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Original Research Article

Effect of Some Environmental Factors on the Survival Time of *Escherichia coli* in Human Feces Deposited on the Soil

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Indiscriminate defecation in open places is still a common practice in Agbani, Nkanu West Local Government Area, and Enugu State Nigeria. It is obvious that the practice does not only pollute the environment but also some of these people that defecate in the open places may be carriers of infections and may deposit enteric pathogens into the agricultural soils and in the process infecting the healthy community. Effect of some environmental factors on the survival time of *Escherichia coli* in human faces deposited in the soil was studied. Early morning fresh faecal samples were collected randomly in Agbani Nkanu West L.G.A. Enugu State. The enteric organisms were isolated from the faecal sample using standard cultural techniques and characterized, morphologically and molecularly and identified as Escherichia coli. The survival time of *Escherichia coli* exposed to sunlight, shade, pH and temperature was monitored for fifty days and the results showed that there was a decrease in number of *Escherichia coli* exposed to sunlight, shade, pH and temperature from day seven. *Escherichia coli* showed little or no growth on day fifty Hence, sunlight, shade, pH and temperature had a lethal effect on the survival of *Escherichia coli* that had stayed up to fifty days in fecal soil. However, elimination of enteric pathogens present in open defecated feces in soil by sunlight and other environmental factors may be slow, but it is cheap and lead to environmentally friendly agricultural products. However, Open defecation is not recommended because of the health hazards associated with it.

Keywords: Environment, open defecation, survival time, enteric organisms, human faces, soil, sunlight, shaded area, *Escherichia coli.*

INTRODUCTION

Advocacy on the dangers of open defecation practices is the main aim of improving access to sanitation worldwide and it is a proposed indicator for sustainable development goals. When large numbers of people are engaged in open defecation, it is extremely difficult to avoid ingesting human waste, either because it has contaminated the food through soil or water supplies or because it has been spread by flies and dust. Open defecation can also lead to water pollution, when rain flushes feces that are dispersed in the environment into surface water or unprotected well water. *Escherichia coli* are found to be present in human and animal feces, sewage, soils and water. The aim of this study was to determine the effect of some environmental factors on the survival time of *Escherichia coli* in human faces in the soil. It is expected that the results of this research work would provide adequate information on the effect of environmental factors on soil enteric organisms. It would also serve as a surveillance guide to health care providers and pharmaceuticals in determining the time frame some of these enteric pathogens would survive in the soil especially in the area of disease outbreak caused by these enteric pathogens. It is also expected that the results of this research work if not effective would discourage open defecation practices in Nigeria and enlighten the public on the dangers of open defecation practices if the exposed factors were not effective as

cidal agents and aside from aesthetic reasons, it would also discourage soil fertilization with human faces which has been the potentialities of transmission of human diseases and parasitic infections through the consumption of raw fruits and vegetables grown on such soil. The soil environment consists of a variety of physical, biological and chemical factors that affect the abundance and diversity of microbes found in the soil. Sunlight, temperature and soil pH have been reported to be detrimental factors to the survival time of some enteric organisms in the soil environment (Meays *et al*, 2004). Understanding the potential of fecal bacteria to survive and grow under certain circumstances is critical for managing areas that have chronic high fecal counts. Microbial contamination of agricultural soils and drinking water is a major environmental and health issue worldwide. There have been studies supporting the idea that fecal bacteria can survive and grow in the soil environment. Some enteric pathogens deposited in farm soils through human defecation practices such as coliforms are monitored most frequently as total or fecal coliforms. However, *E. coli* can also originate in other warm-blooded animals, thus it cannot solely be diagnostic of human fecal contamination. Typically, *E. coli* constitutes about 20 to 30 percent of the total coliforms found in raw and treated domestic wastewater. Enteric bacterial pathogens die off very rapidly outside of the human gut whereas indicator bacteria such as *E. coli* persist for longer periods of time (Sped *et al*, 2000).

The survival of *E. coli* and their related pathogens in surface water is most greatly impacted by temperature. Elevated temperatures cause rapid inactivation and die-off of many microorganisms, especially in concert with desiccation. It has been shown that coliform bacterial survival is inversely proportional to temperature in the range of 5 to 15^{o}C. Furthermore, both visible and ultraviolet wavelengths are lethal to enteric pathogens and their indicators (including *E. coli*) after sufficient exposure (Sped *et al*, 2000).

However, some cells have UV repair mechanisms that can reduce impact and promote survival by some of the exposed population a trait that is particularly prevalent among viruses.

The die-off rate of pathogenic *E. coli* strain O157 is approximately equivalent to that of other strains; therefore, the evaluation of the total *E. coli* concentration is a suitable indicator for changes in pathogenic concentrations. Other faecal coliforms are less stressed by visible light when in the presence of humic substances, but may be more sensitive to lowered pH. However, most fecal coliforms and their potentially-associated pathogens are generally pH-tolerant, as demonstrated by their survival and proliferation in gastrointestinal systems of host organisms (Ferguson *et al*, 2003).

Open defecation and lack of sanitation and hygiene in general is an important factor in causing various diseases, most notably diarrhea, typhoid, cholera, urinary tract infection and dysentery which are caused by enteric pathogens.

MATERIALS AND METHODS

Study Area

The study was carried out at Agbani Nkanu in Enugu State Nigeria. Agbani is located in Nkanu West Local Government area of Enugu State, Nigeria. It is home to the diverse Eke Market and several institutions of learning such as the Nigerian Law School, Enugu State University of Science and Technology, Renaissance University, Mea Mater School, Airforce School among others. It is the home town of Dr Chimaroke Nnamani, a former executive governor of Enugu State Nigeria. Nkanu people live contigually within the Enugu East Senatorial zone of the present-day Enugu Sate of south-east Nigeria. They are predominantly farmers. In Agbani, the temperature typically varies from *64°F* to *88°F* and is rarely below *58°F* or above *91°F*. The area lie approximately between latitude 60 30’ North and longitude 70 30’ East, and stands on an estimated excavation of about 763 feet above sea level. According to sources from the defunct State Ministry of Works, Land and Transport, Nkanu clan occupies an area of about 1602.22 square kilometers. A colonial officer described the soil of the area as “fairly fertile with a light sandy soil made up of outcrop of laterite nature.” Indeed, the area is endowed with such mineral resources as bindle, salt, copper, and notably coal. Its population was estimated to 888,524 in 1990. At present it is presumed to be nearing almost 2 million.

Study Design

Fresh human faces deposited in open places were collected. The effect of sunlight, shade, pH and temperature on the survival time of *Escherichia coli* in the feces was determined. The study was designed, to take place during the dry season when there is abundant sunlight.

Ethical Consideration

No ethical consent was sought for because the faces were indiscriminately deposited and the identities of the depositors were not known.

Collection and Transport of Feces Samples

Fresh different faces samples in Agbani Nkanu, Enugu State were collected in the early morning. The freshly deposited faces were collected using stainless spoons that were sterilized in the hot air oven and were transferred into sample containers with leak-proof lids. The lids were sealed once the sample had been placed in the ice-packed containers. The samples were delivered to the Microbiology laboratory of Enugu State University of Science and Technology Enugu State Nigeria immediately after collection for culturing.

Isolation of Some Enteric Organisms from the Human Feces Deposited on the Soil

The isolation was carried out as done by (Conboy *et al*, 2000). One gram of the fecal samples was measured using electronic weighing balance and suspended in a beaker containing 9ml of sterile distilled water which was properly mixed and allowed to stand for 1 minute. The fecal suspension was streak plated in duplicates on MacConkey agar, Salmonella Shigella agar (SSA) and Nutrient agar and incubated for 24 hours at 35ºC. Bacterial colonies that developed after incubation were counted and the results were recorded. The isolates were severally sub cultured on Nutrient Agar plates to obtain pure culture: The sub culturing of the isolates was done by placing each colony in the middle of the agar plate and streaked up and down and across the plate. After inoculation, the plates were incubated for about 48 hours at 35^{o}C.

Morphological and Biochemical Identification of the Isolates

The isolates were identified morphologically according to (Cheesbrough, 2000) and also molecularly. The tests carried out were microscopic observation using compound microscope. Other tests carried out were Gram staining, Citrate utilization, Sugar fermentation, catalase, oxidase, Methyl red, urease, Indole and Voges proskaeur tests which were also carried out according to (Cheesbrough, 2000).

Antibiotics Sensitivity Test of the Isolates

The sensitivity test was carried out on the isolates to determine their susceptibility patterns to antibiotics using discs diffusion method as described by (Jorgensen *et al*, 2007).

Five milliliters of sterile water was added into a sterile test tube and a colony of the isolates was transferred into the tube of sterile water and was diluted to obtain a turbidity equivalent to 0.5 McFarland test standard. The diluted tube and 0.5 McFarland test standard was held against the black lined McFarland reference card to accurately rate the turbidity. Sterile swab stick was dipped into the properly adjusted inoculum and was streak- plated on the Mueller Hinton Agar plate. After 5 minutes, antibiotics sensitivity discs were placed on inoculated Mueller Hinton Agar plate using sterile forceps and the plate was incubated in an inverted position for 24hrs at 37^{o}C. The sensitivity pattern of the isolates were examined by measuring the inhibition zone with meter rule in millimeter and the values was compared with those on the disk diffusion zone diameter chart to determine the susceptibility level to the antibiotics.

Determination of the Effect of Sunlight on Survival of the *Escherichia coli* in the Feces

This was carried out using a method described by (Mubiru *et al*, 2000). A sterilized trowel was used to excavate soil with fecal deposit exposed to sunlight at a depth of 2 cm and was transferred into sterile cups for monitoring. On the day 1, One gram of the exposed fecal samples containing the isolate was measured using electronic weighing balance and was suspended in a beaker containing 9ml of sterile distilled water which was properly mixed and allowed to stand for 1 minute. The fecal suspension was serially diluted in 10-fold and thereafter streaked- plated in duplicates on sterile Eosin Methylene Blue agar (EMB) and was incubated for 24 hours at 37ºC. After incubation, the colonies of *E. coli* were counted using colony counter and the results were recorded. This test was carried out at weekly intervals for 50 days.

Determination of the Effect of Shade on Survival of *Escherichia coli* in the Feces

This was carried out using a method described by (Mubiru *et al*, 2000). A sterilized trowel was used to excavate soil with fecal deposit exposed to shaded area at a depth of 2 cm and was transferred into sterile cups for monitoring. One gram of the exposed fecal samples containing the isolate was measured using electronic weighing balance and was suspended in a beaker containing 9ml of sterile distilled water which was properly mixed and allowed to stand for 1 minute. The fecal suspension was serially diluted in 10-fold and thereafter streaked-plated in duplicates on Eosin Methylene Blue agar (EMB) and was incubated for 24 hours at 37ºC. After incubation, the colonies were counted using colony counter and the results were recorded. This was done at 7th day intervals for fifty days.

Determination of the Effect of Soil Temperature on the Survival of *Escherichia coli* in the Feces

The pH of the soil was determined using the method described by (Hamarshid *et al*, 2012). The soils pH on which the faces were deposited under sunlight and shade was determined and the results were recorded before collection of the fecal deposit for culture. A depth of 15cm of the soil was dug and distilled water was poured into the hole to make the soil moist. The pH probe was immersed into the soils and the pH of the soil were determined and recorded after 60 seconds before the fecal soil sample were cultured on EMB agar and incubated for 24 hours at 37ºC. After incubation, the colonies of isolates were counted using colony counter and the results were recorded. The test was repeatedly done at weekly intervals for 50 days.

Determination of the Effect of Soil Temperature on the Survival of *Escherichia coli* in the Faces

The temperature was determined as described by (McQuestin *et al*, 2009). The silver part of the thermometers was inserted into the faecal soil exposed to sunlight and the same was done on the soil under the shade and the temperatures were determined after 30 seconds before the fecal soil sample were cultured on EMB agar and incubated for 24 hours at 37ºC. After incubation, the colonies of isolates were counted using

colony counter and the results were recorded. The analysis was carried out every seven days for 50 days.

Determination of the Number of *Escherichia coli* in the Feces

The number of the enteric pathogens exposed to sunlight and shade was determined using the formula:

$$\text{No of isolates} = \frac{N}{V \times 10^{\wedge 5}}$$

Where N= Average Number of colonies obtained from the plates

V = Volume of the inocula (ml)

D = Dilution factor ($10^{\wedge 5}$)

RESULTS

The morphological characteristics of the *Escherichia coli* from the feces exposed to sunlight and shade are shown in Table 1.

Table 1: Morphological Characteristics of the Escherichia coli

Bacterial Isolate	Macroscopic Characteristics	Microscopic Characteristics
Escherichia coli	Consisted of thick, grayish-white, moist and smooth large colonies on Nutrient agar plate and also appeared mucoid bright pink colonies on MacConkey agar medium.	Consisted of Gram negative single short rods which appeared pink.

The biochemical characteristics of the *Escherichia coli* from the faces exposed to sunlight and shade are presented in Table 2.

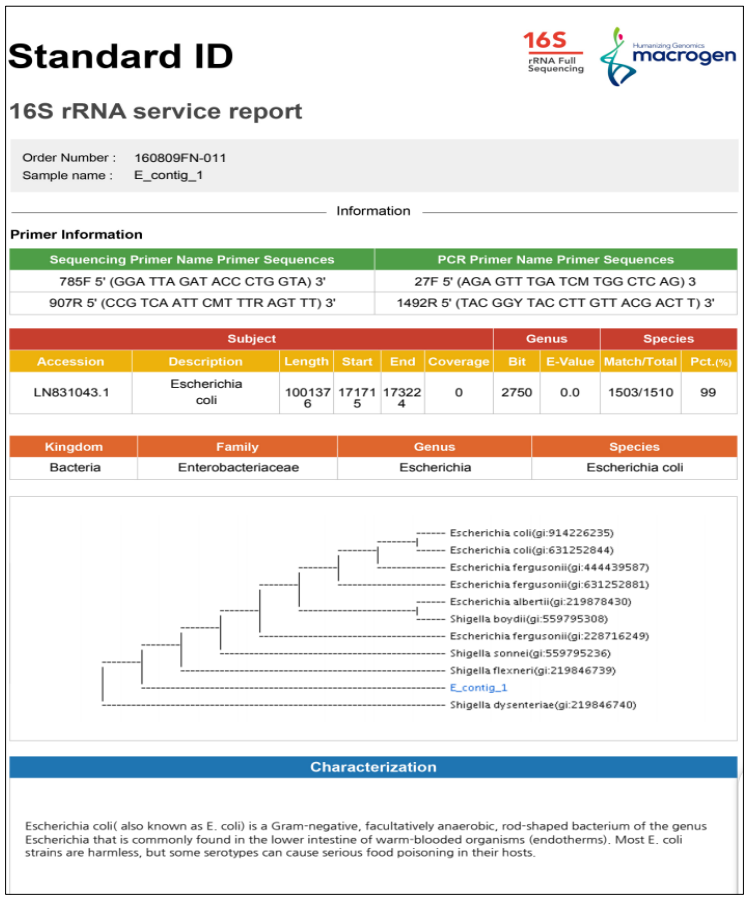
They were biochemically identified as *Escherichia coli* by their unique reactions to the chemical reagents.

Table 2: Biochemical Characteristics of the Escherichia coli

Bacterial isolate	Gram Stain	Indole test	Catalase test	Oxidase test	Urease test	Citrate Utilization test	Methyl red test	Glucose fermentation test	Lactose fermentation test	Voges-proskaur test
Escherichia coli	Negative	Positive	Positive	Negative	Negative	Negative	Positive	Positive	Positive	Negative

The molecular identification of the *Escherichia coli* from the feces exposed to sunlight and shade are shown in Table 3.

Table 3: Molecular identification of the *Escherichia Coli*



Standard ID

16S rRNA Full Sequencing

macrogen

16S rRNA service report

Order Number : 160809FN-011
Sample name : E_contig_1

Information

Primer Information

Sequencing Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'	27F 5' (AGA GTT TGA TCM TGG CTC AG) 3
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Subject						Genus		Species	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
LN831043.1	Escherichia coli	1001376	171715	173224	0	2750	0.0	1503/1510	99

Kingdom	Family	Genus	Species
Bacteria	Enterobacteriaceae	Escherichia	Escherichia coli

Characterization

Escherichia coli(also known as E. coli) is a Gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in their hosts.

The antibiotics sensitivity test of the *Escherichia coli* from the faces exposed to sunlight and shade was determined using Gram negative antibiotics sensitivity discs. The organism was observed to be susceptible to Ciprofloxacin, Gentamicin and resistance to Neomycin, Streptomycin, Amoxicillin, and Chloramphenicol as shown in Table 4.

Table 4: Antibiotics Sensitivity Test of the *Escherichia Coli*

Bacteria Isolates	Ciprofloxacin 10ug (mm)	Gentamicin 10ug (mm)	Neomycin 100ug (mm)	Streptomycin 30ug (mm)	Amoxicillin 30ug (mm)	Chloramphenicol 10ug (mm)	Ofloxacin 10ug (mm)	Meropenem 10ug (mm)
Eschericia coli	≥21	≥15	≤13	≤13	≤12	≤14	≥15	≥15

The diameter zone of inhibition:
≤12- ≤14= Resistance
≥15- ≥21= Susceptible.

The effect of sunlight on the survival of *Escherichia coli* from the faces was observed for the period of 50 days. The result showed a decrease in the growth peak of *Escherichia coli* from day 7 to 50 days as shown in Figure 1.

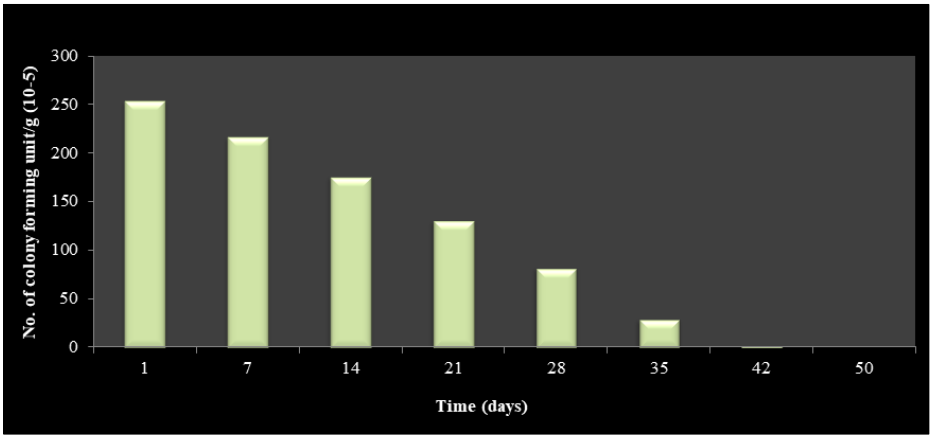


Figure 1: Effect of Sunlight on the Survival of *Escherichia coli* in the Feces

The effect of shade on the survival of *Escherichia coli* from the fecal soil was also observed for the period of 50 days. The result also showed a decrease in the growth peak of *Escherichia coli* from day 7 to 50 days as shown in Figure 2.

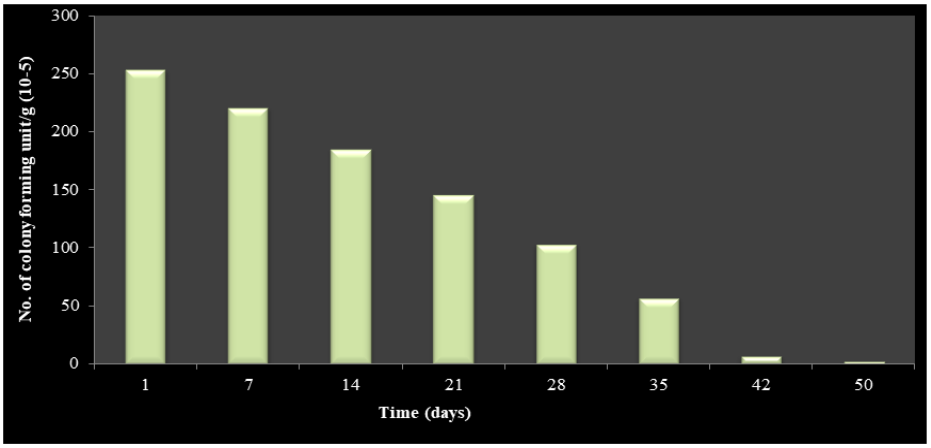


Figure 2: Effect of Shade on the Survival of *Escherichia coli* in the Feces

The effect of pH on the survival of *Escherichia coli* from the faces exposed to sunlight was determined for the period of 50 days. The result showed a decrease in the growth peak of *Escherichia coli* from day 7 to 50 days as shown in Figure 3.

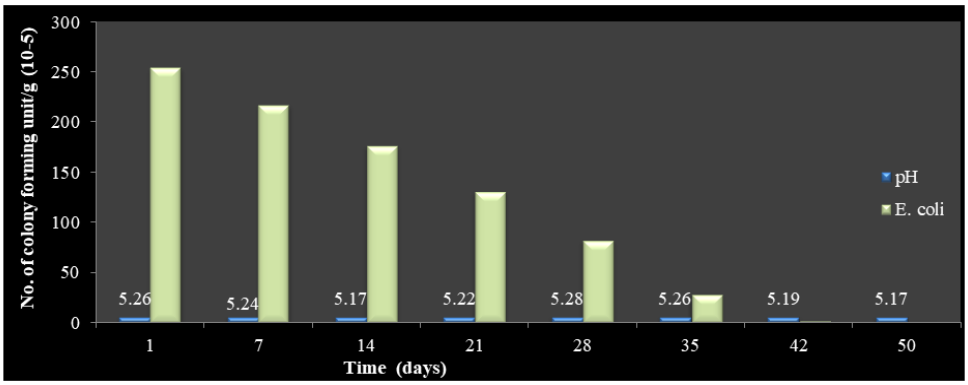


Figure 3: Effect of pH on the Survival of *Escherichia coli* in Feces exposed to Sunlight

The effect of pH on the survival of *Escherichia coli* from the faces exposed to shade was observed for the period of 50 days. The result also showed a decrease in the growth peak of *Escherichia coli* from day 7 to 50 days as shown in Figure 4.

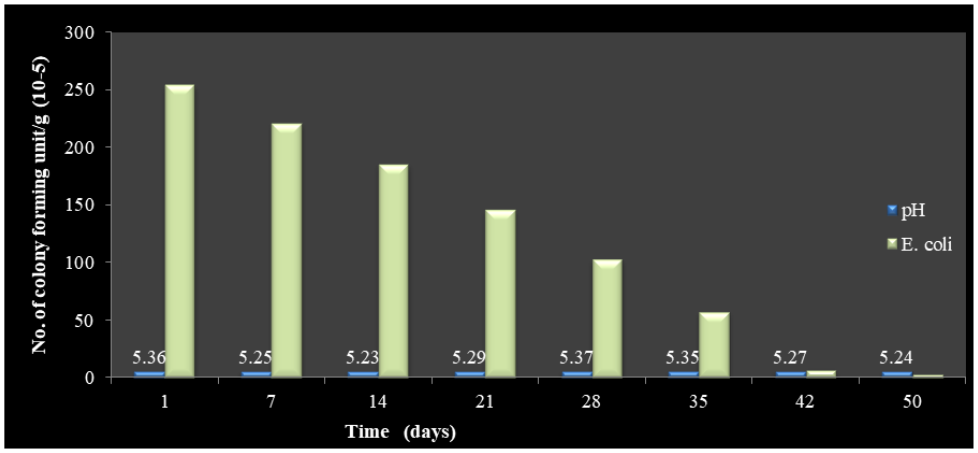


Figure 4: Effect of pH on the Survival of *Escherichia coli* in Feces exposed to shade

The effect of temperature on the survival of *Escherichia coli* from the faces exposed to sunlight) was determined for the period of 50 days. The result showed a decrease in the growth peak of *Escherichia coli* exposed to sunlight from day 7 to 50 days as shown in Figure 5.

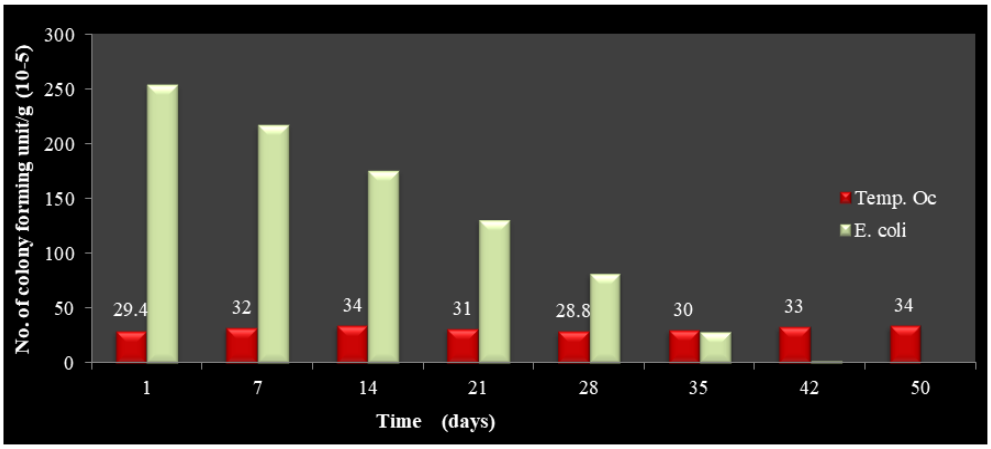


Figure 5: Effect of Temperature on the Survival of *Escherichia coli* in Feces exposed to Sunlight

The effect of temperature on the survival of *Escherichia coli* from the faces exposed to shade was also determined for the period of 50 days. The result also showed a decrease in the growth peak of *Escherichia coli* from day 7 to 50 days as shown in Figure 6.

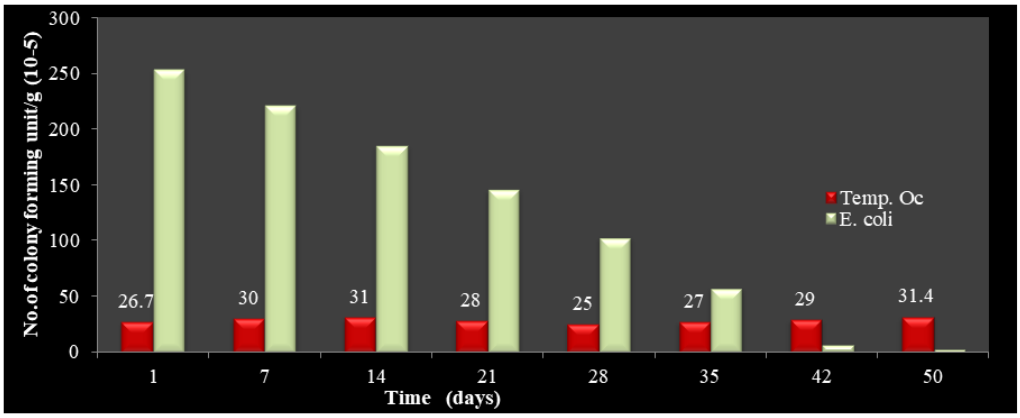


Figure 6: Effect of Temperature on the Survival of *Escherichia coli* in Feces exposed to Shade

DISCUSSION

Experiments were conducted on the open defecated human faces deposited in soil of Agbani Nkanu West L.G.A, Enugu State. The enteric organisms isolated from the human faces were identified morphologically and molecularly as *Escherichia coli* (Tables 1-3). This result agreed with the work of (Avery *et al*, 2004) which identified *Escherichia coli* that originated from livestock faces deposited in agricultural soil. Antibacterial sensitivity test was carried out and the inhibition zone diameters of the bacteria isolates were determined. The bacteria isolates were susceptible to Ciprofloxacin and Gentamicin, but resistance to Neomycin, Streptomycin, Amoxicillin, and Chloramphenicol (Table 4). This corresponds to the work of (Jorgensen *et al*, 2007). Effect of sunlight on the survival of *Escherichia coli* in faces showed a decrease in the growth peak of *Escherichia coli* exposed to sunlight for 50 days. It showed that sunlight was effective in inactivating or killing the fecal indicator bacterium *E. coli* in soil (Figure 1). Effect of shade on the survival of *Escherichia coli* in soil also showed a decrease in the growth peak of *Escherichia coli* exposed to shade for 50 days. It showed that even shade was effective in inactivating *E. coli* in soil (Figure 2). Effect of pH on the survival of *Escherichia coli* in soil exposed to sunlight showed a decrease in the growth of *Escherichia coli* at a given pH for 50 days. It showed that soil at pH 5.17 which is slightly acidic was effective in inactivating or killing *Escherichia coli* in soil exposed to sunlight (Figure 3). Effect of pH on the survival of *Escherichia coli* in soil exposed to shade also showed a decrease in the growth peak of *Escherichia coli* at a given pH for 50 days. It showed that soil at pH 5.24 which is slightly acidic was effective in inactivating or killing *Escherichia coli* in fecal soil exposed to shade (Figure4). Effect of temperature on the survival of *Escherichia coli* in soil exposed to sunlight showed a decrease in the growth peak of *Escherichia coli* exposed to sunlight at a given temperature for 50 days. It showed that the daily highest temperature of the soil exposed to sunlight did not reach 40°C in the first 3 weeks and *E. coli* survived over the first 3 weeks. The temperature was < 40°C during the 4^{th} and 5^{th} weeks. *E. coli* became undetectable within 6 weeks in fecal soil at 34°C (Figure 5). This is in line with the work of (Unc and Goss, 2006) who reported that *E. coli* populations decreased faster at a soil temperature of 20°C compared with the relative lower temperatures of 12 and 4°. Effect of temperature on the survival of *Escherichia coli* exposed to shade showed a decrease in the growth peak of *Escherichia coli* at a given temperature for 50 days. In the shaded soil, the temperature never reached 35°C and *E. coli* slightly survived for 6 weeks but not up to 50 days (Figure 6). A faster decrease in the *E. coli* population in the soil exposed to sunlight compared with the shaded soil might be attributed to the higher temperature and also lethal effects of ultraviolet radiation from the sun which can cause cell denaturation of proteins and thermal breakdown of the cells plasma membrane.

CONCLUSION

This study showed that longer exposure time played important role on the lethal effect of some environmental factors such as sunlight, shade, pH and temperature on the test organism. Though these environmental factors were found to be lethal to the *Escherichia coli* in the faces exposed to sunlight and shade, open defecation is not recommended.

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