# Microbial Quality of Well Water in Upland and Riverine Communities of Rivers State, Nigeria


## ABSTRACT

In this study, health risk assessment of well water from twelve communities grouped into upland and riverine in Rivers State was carried out in several categories such as uses of water, skin infections and health assessment via questionnaire distribution. Malaria was recorded to be the most common disease related to water. Furthermore, water samples were collected and analysed for physiochemical, biochemical and pathological characteristics. The average pH was 7.52, an indication of neutrality. Several species of bacterial and fungal organisms were isolated and identified. The total heterotrophic bacteria (THB), total fungal and total coliform counts ranged from $14.0x10^4$cfu/ml to $100.0x10^4$cfu/ml, $3.2x10^3$cfu/ml to $7.4x10^3$cfu/ml and 4 cfu/100ml to ≥2400 cfu/100ml respectively. Morphological and biochemical observations revealed the presence of the following organisms: *Aeromonas* sp*., Alcaligenes sp., Bacillus sp. Citrobacter sp. E.coli, Enterbacter sp, Klebsiella* sp*., Micrococcus sp.,Proteus* sp*., salmonella* sp*.,sarcina* sp., *Shigella* sp., *Staphylococcus* sp., *Streptococcus* sp. *and Vibro* sp. *Staphylococcus* sp. *and Streptococcus* sp. had the least percentage incidence of 8.3% while *Vibro* sp had the most incidence of 100% in all the well water sampled. Well water is a source of pathogenic bacteria; hence, it is recommended that consistent water quality studies should be conducted on all the well water in the communities at least once in a year. Well water should also be treated before use to avoid the outbreak of water borne diseases.


## INTRODUCTION

Water, which is the most abundant substance in nature, is very essential to life, well-being, food security and socio-economic development of human being. In many developing nations of the world, the unavailability of water has become a life-threatening and serious problem and presently, it is a matter of great concern to families and communities depending on non-public water supply systems ~~for~~ to meet their daily water demand [1]. According to [2], increase in the population of human has caused a massive pressure on the provision of potable water especially in developing nations of the world in Asia and Africa continents.

Water can be found both underground and on the surface of the earth [3]. The term groundwater is usually reserved for the subsurface water that occurs beneath the water table in soils and geologic formation that are fully saturated [4]. Gradually, groundwater in Rivers state in particular and Nigeria in general are experiencing an increase menace of pollution from petroleum exploration and exploitation, development, industrial growth, agricultural and mining undertakings [5, 6], Other sources include atmospheric fall-out and acid rain.

Microbial analysis of water is used widely to monitor and regulate the quality and safety of numerous kinds of water sources. As various possible pathogens could be related with water, though it is not practical to test samples for all potential disease causing-microorganisms. Alternatively, several indicator micro-organisms have been used as surrogate markers of risks. Most common water borne diseases such as typhoid fever and cholera are associated to faecal pollution of water sources [7]. The presence of coliforms in water indicates contamination with faecal materials which usually pose extreme risk to human and results to severe diseases [8]. ~~The~~ Total coliform analysis remains the standard for determining the microbial quality of drinking water.

Nigeria is situated in the coastal region of West Africa where water is abundant [9, 10], yet the respondents lack adequate and safe drinking water [10]. This has prompted the digging of wells (water wells) to the ever-growing population without any prior form of treatment before use.

The World Health Organisation (WHO) reports that lack of potable water is one of the biggest global problems [11]. The WHO reported that more than one billion people lack safe drinking water and that 46 percent of Africans lacked access to safe drinking water. The organisation stated that the problem had reached such an endemic proportion that about 2.2 million death per annum occurred from unsanitary water related diseases of which more than 90 per cent of these are children under the age of five.

Potable drinking water is a transparent liquid without colour, odour and taste. When infected with organisms like bacteria or fungi, these qualities are lost and such water becomes harmful and unsafe for human and animal consumption [12-15]. Bad tastes in water have been often associated with pipe wall growth of microorganisms (biofilms).

Preliminary investigation revealed that groundwater (e.g. boreholes and open wells) and surface waters (e.g. rivers, streams and ponds), rain-water are the main sources of water available to the dwellers of Rivers state. More so, rural communities in these Rivers state rely mostly on groundwater as the main source of drinking water. Although groundwater is naturally free from disease causing organisms and safe for drinking due to the filtering nature of the overlaying soil, it is however, prone to pollution and contamination from natural disasters and the activities of man.

## MATERIALS AND METHODS

### Sampling Sites

Water samples were collected from twelve (12) wells sited in twelve different locations in Rivers state (Figure 1). The sampling sites (towns) were divided into upland and riverine. The upland towns include Orazi, Rumuigbo, Rumuokoro, Rumuosi and Rukpokwu in Obio/Akpor Local Government Area which is part of the metropolis of Port Harcourt; Emuoha, Ndele, Rumuji and Elele in Emuoha Local Government Area. The riverine communities include Nyogor in Khana Local

Government Area and YooyooYeghe in Gokona Local Government Area and finally from Andoni in Andoni Local Government Area.


**Figure 1. Map of Rivers state showing regions of sampling sites**

## Sample Collection

Water samples were collected in 1 litre bottles tied to ropes, both of which were previously disinfected with 70% alcohol. The rope was lowered to immerse the bottle in into the water until it was filled up. Once the bottle was filled up, the rope was pulled out of the well and the bottle was corked firmly (Figure 2). All water samples were collected in triplicate, labelled appropriately in each case, stored accordingly in a cool box according to [8] and taken to the laboratory for analysis.


**Figure 2: Wells from where water samples were collected**

## Experimental Methods

### Detection of coliform organisms

The technique employed in the detection of the coliform organisms is the multiple tube fermentation (Most Probable Number, MPN) technique according to [8].

### Presumptive test

Five tubes each containing 10 ml of MacConkey broth, fitted cap and inverted Durham's tube were prepared in Triplicates. The Mac Conkey broth contained in the first set of the tubes is double strength while single strength in the other sets. 10 ml of the water sample was added to each of the five tubes of the first set and labelled. 1ml and 0.1ml of the sample were added to each of the five tubes of the second and third sets respectively; and labelled accordingly. The tubes were loosely capped and incubated at 35-37 $^{o}$C for 24 hours after which were examined for gas and acid productions. Positive tubes were identified by both gas production shown by collection of bobbles in the inverted Durham's tubes; and acid production shown by change in colour of MacConkey broth from purple to yellow. Positive tubes were subjected to confirmatory test. The negative tubes were re-incubated at the same condition for total of 48 hours and re-examined for gas and acid productions.

### Confirmatory test

Positive tubes from the presumptive test were paired with new tubes each containing similar content to its positive pair. Using sterile wire loop, the new pairs were inoculated with their corresponding positive pairs and inoculated for 24 hours at 35-37$^{o}$C. Positive tubes confirmed the presence of lactose fermenters in the water sample.

### Completed test

This test is to confirm the lactose fermenters were coliforms not Gram positive bacteria. Positive tubes from confirmatory test were inoculated on Levine's Eosin methylene blue (EMB) agar using streaking method and incubated for 24 hours at 35-37$^{o}$C. Coliforms' presence was confirmed by nucleated (dark centre) colonies as

methylene blue content of the medium inhibits the growth of Gram positive bacteria. The Most Probable Number (MPN) of coliform bacteria in 100ml of water was determined using MPN probability table.

## Microbial Analysis

### Preparation of culture media

All media was prepared according to manufacturer's instruction, these include Eosin Methylene Agar (EMB) Agar, Nutrient Agar**,** MacConkey broth, Salmonella Shigella (SSA) agar. For microbial analysis, 10 fold serial dilution was prepared with peptone water, 0.1ml aliquot was inoculated into the different culture media and plates were incubated for 24 and 78 hours respectively. On completion of the culture, microbial species were identified using biochemical tests such as Urease, Catalase, Coagulase, Gram staining and Indole. Stock cultures of the identified organisms also prepared and preserved.

### Identification of bacteria

Pure culture of bacterial isolates were obtained by sub-culturing colonies from positive completed test on nutrient agar (NA) and incubated for 24 hours at 35-37 $^{o}$C and discrete colonies were stored in nutrient agar (NA) slants for further characterization and identification. The colonial morphology on growth medium and cellular morphology under a light microscope were examined.

### Nutrient agar (NA)

Exactly 28 g of the medium was dissolved in 1000 ml of distilled water. The suspension was first dissolved completely by shaking and then sterilized by autoclaving at 121°C for 15 minutes. The molten medium will then be allowed to cool at 45 $^{o}$C before dispensing into sterile Petri dishes.

### MacConkey broth

This broth contains lactose which, when degraded, gives acid and gas, according to the definition indicating the presence of E. coli. The gas formed is collected in DURHAM tubes and acid production is detected by the indicator bromocresol purple, which turns yellow. Ox bile promotes the growth of several species of intestinal bacteria and inhibits that of microorganisms, which do not inhabit the intestine. Typical Composition (g/L): Peptone from gelatin 20.0; lactose 10.0; ox bile, dried5.0 bromocresol purple 0.01.

Preparation; Suspend 35 g/litre or more (see Table below) in purified water, fill into test tubes, if desired insert Durham tubes, autoclave (15 min at 121 °C). pH: 7.3±0.2 at 25 °C. The prepared broth is clear and purple.

**Gram's staining**

A smear of the test organism was prepared on a slide, heat fixed and covered with crystal violet stain for 30-60 seconds. It was washed with clean water. The water was tipped off, covered with iodine for 30-60 seconds and washed with water. It was then decolorized with 95 % alcohol and washed with water immediately. It was covered with safranin (counter stain) for two minutes and washed with water. The back of the slide was wiped, dried on staining rack and observed under microscope. Gram positive organisms appeared purple while negative appeared red.

**Biochemical Analyses**

In order to further identify the isolated organisms, the following biochemical tests were carried out using methods described in [4].

**Indole test**

This test is based on identifying enterobacteria with the ability of producing enzyme tryptophanase. The test organism was inoculated in 3ml of peptone water and incubated at 35-37 $^{o}$C for up to 48 hours. 0.5 ml of KOVAC'S reagent was added and shook gently. Red colour in the surface layer was examined within 10 minutes, the presence of which indicated that the test organism produced an enzyme tryptophanase which broke down tryptophan contained in the peptone water to indole, pyruvic acid and ammonia. The compound p-dimethylamino-benzaldehyde in the KOVAC'S reagent then reacted with the indole and produced red compound, hence the organism indole positive.

**Methyl Red – Voges Proskauer (MR - VP) test**

The methyl-red (MR) test is based on identifying mixed acid fermenting bacteria that yield a stable acid end product. The Voges-Proskauer (VP) test is based on identifying bacteria capable of 2, 3butanediol fermentation following mixed acid fermentation. Sample was inoculated into 5ml of MR –VP broth and incubated for 48-37 hours at 35-37 $^{o}$C. 1 ml of the broth was transferred into a small serological test tube to which 2-3 drops of methyl red was added. Red colour on addition of the indicator indicated positive methyl red test. Five drops of 40 % potassium hydroxide (KOH) was added to the remaining 4ml of the broth followed by 15 drops of 5 % naphthol in ethanol. It was then shook, the cap was loosed and placed in a sloping position. Development of a red colour starting from the liquid – air interface within 1 hour indicated Voges-Proskauer positive test.

**Citrate utilization test**

The test is based on the ability of an organism to utilize citrate as its only source of carbon. A slope of Simmon's citrate agar was produced. The sample was inoculated by streaking the slope with saline suspension of the test organism and stabbing the butt. It was then incubated for 48 hours at 35-37 $^{o}$C. Bright blue colour in the medium indicated positive test while negative test was indicated by no change in colour.

**Oxidase test**

This test was performed using the test oxidase reagent (PL.390) from Mast Diagnostics (Nesto, Wirral, UK) in accordance with the manufacturer's published

protocol. A well-isolated pure colony was placed on a filter paper using a sterile wire loop. A drop of test oxidase reagent was added on to it and mixed. After 30 seconds, the filter was observed for a colour change with oxidase positive isolates producing a purple colour being taken as presumptive *Aeromonas* and *Pseudomonas* isolates. Oxidase negative colonies were colourless and were presumptively considered to be *E. coli.*

**Triple sugar iron (TSI) agar**

**Composition of Triple Sugar Iron (TSI) Agar: Lactose**, Sucrose and **Glucose** in the concentration of 10:10:1 (i.e. 10 part Lactose, 10 part Sucrose and 1 part Glucose). **Iron:** Ferrous sulphate: Indicator of $H_2S$ formation **Phenol red:** Indicator of acidification (It is yellow in acidic condition and red under alkaline conditions).It also contains Peptone which acts as source of nitrogen. (Remember that whenever peptone is utilized under aerobic condition ammonia is produced) Other basic understanding is TSI Tube contains butt (poorly oxygenated area on the bottom) slant (angled well oxygenated area on the top).

**Oxidative/fermentation (OF) glucose test**

Oxidative/Fermentation (OF) glucose test is a biological technique utilized in microbiology to determine the way a microorganism metabolizes a carbohydrate such as glucose (dextrose). OF-glucose deeps contain glucose as a carbohydrate, peptones, bromothymol blue indicator, and 0.5% agar. To perform the OF-glucose test, two tubes of OF-glucose medium are inoculated with the test organism. A layer of mineral oil is added to the top of the deep in one of the tubes to create anaerobic conditions. Oil is not added to the other tube to allow for aerobic conditions. The tubes are then incubated for 24–48 hours. If the medium in the anaerobic tube turns yellow, then the bacteria are fermenting glucose. If the tube with oil doesn't turn yellow, but the open tube does turn yellow, then the bacterium is oxidizing glucose. If the tube with mineral oil doesn't change, and the open tube turns blue, then the organism neither ferments, nor oxidizes glucose. Instead, it is oxidizing peptones which liberate ammonia, turning the indicator blue. If only the aerobic tube has turned yellow then the organism is able to oxidase glucose aerobically ("O") By-products: $CO_2$ and although organic acids may be present at low rate, If both tubes are yellow then the organisms is capable of fermentation ("F") If there is however growth is evident on the aerobic tube however the medium has not turned yellow a) Either glucose has been respired $CO_2$ without significant production of acid production b) or is respiring the peptone.

**Physico-Chemical Analyses**

The physico-chemical parameters such pH, Temperature and Total Dissolved Solids (TDS in mg/L) were measured. A multi-purpose PH meter model D46 (PH\MV\OC meter) were used to determine the pH of the Well water sample. Total

dissolve solid (TDS) meter – 4-HMD was used to determine the Total Dissolved Solids in the well water. All the physical parameters were measure on site by dipping the respective instruments into the bucket. Nitrate ($NO_3$-N), Calcium hardness as calcium carbonate ($CaCO_3$), Iron LR, and Fluoride (F-) were the chemical parameters analysed using the Wagtech test instructions. Palin test kit and Wagtech photometer 5000 was used to determine the frequency readings. Respective calibration charts were then used to determine concentrations of these parameters. 10 well water samples were analysed in the laboratory of University of Port Harcourt Microbiology. All parameters measured on the same day of sampling. Safety and complementary instructions were also adhered to [16].

## Bacteriological Analysis of Well Water

Heterotrophic bacteria were enumerated using the spread plate method with Plate Count Agar (Bio-Rad, France), incubated at 37 °C for 72 hours. Membrane filtration was used to enumerate qualitative microbial indicators (total *coliforms*, faecal coliforms, *Escherichia coli,* and faecal *streptococci*) according to the standard methods [17]. The m-Endo LES (Difco Laboratories, Detroit, MI, USA) agar was used for the enumeration of Total Coliforms, Faecal Coliforms and E. coli. Slanetz-Bartley and Bile EsculinAzide (BEA) agars (Biokar Diagnostics, Beauvais, France) were used for faecal streptococci counts. All analyses were done out in triplicate.

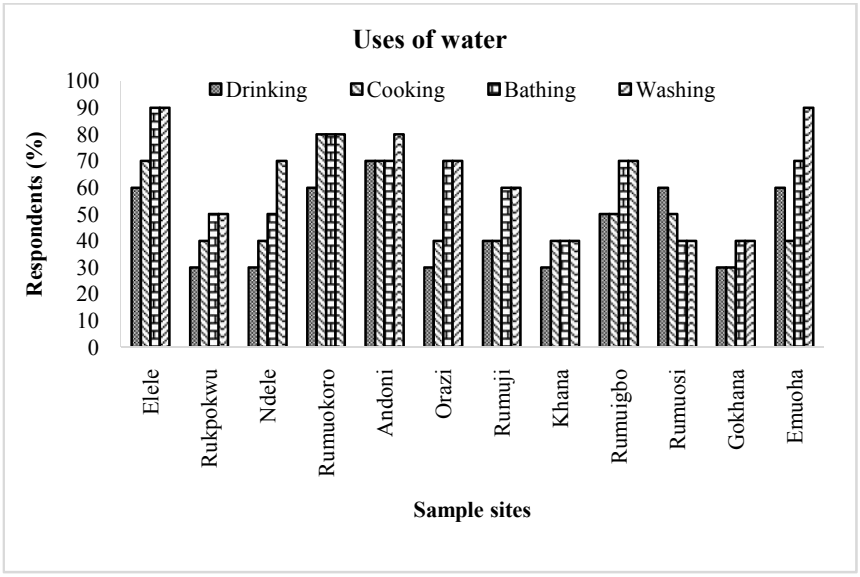
## Preparation of bacterial stocks

For the preparation of bacterial stocks, a colony forming unit (CFU) of each strain from standard agar medium was inoculated into 100 mL of nutrient broth for 24 hours at 37 °C. The strain of V. *choerae* was grown on alkaline nutrient agar and each of the other strains on standard no selective Plate Count Agar (Bio-Rad) for later use. Cells were then harvested by centrifugation at 3000 g for 10 min at ambient temperature and washed twice with sterile NaCl solution (8.5 g/L).

# RESULTS AND DISCUSSION

## Assessment of Well Water

Questionnaires were administered to two hundred and forty (240) adult residents of the different locations where the wells were located by simple random techniques. Residents were asked to identify various uses of well water, common skin infections and common water borne diseases. Their responses revealed that well water is used for various purposes in both the upland and riverine communities of Rivers State. Figures 3- 8 represents the responses from residents on the use of well water and diseases common to residents as diagnosed.

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307 **Figure 3. Various uses of well water**

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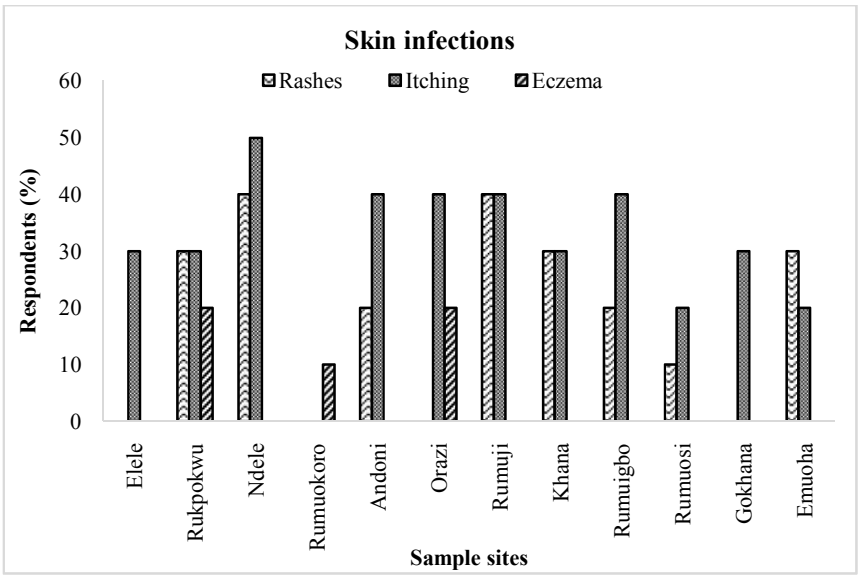


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312 **Figure 4. Skin infections associated with well water**

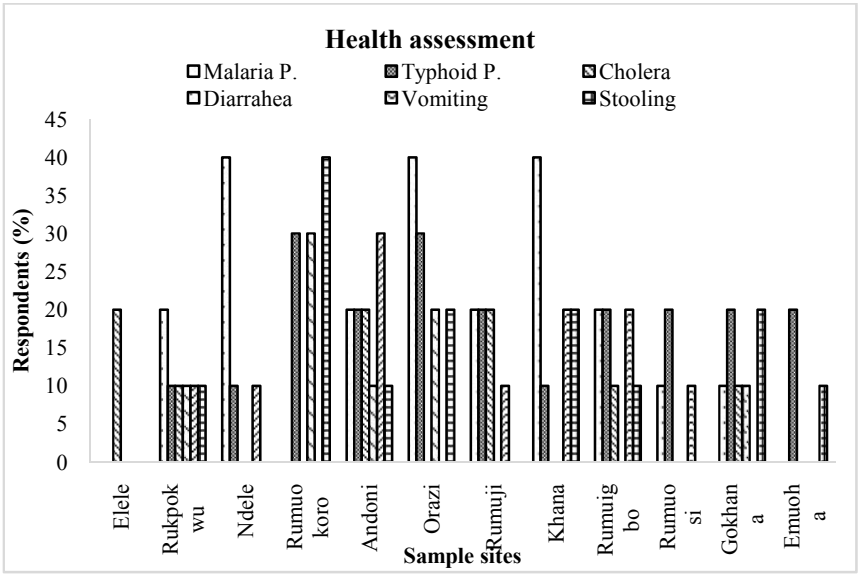



**Figure 5. Health assessment from uses of well water**

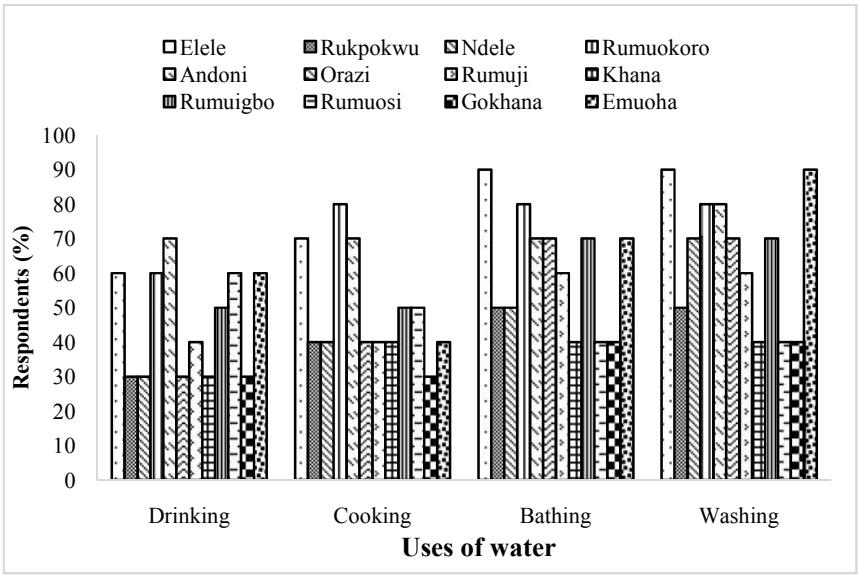
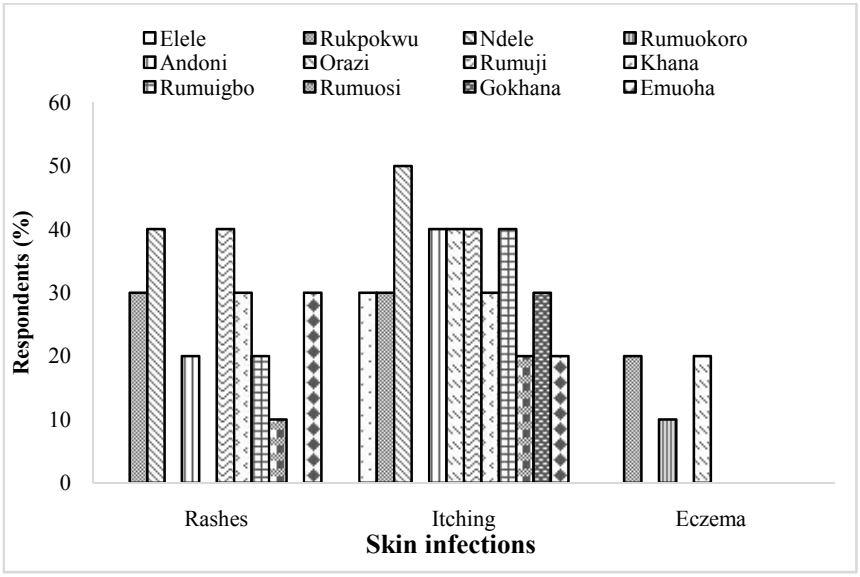



**Figure 6: Community comparative assessment of water use**

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321 **Figure 7. Community comparative assessment of skin infections**

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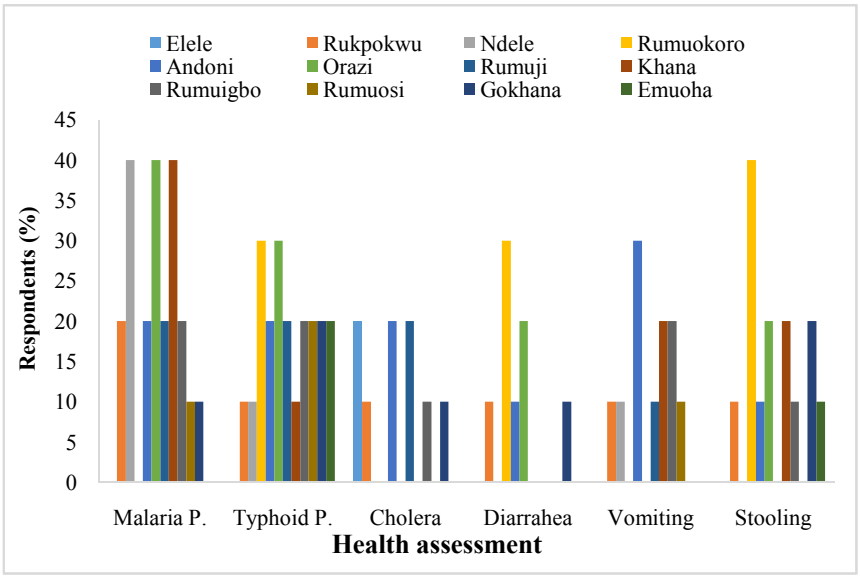


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326 **Figure 8. Community comparative health assessment**

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## Evaluation of Well Water Quality

### *Physicochemical properties of well water*

Water samples differ considerably among the twelve wells as shown in Table 1. These variations were observed by [18, 19] in the study of groundwater quality in some parts of Nigeria. The changes in human population, occupation, spatial heterogeneity of the soil of the area, and the variability of retention of microbes and chemicals by this soil could be the causes. The pH values from this research ranged from 7.02 to 8.50, this is within the WHO limit of 6.50-8.50. Drinking water with a pH between 6.5 to 8.5 is considered satisfactory. Acid water tend to be corrosive to iron. Chloride value ranged from 1008mg/L to 1991mg/L, the values obtained exceeded WHO maximum limit, the concentration of chloride indicates sewage pollution and has laxative effect. Atmospheric sources or sea water contamination accounts for increase of the chloride concentration in groundwater which may exceed due to base-exchange phenomena, high temperature, domestic effluents, septic tanks and low rainfall [20]. TDS indicates general water quality as it increases turbidity. High concentrations of TDS make the water unsafe for drinking, TDS values ranged 3312 – 7566mg/l, the values exceeded WHO limits of 500mg/l, calcium and manganese were within WHO limits. The result is similar to the report of [21]. Electrical conductivity (EC) is an important tool for assessing the purity of water and the EC values obtained ranged between 1003-1972 uS/cm.

### *Microbiological quality of Well Water*

The result of microbial counts of well water in all sites as represented in Table 2 shows that Total Heterotrophic bacteria ranged from $1.1x10^5$ to $1x10^6$ while Total Fungal count ranged from $3.2x10^3$ to $7.4x10^3$, *Salmonella*, *Shigella* and *Vibrio* count ranged from 3.2 to $3.9x10^3$, 3.6 to $4.9x10^3$ and 1.0 to $8.0x10^3$. Total coliform ranged from 4 - 140/100 ml. The microbial load exceeded WHO standard of $1x10^2$ for THB, and 0 for total fungi, total coliform and faecal coliform. Bacterial isolates represented in Table 3 shows the presence of *Vibro* sp in all well sampled with relative abundance of 100 %, while *Streptococcus* and *Staphylococcus* species were least dominant at 11.1 %. All examined well water samples contain substantial numbers of total heterotrophic bacteria (THB), total coliforms (TC), *Salmonella, Shigella* and *Vibrio* (established indicators of pollution) which exceeds WHO permissible limit. The order of bacterial abundance in all sites ranged from Vibrio > Bacillus sp/Citrobacter sp > Enterobacter > Shigella > Proteus sp > Salmonella sp > Klebsiella >Aeromonas > Alkaligens > Micrococcus > E. coli > Sarcina > Staphylococcus/Streptococcus sp. All samples showed the presence of coliforms, which exceeded WHO acceptable limit of Zero. This finding agrees with the report of [19, 21, 22] in their works on bacterial quality of well water. Figures 9 – 10 presents *Aspergillus* and *Vibrio* as the most dominant fungi and bacteria in well water sampled

The presence of faecal coliforms such as *E. coli* and *Klebsiella* sp. is of public health importance because they actually indicate recent pollution of water bodies by human/animal faecal wastes and sewage [12, 23, 24]. The presence of *Proteus* sp., *Streptococcus* sp, *Enterobacter* sp and *Staphylococcus* sp are implicated for causing diseases [12]. Obviously, the well water samples are not safe for drinking

and could be implicated for the diseases frequently diagnosed on the residents as reported in Figure 4, 5 and 8.

The result of fungal cultures as represented in Table 4 shows the relative abundance of fungal in order *Aspergillus* > *Penicillin*> *Saccromyces* > *Fusarium* and *Rhizopus* sp. With relative abundance of 66.7%, 55.6%, 33.3%, 22.2% and 11.1% respectively. The presence of the fungi, *Penicllium* sp in the water sources are also of public health significance because studies have implicated them in cases of allergy, asthma and some respiratory problems through drinking of contaminated underground water sources [25].

This finding agrees with previous reports which showed that hand dug wells and borehole waters in Nigerian communities were microbiologically poor [18,19, 26-29]. Poorly constructed latrines, improper disposal of wastes, open drainage systems, construction of well water close to latrine could be responsible for the contamination of well water with microorganisms as reported by [21].

According to WHO/UNICEF (2000), enteric bacteria such as *Escherichia coli, Shigella* species, *Salmonella* species, among others, are the most frequently implicated microorganisms in waterborne diseases and have been associated with the estimated 80% diseases affecting developing countries. Filtration and/or boiling are water treatment methods widely accepted as a solution to compensate the lack of potable drinking water in underprivileged communities in developing countries [30].

398 **Table-1: Physico-chemical Analysis of well water**

| Parameters | Method | Unit | Gokana | Khana | Rumuokoro | Rumuigbo | Orazi | Rumuosi | Elele | Rumuiji | Ndele | Andoni | Rukpokwu | Emuoha |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | ASTM | Nil | 7.02 | 7.22 | 7.41 | 7.21 | 7.32 | 7.52 | 7.39 | 7.66 | 7.46 | 7.65 | 7.50 | 8.50 |
| Temperature | ASTM | $^{o}$C | 22.40 | 25.80 | 23.40 | 24.21 | 28.93 | 29.21 | 26.44 | 27.89 | 28.72 | 27.30 | 27.40 | 31.21 |
| Conductivity | ASTM | µS/cm | 1434 | 1284 | 1905 | 1110 | 1423 | 1003 | 1242 | 1972 | 1215 | 1894 | 1635 | 1023 |
| Chloride | ASTM | mg/L | 1091 | 1844 | 1551 | 1723 | 1702 | 1268 | 1315 | 1991 | 1450 | 1882 | 1091 | 1008 |
| Bicarbonate | ASTM | mg/L | 788 | 506 | 812 | 678 | 976 | 705 | 880 | 943 | 752 | 646 | 907 | 855 |
| Barium | ASTM | mg/L | 12.12 | 10.24 | 12.00 | 13.82 | 10.44 | 10.28 | 10.01 | 9.22 | 8.42 | 10.23 | 16.00 | 11.88 |
| Sodium | ASTM | mg/L | 4445 | 3001 | 2724 | 3451 | 3201 | 3443 | 3280 | 2880 | 3542 | 3881 | 3984 | 3576 |
| Calcium | ASTM | mg/L | 17.10 | 15.22 | 14.20 | 10.98 | 13.44 | 9.23 | 10.67 | 10.25 | 9.56 | 8.21 | 12.70 | 9.13 |
| Magnesium | ASTM | mg/L | 12.22 | 9.73 | 9.00 | 7.07 | 9.26 | 12.73 | 11.10 | 9.22 | 9.14 | 8.65 | 8.00 | 12.73 |
| Manganese | ASTM | mg/L | 11.44 | 10.20 | 9.23 | 8.66 | 9.15 | 10.00 | 9.23 | 6.70 | 6.33 | 7.98 | 13.78 | 11.00 |
| Total Iron | ASTM | mg/L | 1.70 | 1.00 | 1.05 | 1.50 | 1.50 | 1.00 | 0.85 | 0.55 | 1.62 | 1.54 | 1.00 | 2.00 |
| Carbonate | ASTM | mg/L | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TDS | ASTM | mg/L | 7500 | 5441 | 3312 | 6324 | 6544 | 6660 | 7421 | 6661 | 6112 | 6350 | 6445 | 7566 |
| Sulphate | ASTM | mg/L | 10.00 | 5.20 | 6.60 | 5.32 | 5.00 | 5.32 | 5.10 | 9.00 | 6.30 | 8.12 | 5.32 | 5.32 |
| $H_2S$ | ASTM | mg/L | 0.20 | 0.10 | 0.15 | 0.10 | 0.10 | 0.13 | 0.14 | 0.15 | 0.10 | 0.15 | 0.13 | 0.15 |
| Carbon dioxide | ASTM | mg/L | 16.00 | 18.00 | 17.00 | 18.00 | 18.00 | 19.00 | 16.00 | 20.00 | 15.00 | 17.00 | 17.00 | 17.00 |

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405 **Table 2. Enumeration of microorganisms in well water**

| S/N | Location | THB (cfu/ml) x$10^4$ | Total Fungi (cfu/ml) x$10^3$ | Salmonella count (cfu/ml) x$10^3$ | Shigella count (cfu/ml) x$10^3$ | Vibrio count (cfu/ml) $10^3$ | Coliform (cfu/100 ml) |
|---|---|---|---|---|---|---|---|
| 1 | Khana | 20.0 | 3.9 | - | - | 1.0 | 33 |
| 2 | Gokana | 20.8 | 3.7 | - | - | 2.0 | 17 |
| 3 | Orazi | 16.0 | 3.2 | - | - | 4.1 | 33 |
| 4 | Rumuigbo | 14.0 | 4.3 | - | - | 4.0 | 26 |
| 5 | Rumuokoro | 11.0 | 4.8 | - | - | 1.4 | ≥2400 |
| 6 | Elele | 72.0 | 7.2 | 3.2 | 3.6 | 35 | 6 |
| 7 | Emuoha | 82.0 | 7.4 | - | 4.6 | 32 | 9 |
| 8 | Andoni | 88.0 | 4.0 | 3.9 | - | 46 | 12 |
| 9 | Ndele | 71.0 | 7.2 | - | 4.0 | 8.0 | 4 |
| 10 | Rumuosi | 100 | 5.8 | - | 4.9 | 7.6 | 11 |
| 11 | Rumuji | 59.0 | 4.4 | 3.3 | 4.9 | 6.0 | 4 |
| 12 | Rukpokwu | 89.0 | 6.4 | - | 4.6 | 7.1 | 9 |

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408 **Table 3. Characterization of Fungal cultures from well water**

| S/N | Sample sites | *Aspergillum* sp. | *Fusarium* sp. | *Penicillum* sp. | *Rhizopus* sp. | *Saccharomyces* sp. | No. of organisms | % occurrence of organisms |
|---|---|---|---|---|---|---|---|---|
| 1 | Gokana | + | | | | | 1 | 20.0 |
| 2 | Khana | | + | | | | 1 | 20.0 |
| 3 | Orazi | | | + | | + | 2 | 40.0 |
| 4 | Rumuokoro | + | | | | | 1 | 20.0 |
| 5 | Rumuigbo | + | | | | | 1 | 20.0 |
| 6 | Elele | | | + | + | | 2 | 40.0 |
| 7 | Ndele | + | + | + | | + | 4 | 80.0 |
| 8 | Rumuji | + | | + | | | 2 | 40.0 |
| 9 | Emuoha | + | | + | | + | 3 | 60.0 |
| | No. of occurrence | 6 | 2 | 5 | 1 | 3 | | |
| | % incidence of organisms | 66.7 | 22.2 | 55.6 | 11.1 | 33.3 | | |

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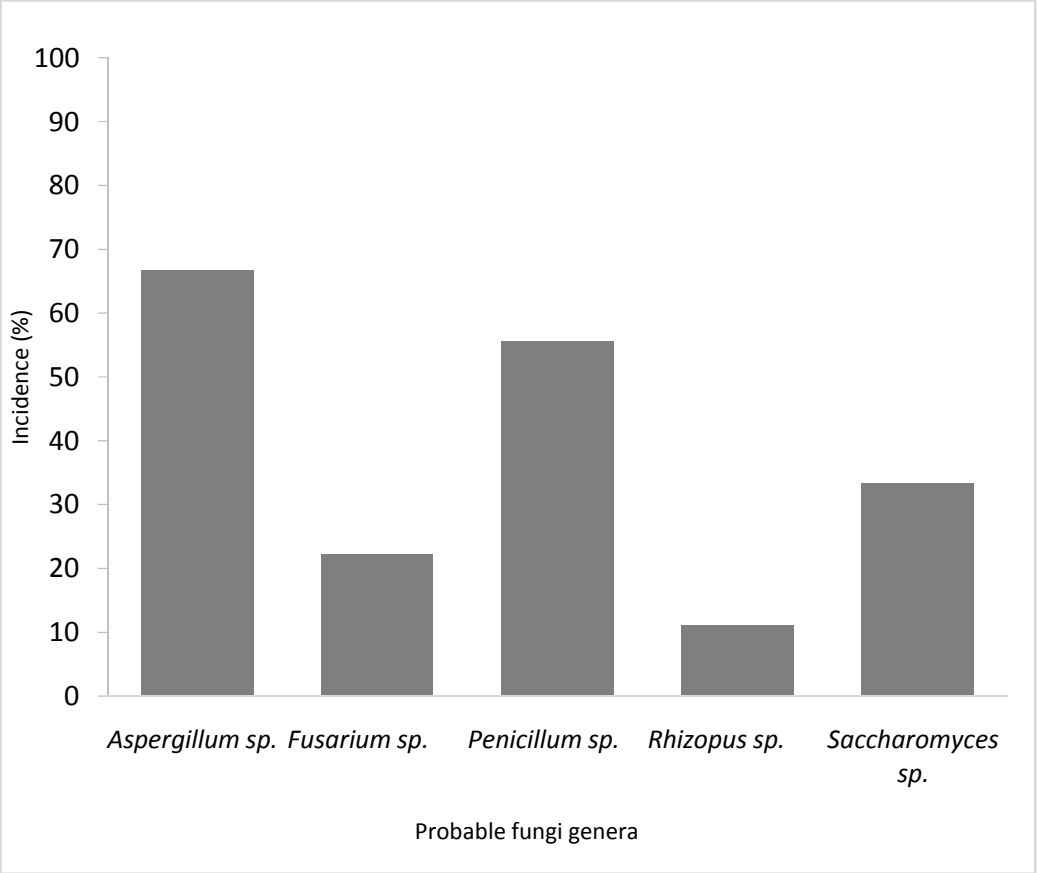


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415 **Figure 9. Percentage incidence of fungal cultures from well water**

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423 **Table 4. Characterization of Bacterial isolates from well water**

| S/N | Sample sites | Organisms Isolated | | | | | | | | | | | | | | | No. of organisms | % occurrence of organisms |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Aeromonas sp.* | *Alcaligenes sp.* | *Bacillus sp.* | *Citrobacter sp.* | *E. Coli* | *Enterbacter sp.* | *Klebsiella sp.* | *Micrococcus sp.* | *Proteus sp.* | *Salmonella sp.* | *Sarcina sp.* | *Shigella sp.* | *Staphylococcus sp.* | *Streptococcus sp.* | *Vibro sp.* | | |
| 1 | Gokana | - | - | + | + | - | + | - | - | + | - | - | - | - | + | + | 6 | 40.0 |
| 2 | Khana | + | - | - | + | - | - | + | - | - | + | + | - | - | - | + | 6 | 40.0 |
| 3 | Orazi | - | + | + | + | + | + | - | - | - | - | - | - | - | - | + | 6 | 40.0 |
| 4 | Rumuigbo | - | - | + | + | - | + | - | - | + | - | + | - | - | - | + | 6 | 40.0 |
| 5 | Rumuokoro | - | - | + | + | - | + | + | + | - | - | - | - | - | - | + | 6 | 40.0 |
| 6 | Elele | + | - | + | + | - | + | - | - | - | + | - | + | - | - | + | 7 | 46.7 |
| 7 | Emuoha | - | - | + | + | - | - | - | + | + | - | - | + | - | - | + | 6 | 40.0 |
| 8 | Andoni | - | + | + | - | - | + | + | - | - | + | - | - | - | - | + | 6 | 40.0 |
| 9 | Ndele | + | - | + | + | - | - | - | - | + | - | - | + | - | - | + | 6 | 40.0 |
| 10 | Rumuosi | - | + | + | + | - | - | - | - | + | - | - | + | - | - | + | 6 | 40.0 |
| 11 | Rumuji | - | - | + | + | + | - | + | + | - | + | - | + | - | - | + | 8 | 53.3 |
| 12 | Rukpokwu | - | - | + | + | - | + | - | - | - | - | - | + | + | - | + | 6 | 40.0 |
| | No. of occurrence | 3 | 3 | 11 | 11 | 2 | 7 | 4 | 3 | 5 | 4 | 2 | 6 | 1 | 1 | 12 | | |
| | % incidence of organisms | 25.0 | 25.0 | 91.7 | 91.7 | 16.7 | 58.3 | 33.3 | 25.0 | 41.7 | 33.3 | 16.7 | 50.0 | 8.3 | 8.3 | 100.0 | | |

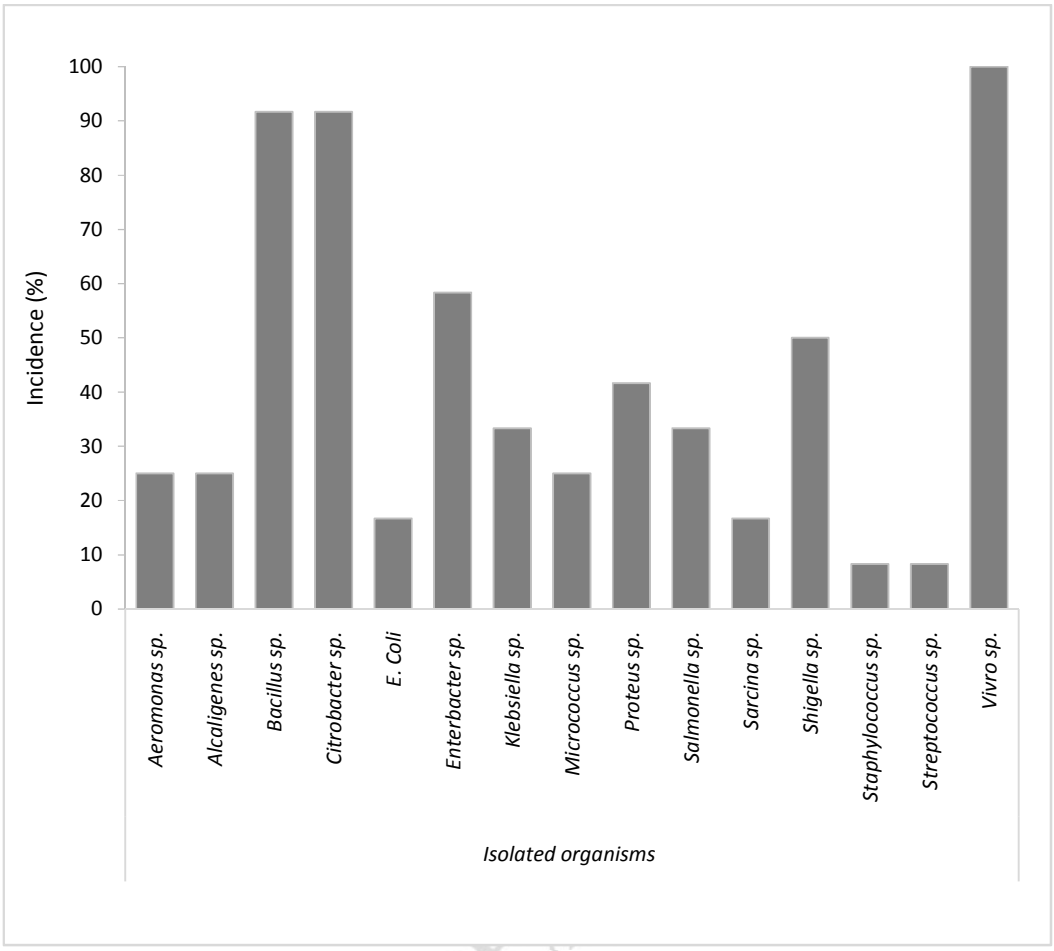


**Figure 10. Percentage Incidence of Bacterial isolates from well water**

## CONCLUSIONS

This study shows high level of microbial contamination in all well water samples: microbial load and coliform content exceeded WHO standard. Therefore suggests that well water sampled are not safe for drinking and could be implicated for the diseases frequently diagnosed on the residents as reported in the questionnaire. There is a need to enlighten the general public about the quality of their water sources and the importance of clean and healthy environments close to water sources The importance of simple water treatment should be advocated such as boiling, The respondents should be educated on proper disposal of wastes, and wells should be dug deep and wells must be dug at distances away from the latrine or suck away by the users and also by simple treatment methods such as boiling by the consumers.

## RECOMMENDATIONS

In view of the outcome of this study, the following recommendations are made:

i. Water quality analysis should be carried out on all the well water in the communities at least once every year. This will ensure that incidences of contamination are noticed earlier for remedial action to be taken.
ii. The treatment of these well water and their wells especially those in rural/riverine communities by the appropriate body should be done on a regular basis and also by simple treatment methods such as boiling by the consumers.
iii. The communities should not compromise on their sanitary practices as a dirty environment could serve as source by which groundwater gets contaminated.
iv. Regular physical and health examination should be carried out on these community dwellers who make use of well water to meet their daily water demand.

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