Effect of Soil Types on Infiltration Pattern of Antibiotic Resistant *Escherichia Coli* From Hospital Wastewaters Into Surrounding Groundwater

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Abstract: This study investigated the vulnerability of six groundwater sources to contamination by *Escherichia coli* using Vertical Electrical Sounding (VES) and standard Microbiological procedures. Hospital wastewater and groundwater samples were collected from five hospitals. VES was accomplished using Schlumberger electrode configuration. All and four of the wastewater and groundwater sources sampled were contaminated with *E. coli.* Bacterial isolates (n=118). Areas of lowest vulnerability to infiltration had clay overburden reflected by resistivity value of <100flm and 0% *E. coli* isolation, while highly vulnerable areas were underlain by sand or clayey sand. Results showed that groundwater sites underlain by coarse grained permeable soil strata were negatively impacted by unhygienically disposed hospital wastewater.

Keywords: *Escherichia coli,* Hospital wastewater, Multiple Antibiotic Resistance, Vertical electrical sounding.

Introduction

Groundwater is a major source of drinking water worldwide and unquestionably one of the most essential resources for life sustainability. Protection of this resource is of paramount importance because the effects of contamination are often very reaching and of serious health consequences (Adelekan 2010; WHO 2014). Depending on the geology of the area, it may be deeply buried from contamination or more often shallowly harboured within the saturated zone of diverse earth materials with varying protective capacity to attenuate microbial contaminants. However, certain factors may make the groundwater vulnerable (Krauss and Griebler, 2011). Groundwater vulnerability is defined as its sensitivity to an imposed contamination load; influenced among other factors by depth to water table, rate and composition of groundwater recharge and the thickness of the near surface clayey layer (Morris *et al.,* 2003). It was reported as a function of protection nature of the strata between the pollution source/load applied on the land surface and water table (Liggett and Talwar, 2009). The natural maintenance of a groundwater source requires its replenishment from its environment and this may arise from natural sources like rain or various other sources (Vazquez-Sune *et al.,* 2010). Groundwater especially located in the surrounding of healthcare facilities may inadvertently be recharged by disposed wastewater from hospitals. The transport of anthropogenic contaminants to groundwater via recharge waters have been reported to be enhanced where permeable soil allows water to seep in or when a confining layer is absent (Knappett *et al.,* 2012). Hospital wastewater has been reported as one of the most important source of antibiotic resistance genes due to the presence of antibiotics which exerts a selection pressure on the wastewater microflora. Also significant amount of antibiotics finds their way into hospital wastewater through faeces and urine, creating a unique ecology which favours the spread and transfer of antibiotic resistant bacteria and their resistance genes (Davies and Davies 2010, Munir *et al.,* 2011).

In Nigerian, most health care facilities lack wastewater treatment facilities, hence, the unhygienic discharge of hospitals wastewaters into the environment through drains which open directly into municipal drainage system or just left to percolate into the soil is a common occurrence (Ekhaise and Omavwoya 2008). The choice of *E. coli* as bacteria of interest stems from its importance as an indicator of microbial quality of water. Though its primary habitat is human gut, it's able to persist in natural environment such as soils and water and migration between these habitats has also been reported (Vital *et al.,* 2008; Van Elsas *et al.,* 2011). Moreover, the ease of acquisition of antibiotic resistance and dissemination of resistance determinance in this bacterium has been reported (Forsberg *et al.,* 2012). In many parts of Nigeria and several other African countries ground water sources are readily explored to meet community need for water hence the need to protect it (Adelekan 2010). The application of geophysical method has been previously used to explain processes of groundwater pollution (Adelowo *et al.,* 2008; Ariyo *et al.,* 2015).

There is still a paucity of reports on the proper understanding of the processes of attenuation carried out by successive layers of soil strata as wastewater travel through the subsurface and possible contribution of unhygienic disposal of hospital wastewater to the spread of multiple antibiotic resistances via

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groundwater contamination. Most studies in Nigeria have hitherto been limited to effects of improper disposal of hospital wastewater into surface water. Thus the present study aimed to investigate infiltration pattern of *E. coli* through soil overburden of groundwater sources, and determine their capacity to limit or permit infiltration from hospital wastewater to groundwater samples within study area.

Sampling Sites and Geology of Study Sites

Five hospitals are designated (A, B, C, D and E) were selected for this study within Ogun State of South-western Nigeria in Ijebu Ode, (N003 55 34 9, E06 48 44.7), Ago Iwoye (N003 54 46.7 E 06 56 49.4), Ijebu Igbo (N003 59 34.9 E 06 57 59.5), Ibiade (N004 20 39.6 E 06 32 14.2) and Shagamu (N 003 37 56.4 E 06 51 17.3) towns of Ogun State. Geologically, Ago-Iwoye and Ijebu Igbo fall within the Basement complex and are mainly underlain by undifferentiated magmatite gneiss complex, schitz, quartzize and granite, Shagamu, Ijebu Ode and Ibiade towns are within the sedimentary terrain classified as Abeokuta group of the Dahormey basin (Omatsola and Adegoke 1981). All the hospitals are located within the metropolis which brings them into a close proximity with residential groundwater sources. Wastewaters are directly discharged through drainage unto the ground. In addition some wastewater drains empty into a poorly constructed unlined municipal drainage in Ijebu Igbo.

Materials and Methods

Sample collection

In order to have homogenous representation of the hospital wastewater, samples were collected from three draining points into sterile sample bottles and thereafter pooled to make a composite sample. Thirty millimetres were collected at each sampling time. Similarly, composite sampling of groundwater was also done. The samples were placed on ice and transported to the laboratory for analysis within 24 h. Hospital wastewater samples were collected between September 2011 and August 2013. Sampling was done once monthly for 24 months. In addition, a control well located at about 500m from the nearest hospital in order to dispense off the possibility of hospital wastewater influence was sampled.

Isolation of microorganisms

Escherichia coli was isolated using the surface spread method; 0.2 ml each of thoroughly mixed samples were plated on the surface of already prepared MacConkey agar (MAC) (Oxoid, Basingstoke Hampshire, UK) and Eosin Methylene blue agar (EMB) (Oxoid, Basingstoke Hampshire, UK). Plates were incubated aerobically at 37°C for 24–48 hours and distinct colonies sub cultured until pure colonies were obtained. Relevant biochemical tests were carried out using the protocol of (Barrow and Feltham, 1999). The presumed *E. coli* isolates were confirmed by streaking on Brilliance *E. coli*/coliform agar (Becton Dickinson Diagnostics, GmBH Heidelberg, Germany). Confirmed distinct colonies were stored on slants for further analysis.

Total Heterotrophic and Fecal Coliform Test

Water samples from the wells and boreholes of studied hospital as well as wastewater samples were tested for the presence of fecal coliforms using the American Public Health Association method 9221B (APHA. The most probable number (MPN)was determined from standard tables following APHA 9221C (APHA 1998).The total heterotrophic counts (THC) were estimated using the pour plate method on nutrient agar. One ml of appropriate dilutions of the effluents and sampled groundwater was used to inoculate the plates in duplicate, the plates were incubated at 37□C for 24–48 h and the colony forming units estimated.

Vertical Electrical Sounding

Vertical Electrical Sounding (VES) was carried out in the sites investigated using Schlumberger electrode configuration. The spread of each traverse (electrode spacing) was 100m. The graphical plot of apparent resistivity against electrode separation (AB/2) on a bi-logarithmic paper generated the intrinsic resistivity curve of the area. Curve standardization was achieved via the use of partial curve matching technique. The generated layering parameters during matching processes were fed as input model for computer iteration, from which the true resistivity model of the area was established.

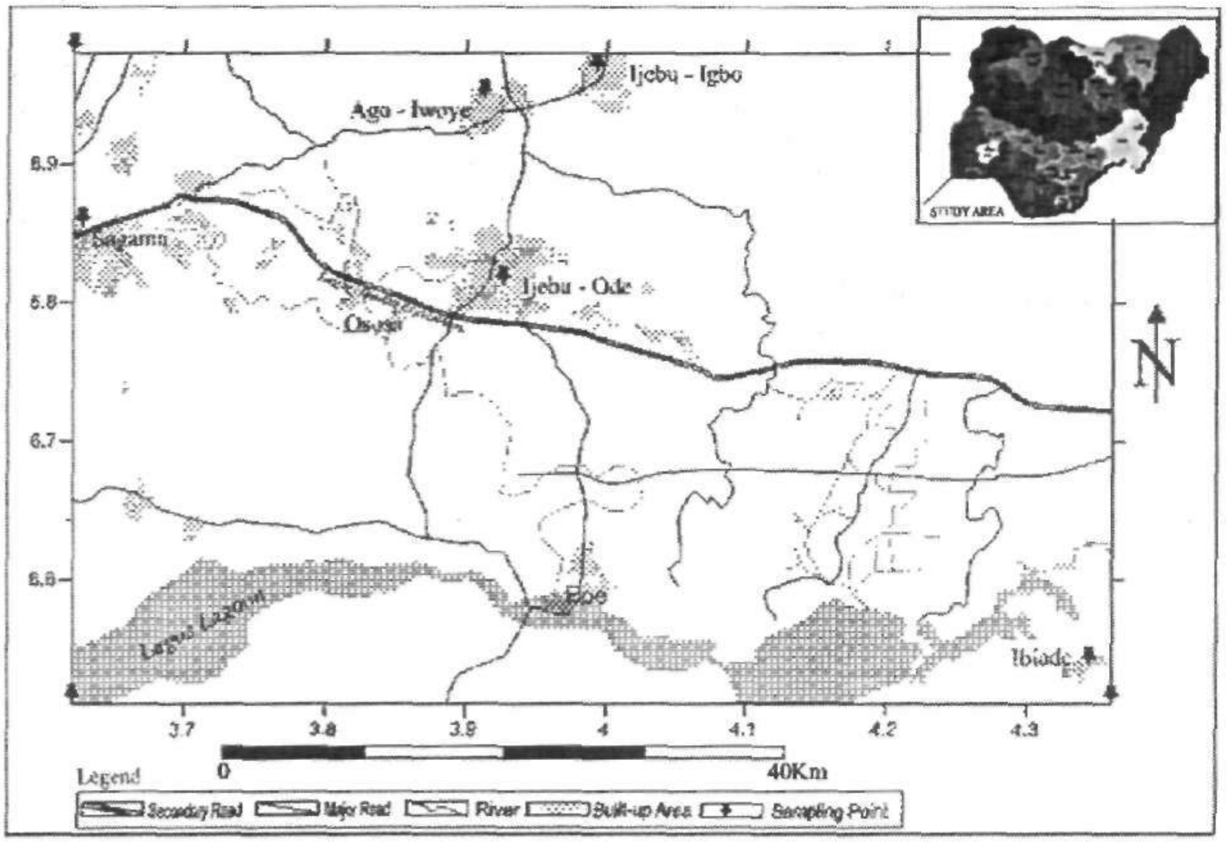


Fig.1. Map of Parts of South-western Nigeria showing the Study Sites

Results and discussion

Bacterial isolates

Escherichia coli were detected in all sampled hospital wastewaters in varying concentrations and in four of the groundwater sites. Overall, 70.8% (n=80) and (n=38) of the total isolates were recovered from wastewater and groundwater respectively. The presence of *E. coli* in hospital wastewaters in the studied sites is not surprising as the wastewaters arising from wards laboratories bathrooms and operation theatres were simply disposed of on the ground without any form of treatment. Related studies also affirm the high prevalence of E. coli from untreated hospital wastewater (Gundogdu *et al.*, 2013; Akther *et al.*, 2018). The presence of *E. coli* in the groundwater samples is however worrisome as *E. coli* is recognised as indicator organism and their presence suggest contamination which could have resulted from disposed hospital wastewater within sampled area. The organism may have impacted negatively on these groundwater sources as the highest isolation were from shallow wells within 5m depth. Moreover, the minimum horizontal separation distances as set by United State Environmental Protection Agency (USEPA, 2009) standard, required to be in between a well and known or potential source of contamination which is given as 50 ft (15.24m) was compromised at most of these study sites (Table 1).

Previous literature have demonstrated the capacity of this organism to move from soil surface to groundwater and even transfer resistance genes in diverse environments (Forsberg *et al.*, 2012; Gundogdu *et al.*, 2013). Microbial contaminant leaching from pit-latrines, unhygienically disposed wastewater and run off into shallow wells has also been reported (Adelowo *et al.*, 2008; Efuntoye and Apanpa, 2010)

All the sampled wastewater displayed high bacteria load with mean total heterotrophic counts ranging from 6.8 x 10^7 to 10.2 x 10^7 cfu/ml. The THC from the wells and boreholes ranged from 22cfu/ml from the borehole sample of Ijebu Ode Hospital to 28.4x10^2 respectively. The high microbial load of the hospital wastewaters across the sampled sites is clearly an indication that the microbiological quality of the hospital wastewater disposed into the environment is very poor. Such areas have been as possible route for the spread of antibiotic resistant propagules in the receiving environment (Ekhaise and Omavwoya, 2008; Brechet *et al.* 2014). Results in this study agreed with reports of other investigations on the poor bacteriological quality of hospital effluent being disposed into the Nigerian environment (Ekhaise and Omavwoya, 2008; Alam *et al.*, 2013).

The TVC of the control well (2.4 x 10^2cfu/ml) was the lowest among groundwater sampled, and no faecal coliform was detected. Among experimental wells, the highest TVC were observed in shallow wells of Ago-Iwoye, Ijebu Igbo and a borehole at Ibiade; all bearing feacal coliform. No faecal coliform was found in borehole samples of Ijebu Ode and Ijebu-Igbo (Table 1). Depth to groundwater can impact on their quality because wastewater bearing pathogens will require a longer travel time to reach water table during which attenuation may have occurred. The MPN values of other groundwater sampled ranged from 28-350MPN/100ml, the highest MPN/100ml were found in shallow wells of Ago Iwoye and Ijebu Igbo (Table 1). The high microbial load of the wastewater may have impacted on the groundwaters through infiltration because there w continuous contamination load of hospital wastewater applied on the ground by the day to day running of the hospitals. Moreover, the shallow depths of the wells put them at the high vulnerability class as shallow groundwater are generally classified as vulnerable due to their proximity to land surface.

Table 1: Description of Studied Groundwater

Hospitals	Location	Description of Hospital	Source	Groundwater depth(m)	separation distance (m)
A	Ijebu Ode	State	Borehole	82	42.0
B	Ago Iwoye	Private	well	3-5	12.3
C	Ijebu Igbo	P.S	well borehole	5.2; 60.0	10 18
D	Ibiade	State	Borehole	ND	28
E	Shagamu	Private	Borehole	45	20.4

S=state owned hospital, P=private hospital, P.S= private specialist.

Table 2. Total viable and coliform counts of wastewaters and groundwater samples

Hospitals	Source	Total Viable count ($TVC x 10^7$ cfu/ml	Coliform count (MPN/100ml	Source	Total Viable count	Coliform count (MPN/100ml)
Ijebu Ode	Ww	10.2	>1600	w	22	0
Ijebu Igbo	Ww	9.4	>1600	w	28.4×10^2	280
Ijebu Igbo	Ww	9.4	>1600	bh	26	0
Ago Iwoye	Ww	8.8	1600	w	3.8×10^2	350
Ibiade	Ww	7.9	>1600	bh	9.1×10^2	28
Shagamu	Ww	6.8	>1600	w	18.0×10^2	24

ww=wastewater; w=well; bh= borehole

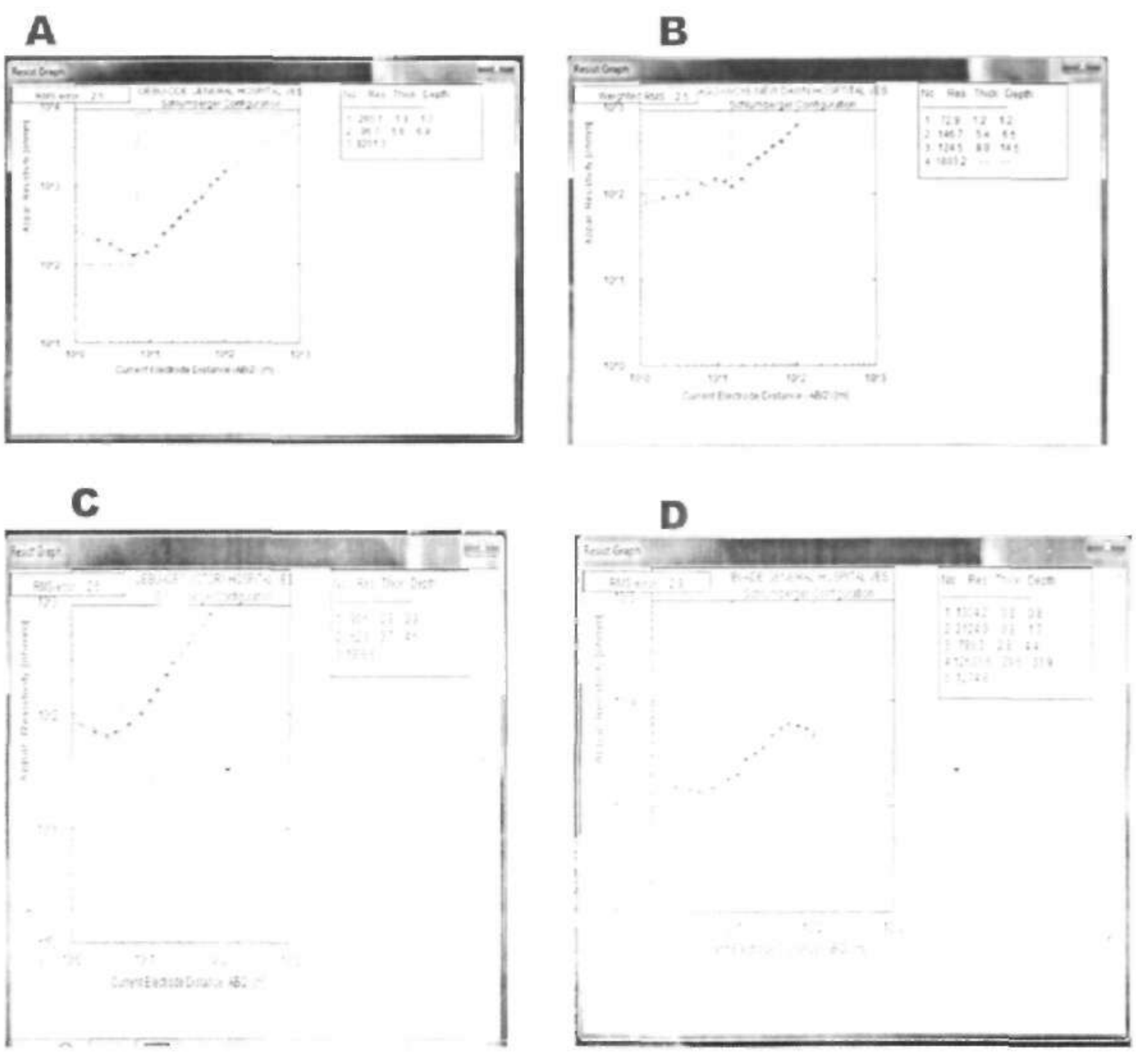
Result of the geophysical survey

The result of the iteration curves are shown in Fig 3. It reveals the resistivity, thickness and depth of the delineated geo-electric layers. Three geo-electric layers were delineated at Ijebu Igbo; and five, four, four, three at Ibiade, Shagamu, Ago Iwoye and Ijebu Igbo respectively. Fig. 4 depicts the lithological cross sections of the area. A resistivity value range between 52.8Ωm and 96Ωmfor the second geo-electric unit around Ijebu Igbo and Ijebu Ode borehole site is assertively indicative of an absolutely clay constituted lithologic unit. The appreciable presence of thick clay acquitard effectively repels contamination, and the boreholes water samples at these sites were free of *E. coli*, recording the lowest heterotrophic count. However, the well water in Ijebu-Igbo though with the same lithology had high isolation rate of *E. coli*. The marked difference in result observed at this site may be attributed to the well which was unlined, hand-dug and less than 5m deep and continually open to pollution load from a close unlined municipal drain bearing hospital wastewater. To buttress this, Morris *et al.*, (2003) classified shallow groundwater with low resistivity but with water table within few meters of ground surface as highly vulnerable to microbial pollution.

At Ago Iwoye and Sagamu the resistivity values at the second layer were indicative of clayey/sand overburden. Presence of sand in this layer is thought to reduce its protective capacity which may account for the high isolation of *E. coli* in Ago Iwoye site. The Ago Iwoye shallow well was observed to have reduced depth to water table during rainy season. This observation further suggests excess recharge from rain. At the Ibiade site however, the resistivity value indicate a lateritic layer of 0.8m thickness which may not be thick enough to act as a confining layer, the third to fifth layers made of sand and sandstone are highly vulnerable to microbial infiltration. Consequent contamination that may arise from groundwater in sandy to sandstone aquifers have been previously reported Powell *et al.*, (2001; Knappett *et al.*, 2012). Microbiological contaminants are able to penetrate these aquifers to significant depths.

Conclusion

Result from this study revealed the poor state of untreated hospital wastewater disposed into the Nigeria environment. The microbial quality of groundwater with vulnerable strata suggests they have been impacted. The potential risk to public health is heightened considering residential buildings which utilize the contaminated groundwater sources for domestic purpose. The use of the VES gives a moderate qualitative assessment of the protective capacity of the subsurface to water table may be incorporated into environmental impact assessment prior to sitting boreholes within healthcare facilities. Though further exhaustive hydrogeological assessments can be explored for mapping aquifer vulnerability. Ensuring proper treatment and disposal of hospital wastewater, coupled with proper enforcement in hospitals may be a step in good direction in reducing spread of MAR bacteria in hospitals.



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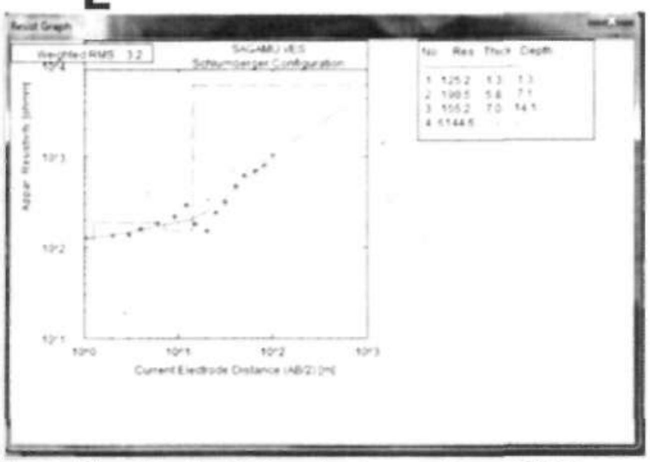

Fig.3. The Vertical electrical sounding (VES) curve of Hospital A, B, C, D and E

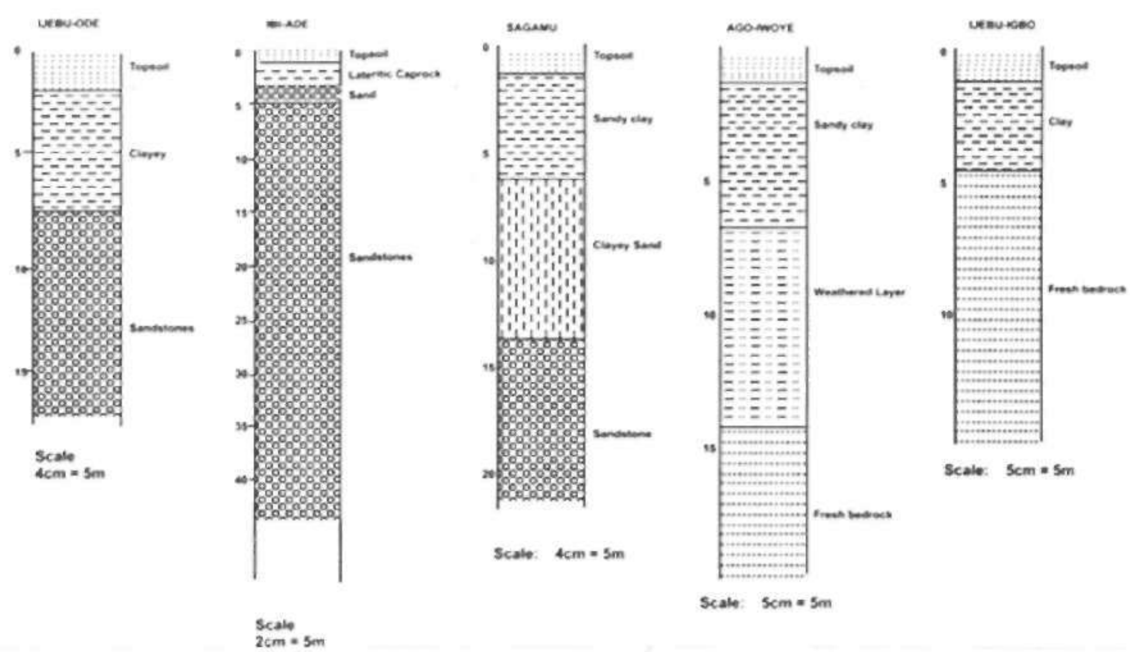

Fig 4. lithological cross-sections for the five locations

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