form the 'simulated wastewater', which is close to the concentration found in regular household wastewater. Cell culture densities were determined by serial dilution in autoclave-sterilized aqueous solutions of saline (NaCl, 0.85%). *E. coli* density was determined by spread-plate using a Quebec Darkfield Colony Counter (Reichert Technologies, Buffalo, NY, USA), and bacterial cell biomass was determined by turbidity using a Genesys 10S UV-VIS spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) read at OD 600 nm wavelength.

For the experiments, 215 mL of the *E. coli* suspension was applied to each column daily from Monday to Friday. Weekend applications were not done to allow resting as may occur in some OSWMS such as low-pressure pipe or drip irrigation systems. This application rate simulated the average loading rate of 0.3 gal/(ft^2 d) specified in the proposed NC regulations for saprolite with a loam texture. Following the addition of *E. coli* suspension to the top of each column, approximately 215 mL of effluent was collected from the bottom of each column daily.

The *E. coli* suspensions were added to the columns until no *E. coli* were detected in the outflow from any column for a period of 6 continuous weeks. This was deemed adequate as indicating that no *E. coli* was likely to be exiting the columns following additional applications of *E. coli* suspensions. Using this criterion, *E. coli* suspensions were applied to the columns for 13 consecutive weeks from the beginning of the applications. It is important to note that although the study focus was not to determine *E. coli* survival under the experimental conditions, previous studies have shown that *E. coli* have the ability to survive for extended

periods of time in supplemented and unsupplemented waters (e.g. tap water) in both natural and laboratory settings (Smith & Badawy 2008; Mosaddeghi *et al.* 2009). This is especially the case when biofilm communities attached to particle surfaces are formed

Effluent samples from all columns that were collected from Monday to Thursday were stored under 4 °C refrigeration. The effluent samples that were collected on each Friday were examined for the presence/absence and quantity of *E. coli* as soon as possible using the Colilert Test Kit and Quanti-Tray enzyme-substrate system (IDEXX Laboratories, Westbrook, ME, USA) following the manufacturer's instructions. Briefly, after the addition of the effluent to the enzyme-substrate system, *E. coli* metabolized the Colilert nutrient-indicator. The positive samples fluoresced under a 6-watt, 365-nm UV light. Colilert was detectable when *E. coli* at 1 CFU/100 mL were present within 24 hours following incubation at 35 ± 0.5 °C. As the upper limit, as many as 2×10^6 *E. coli* per 100 mL could be detected if present. All samples were disinfected and discarded at the end of the experiments.

Statistical analyses

E. coli concentrations in outflow samples were compared to the control concentrations using a Student's *t*-test (1-tailed, two-sample with unequal variance). A significant difference was assumed when the probability values of finding a higher *t* value were ≤0.10.

RESULTS AND DISCUSSION

Selected physical properties of the saprolite are shown in Table 1. Based on the texture, saturated hydraulic conductivity, and bulk density, this saprolite represented a typical material formed from felsic gneiss rocks in NC (Daniels *et al.* 1999).

Water retention characteristics are shown in Table 2 for soil water pressure heads between 0 and −400 cm of water. Water content values are reported separately by replicate for each water pressure. The water contents were similar across replicates for a given pressure indicating the core packing and pore size distribution were similar among the replicates. Saturation percentages are also shown. The 30-cm long columns would have had a soil water pressure head of −30 cm (unsaturated, aerated conditions) at the top of the column and a soil water pressure head of nearly 0 cm (saturated conditions) at the bottom. As shown in Table 2, this meant the water content within the 30-cm long columns ranged between 90 and 100% saturation from top to bottom. Similar results were found for the other columns with slightly lower water contents near the surface. These

Table 1 | Properties of saprolite that was packed into the cylinders

Saprolite property	Property subdivision	Property value
Textural class		Sandy loam
Texture	Sand	62%
	Silt	30%
	Clay	8%
Coarse fragments (>2 mm diam.) removed before packing		
Sand-size distribution	Very coarse	10%
	Coarse	14%
	Medium	14%
	Fine	15%
	Very fine	9%
Water content at packing		0.06 g/g
Saturated hydraulic conductivity		1.34 cm/hr
Bulk density		1.30 g/cm^3

Table 2 | Water retention characteristics of saprolite cores

	Water content (cm^3/cm^3) for different water pressure heads (cm)							
Rep.	0	−15	−30	−45	−60	−100	−200	−400
1	0.56	0.51	0.50	0.48	0.47	0.41	0.33	0.25
2	0.54	0.50	0.49	0.47	0.47	0.42	0.33	0.25
3	0.54	0.50	0.49	0.47	0.46	0.42	0.32	0.25
Mean	0.55	0.50	0.49	0.47	0.47	0.42	0.32	0.25
Saturation percentage	100	91	90	87	86	76	59	46

Table 3 | Pore size distribution of saprolite cores

Pore diameter (mm)	Volume (cm^3/cm^3)
>0.2	0.05
0.2	0.01
0.1	0.02
0.07	0.01
0.05	0.05
0.03	0.09
0.02	0.07
≤0.01	0.25
Total	0.55

Volumes are means of three replicates.

data indicate the columns were close to saturation during the experiment, which would result in relatively rapid water flow through the materials.

The volume of pores found in the repacked saprolite cores are shown in Table 3 for size classes ranging from ≥0.2 to ≤0.01 mm. Pores with diameters ≤0.01 mm occupied the most substantial volume of pores in the columns. Pores in this class are small enough to filter the *E. coli* (Foppen & Schijven 2006). All pores were formed as spaces between the mineral grains or small aggregates that passed through the 2-mm sieve. Bypass flow through macropores (pores having diameters >1 mm) was not expected.

The concentrations of detected *E. coli* (in most probable number (MPN)/100 mL of sample) from the effluent over the test period are shown in Figure 3. No *E. coli* were detected in any samples for weeks 1 and 2. The absence of *E. coli* during the early stages of the experiment suggests that the solution was moving through the saprolite matrix and not along the wall of the column (i.e., 'wall flow') where saprolite met the side of the PVC cylinder. Movement through the matrix and around mineral grains is termed 'matrix-flow', which would

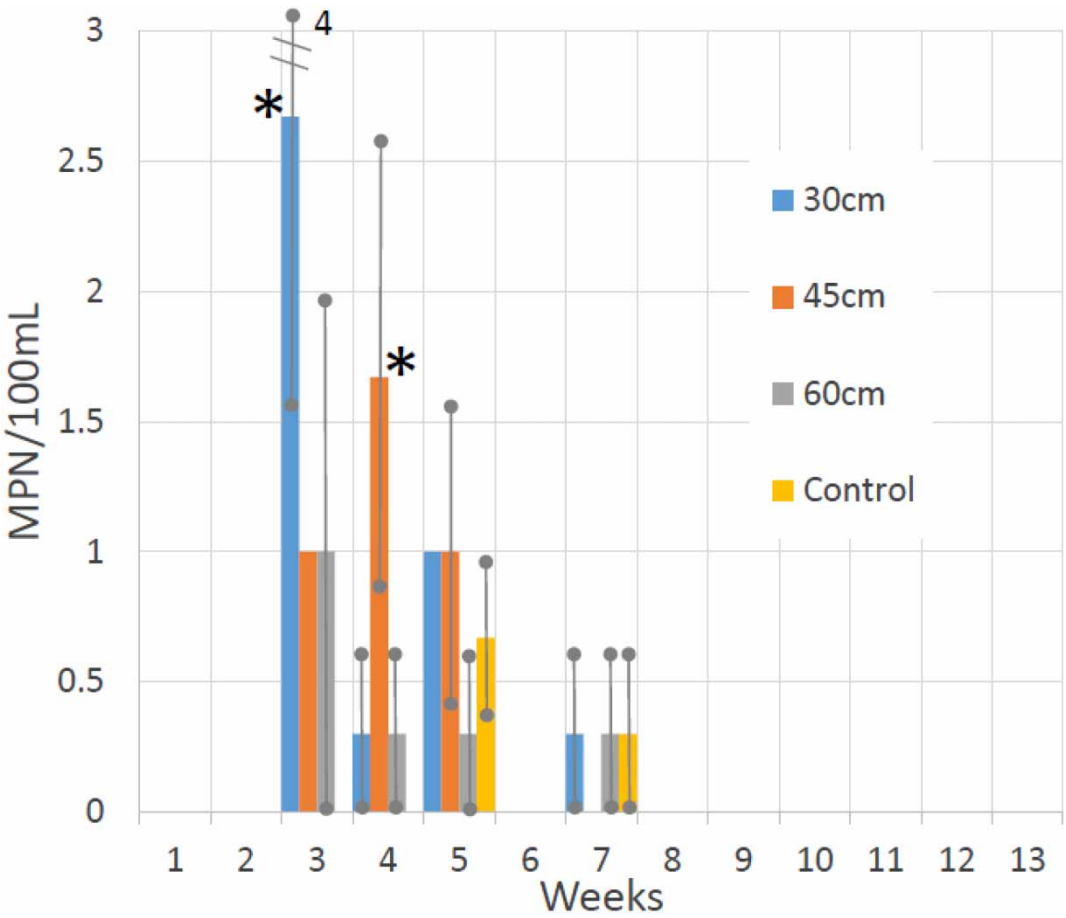

Figure 3 | Concentrations of *E. coli* (means ± standard errors) that were detected in the outflow from the saprolite columns for the weekly periods shown. Control cylinders combined all three saprolite columns as only tap water was applied to them. Asterisks show where *E. coli* concentrations were significantly higher than the controls ($p \leq 0.10$). Significantly higher concentrations of *E. coli* were found for the 30 and 45-cm columns for the weeks shown, but not for the 60-cm columns. One pore volume was estimated to pass through the columns after 13, 19, and 25 d for the 30, 45, and 60-cm long columns, respectively.

allow maximum filtering and adsorption of *E. coli* onto particles. In the absence of filtration or adsorption of the organisms by the saprolite matrix, water containing *E. coli* should pass through the column after 1 pore volume of water displacement (i.e., piston flow). One pore volume displacement was expected to occur at approximately 13, 19, and 25 d for the 30, 45, and 60-cm columns, respectively.

At the end of week 3, the *E. coli* outflow from the 30-cm columns had a mean concentration of approximately 2.7 MPN/100 mL, approximately 0.003% of the concentration initially added, which was significantly higher ($p = 0.08$) than the control (Figure 3). Although this concentration was small, it was too high (>1 MPN/100 mL) for drinking water standard, but suitable for wastewater reuse (<14 MPN/100 mL) (US EPA 2003; Humphrey *et al.* 2011). Considering that 1 pore volume would pass through the 30-cm column in 13 d if piston flow occurred, higher concentrations of *E. coli* in outflow may have occurred between 14 and 20 days. Nevertheless, these results showed that some *E. coli* did pass through the 30-cm long column. The *E. coli* collected in the outflow at the end of week 3 moved slightly slower than what would have been expected if the organisms remained suspended in solution. The outflow *E. coli* concentrations were not significantly different ($p > 0.10$) from the control for the other two column lengths.

At the end of week 4, *E. coli* in the outflow from the 45-cm column averaged 2 MPN/mL, which was significantly higher ($p = 0.10$) than the control. It was estimated that 1 pore volume would pass through the column in 19 d, so the *E. coli* were detected in the outflow after approximately 1.5 pore volumes had drained from the column. *E. coli* concentrations in the 30 and 60-cm columns were not significantly different ($p > 0.10$) than the control at the end of 4 weeks.

At the end of weeks 5 and 7, *E. coli* concentrations from all saprolite columns were ≤1 MPN/mL, and these values were not significantly different ($p > 0.10$) than the controls. No *E. coli* were detected in effluent from any column for weeks 8–13. No *E. coli* were detected in the outflow of the 60-cm long columns at concentrations that were significantly higher than the controls at any time during the experiments.

Although the columns were nearly saturated (Table 2), the saprolite was able to remove most *E. coli* from the solution in the 30 and 45-cm long columns, and all of the *E. coli* in the 60-cm long columns. Most of the *E. coli* were probably removed by filtration, where the organisms were too large to pass through some pores (Brennan *et al.* 2010). As shown in Table 3, most pores in the saprolite had effective diameters of ≤0.01 mm. We do not know what the distribution of pores smaller than this was in the saprolite. However, there were likely sufficient numbers of smaller pores to remove the rod-shaped *E. coli*, which range from 0.002 to 0.006 mm long with diameters of approximately 0.003 mm (Foppen & Schijven 2006). Adsorption of the microorganisms onto the saprolite mineral particles cannot be excluded. These results pertain to sandy loam saprolites. Sandier textured saprolites may give different results as they would have larger pores between mineral grains, which could allow more *E. coli* to pass through.

CONCLUSIONS

E. coli concentrations were significantly higher ($p < 0.10$) in the outflow samples from the 30 and 45-cm long columns compared to the controls for only one sampling period during weeks 3 and 4, respectively. *E. coli* appeared in the outflows approximately 7–11 d after we predicted 1 pore volume had passed through the columns. No significantly higher concentrations of *E. coli* were detected in the 60-cm long columns during any sampling period compared to the controls.

These results suggest that a minimum distance of 60 cm should be sufficient to remove *E. coli* from domestic wastewater in sandy loam saprolite. We recognize that if a biological clogging mat is established in the trenches of the drainfield, then it will provide an additional filtration and remove some *E. coli* before entering the underlying saprolite (Kristiansen 1981). Nevertheless, 60 cm of saprolite below a septic drainfield adds additional confidence that the *E. coli* will be removed, thus minimizing the risk of groundwater contamination. Additional work is needed to determine the most appropriate long-term acceptance rate based on the texture of saprolite and its distance to groundwater or any other sensitive boundary.

ACKNOWLEDGEMENTS

Funding for the research was supplied by the NC Department of Health and Human Services, Division of Public Health under Contract No. 00038125. Laboratory analyses were completed by Mattie Frazier. These contributions are greatly appreciated.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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First received 29 July 2020; accepted in revised form 16 October 2020. Available online 28 October 2020