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Research Article

Single and Synergistic Effects of Solar Radiation, Water guard and Moringa oleifera Treatments on Bacterial Loads and Physicochemical Parameters of Surface Water in Umur and Bele Streams of Benue State

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Abstract
Bacteriological profiles of surface water samples treated with solar radiation, *Moringa oleifera* powder and water guard was carried out to ascertain their effectiveness in water treatment. Bacterial loads of the surface water collected from Umur and Bele streams in Gwer East Local Government area samples were determined before and after treatments and characterised using biochemical and molecular methods. Single and synergistic effects of these treatments on water quality were also examined. Bacteriological analysis showed that Umur stream had the highest bacterial loads of 4.47×10^3 cfu/mL while Bele had the lowest counts. There was significant reduction in the mean viable counts recorded for all the water samples ($p < 0.05$). In the daily bacteria counts, the control water samples gave extremes values. There were no bacteria count recorded following combine treatment in water samples from Bele stream on the fifth day. Molecular analysis based on the *16S rRNA* gene sequence showed bacterial strains to be phylogenetically close to bacterial strains which are capable of causing infectious diseases to man. Normal pH values were recorded in Umur stream while low pH values were recorded in Bele streams. Treatment impacted significantly on the pH of the water samples from Umur stream ($p < 0.05$) while no significant difference was observed with water samples from Bele stream ($p > 0.05$). Treatment impacted significantly on turbidity in water sample from Umur stream ($p < 0.05$). Sulphate was found to be within the permissible limit except for water sample from Bele stream which gave mean values within the range of 51.00 to 68.00 mg/L. Treatments impacted no significant difference on sulphate ($p > 0.05$). Surface water in these rural areas should be thoroughly treated before use.

Keywords: Umur; Bele; Solar radiation; Water guard

Introduction
Water is a vital, indispensable resource that supports all forms of life on earth (Sojobi *et al*., 2014). Nigerians derived their water from surface water (springs, streams, rivers, lakes), hand dug wells, rainwater, pipe borne water and boreholes (FGN, 2000; Gwimbi, 2011). Due to lack of safe public water supply in Gwer East Local Government Area, rivers and the available streams have become a major source of water supply, hence there is need for adequate purification, especially to remove all pathogenic microorganisms before use. Conventional methods of assuring potable water in developing countries are

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unsustainable so there is need to consider the application of sustainable technologies using locally available materials in surface water treatment (Pritchard *et al*., 2009). The use of natural materials that are of plant origin to clarify turbid surface waters is not a new idea according to NRC (2006). Seeds from trees such as *Strychnos potatorum*, roasted grains of *Zea mays* and sap from the 'tuna' cactus (*Opuntia fiscus indica*) have all been used. However, of all the plant materials that has been investigated over the years, the seeds from *Moringa oleifera* have shown to be one of the most effective as a primary coagulant in water purification and disinfectant for water treatment (Raheela *et al*., 2009). *Moringa oleifera* has a great potential in water treatment. Several researchers have reported on its various uses as a coagulant, softening agent, and a bactericidal agent (Montakhab *et al*., 2010).

Water guard use is limited mostly to urban areas and is often unavailable in most rural areas in Nigeria largely due to difficulty in reaching rural areas owing to bad roads and weak advertisement of the product (PATH, 2012).

Materials and Methods

Collection of Water Samples

Surface water samples were collected from Umur and Bele streams in Gwer East Local Government Area of Benue State. The samples were collected where people commonly collect water for their domestic activities. Standard sampling methods of APHA (1999) was adopted in the collection of the water samples. Water samples for physicochemical analyses were collected using transparent sterile containers of 2.0 litres capacity. The plastic containers were thoroughly washed with 5 % nitric acid (HNO_3) and rinsed with tap water (WHO, 2004). They were later rinsed thoroughly with deionized water and allowed to dry before use. Plastic containers for nitrate analysis were washed with boric acids and allowed to dry. Water samples for bacteriological analyses were collected using sterile bottle held at the bottom and inserted into the water with the mouth downward to a depth of at least a foot below the water surface (UNEP/WHO, 2006). Water current was created by dragging the bottle slowly through the water to ensure that the organisms (if any) on the sides of the bottle are washed away and not into the bottle. The bottle was removed from the water as soon as it is full and is immediately stoppered, properly labelled and transported to the laboratory in an ice packed cooler (to maintain the lowest possible temperature) and kept in a freezer until time of analysis (Ademoroti, 1996). Turbidity and pH were measured immediately after sampling to obtain accurate result.

Collection of Plant Samples and Authentication of Plant Seeds

Seeds of *Moringa oleifera* were purchased from Railway market, Makurdi, Benue State. The plant part (seeds) was packed in sterile polythene bags and transported to the laboratory, Biological Sciences, University of Agriculture, Makurdi for identification and analyses.

Conversion of Moringa oleifera Seeds to Powdered Form

Seeds were selected and dried under shade for 10 days. The seeds were de-shelled by hand, crushed and converted to powdered form using a blender and sieved using a strainer with a pore size of 2.5 mm^2 to obtain a fine powder according to Pritchard *et al*. (2009). The powder was stored in a sealed plastic container with cover at room temperature (25ºC) prior to processing and use.

Water Guard

Water guard was purchased from a pharmacy store in Makurdi, Benue State. The expiry date was also ascertained.

Solar Radiation (SODIS)

Solar radiation panel was obtained from Energy Research Centre, University of Nigeria, Nsukka Campus, Nsukka, Enugu State. The solar radiation panel was constructed using a very wide rectangular pan overlaid with a standard reflecting 3 mm glass to concentrate sunlight energy unto the water samples during treatment (EAWAG, 2007).

Treatment with Water Guard

The usual recommended concentration of 4 ml/L of water guard which is equivalent to two capfuls of its container for 25 litres of water was used and after shaking vigorously to ensure uniform mixing was allowed to stand for 1hour before use for bacteria analyses (CDCP, 2007). Daily analyses were carried out for 5 days to obtain total viable count for each day.

Treatment with Moringa oleifera Powder

Exactly 2 g of the prepared powder was mixed with a small amount of sterile distilled water to make a 2 % suspension in a small bottle as described by Ghebremichael *et al*. (2005). The bottle was closed and then shaken after 5 minutes to obtain a good water extract, in order to activate the ingredients present in the powder. This milky extract was then filtered through a clean sterile cloth into 2 litres of the water samples to be treated. After the milky white suspension has been added to the water samples it was stirred rapidly for at least 2 minutes and then slowly for 10-15 minutes. The treated water was covered, left to settle for at least an hour and for treatment to take place. The clean water from the solution was then taken after treatment for analyses. Daily analyses were carried out for 5 days to obtain total viable count for each day.

Treatment with Solar Radiation

Exactly 5 litres of the water samples was measured into clean transparent plastic container of 10 litres capacity and exposed to ultraviolet rays from the sun as reflected from the standard solar panel constructed for a period of 6 hours (EAWAG, 2007). Water samples were taken to the laboratory for daily analyses over a period of 5 days to obtain total viable count for each day.

Bacteriological Analysis of Water

All the media used for this study were prepared according to the manufacturer's specification. Serial dilutions method as described by Nester *et al*. (2007) was used for the water samples by taking 1.0 ml of the original solution with a sterile pipette into the first bottle containing 9 ml of sterile distilled water to make 10^{-1} dilution of the original water samples and the bottle was shaken thoroughly. Exactly 1.0 ml of the prepared 10^{-1} dilution was pipetted into another 9.0 ml of sterile distilled water to obtain 10^{-2} dilution of the original sample. This was diluted further to give 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. The serial dilution was used for total viable count (standard plate count).

For each water sample serially diluted (6-fold), aliquot of 0.1 ml of each dilution was plated out onto nutrient agar, MacConkey agar, Eosin Methylene Blue Agar, SSA and blood agar for bacterial identification (Vandepitte *et al*., 2003).

Standard Plate Count (SPC) for Bacteria Enumeration

Standard plate count for bacteria enumeration was carried out on the water samples before treatment (control) and daily on the water samples after treatment for 5 days. Pour plate method as described by Nester *et al*. (2007) was used. From the serial dilution prepared from the water samples, 1ml of each dilution was introduced into labelled sterile Petri dishes and 15 ml of plate count agar (kept at 45 ºC in a water bath) was added to each plate. The plates were rotated gently 4-6 times clockwise and anticlockwise, allowed to set and incubated aerobically at 37 ºC for 24 hrs in an inverted position. The series of dilutions and plating were done in triplicate. A concentration of 30 – 300 colony forming units (cfu) per plate was targeted to allow for the most accurate enumeration possible. This procedure was carried out on the water samples before and after treatment. After 24 hours of incubation, bacterial colonies for each dilution were counted using automatic colony counter. Counts were recorded as colony forming units per mL (cfu/mL) and bacterial loads were determined by multiplying average counts by dilution factor.

$$\text{cfu/mL} = \frac{\text{No of colonies counted X dilution factor}}{\text{Volume plated (ml)}}$$

Characterization and Identification of Isolates

Characterisation and identification on isolates was carried out adopting methods of Vandepitte *et al*. (2003) and Cheesbrough (2008).

Molecular Analysis of the Bacteria Isolates

Extraction and purification of bacterial genomic DNA was done using methods of Cocolin *et al*. (2000).

Laboratory Methods for Physicochemical Analyses of the Surface Water Samples

Physicochemical analyses of the water samples were determined prior and after treatment with solar radiation, *Moringa oleifera* powder and water guard solution using Standard Methods of Analysis of Water and Wastewater (APHA, 1999)

Data Analysis

Data generated were subjected to analysis of variance (ANOVA) as outline by Steel and Torrie (1980) using MINITAB statistical software version 17.0. Analysis of variance was conducted to assess whether significant ($p < 0.05$) differences existed among the treatment methods given so as to assess their effectiveness at 95.0 % confidence level.

The generated sequences from molecular analysis were identified using the online BLAST search at http://blast.ncbi.nlm.nih.gov/Blast.cgi. (Weston-Hafer *et al*., 2006; Altschul *et al*., 1997). The phylogenetic affiliations of the isolates were then determined with a phylogenetic tree constructed using the MEGA 7.0 software (Kumar *et al*., 2016).

Results and Discussion

Daily effect of treatment on bacteria loads of water samples showed that control water samples from all the locations had much higher bacteria loads than those of the treated samples. This is in line with earlier observation that control water from these locations without treatment is not safe for drinking and is supported by similar studies of Amagloh and Benang (2009) on the effectiveness of *Moringa oleifera* seed as coagulant for water purification. After treatment, the mean bacteria count reduced significantly in the water samples from all the location with the treatment methods giving variable results. The mean bacteria count recorded for the daily observation of treatment were generally high exceeding the limit stated by WHO (2011). However, the value recorded for the combine treatment gave no bacteria counts in water samples from Bele stream which is in accordance with standard set by WHO (2011) (Fig. 1).

With the treatment methods, slight increases in bacteria counts were sometimes seen from the second day of treatment. Despite the reduction in the mean bacteria counts following solar, *Moringa oleifera* and water guard treatment. The profiles of 16S rRNA gene products by DNA electrophoresis showed that the bacterial isolates could be amplified by the universal primers 27F/1492R used (Fig. 3). Exactly 11 bacterial species were detected with a single band observed at around 1500 bp. From this study, bacterial species that belonged to the genera *Klebsiella*, *Pseudomonas*, *Aeromonas*, *Pantoea* and *Kosakonia* were isolated which is supported by similar studies of Ivanova *et al*. (2002) and Felf¨oldi *et al*. (2010) where bacteria species belonging to the genera *Klebsiella*, *Pseudomonas* and *Aeromonas* were reported from drinking water sources.

The bacteria strains isolated from these water samples are phylogenetically close to strains which are pathogenic to man (Fig. 4). The optimum pH range recommended by WHO (2011) is 6.5–8.5. From the results of physicochemical parameters obtained from the water samples, it could be seen that the pH of the control water samples from Umur stream are within the recommended range for drinking water specified by WHO (2011). Low pH values were recorded in water samples from Bele, this is in line with Edema *et al*. (2001) who reported that high levels of free CO_2 in water reduces pH of water (Table 1).

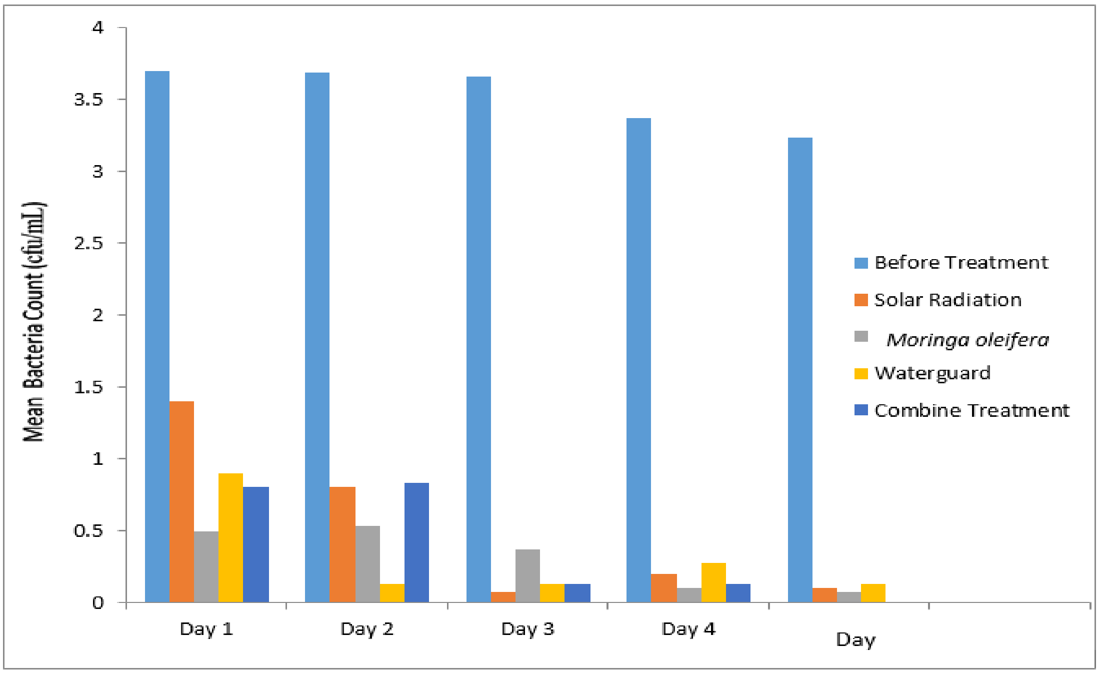

Fig.1: Daily effect of treatment on bacterial loads of water samples from Bele stream.

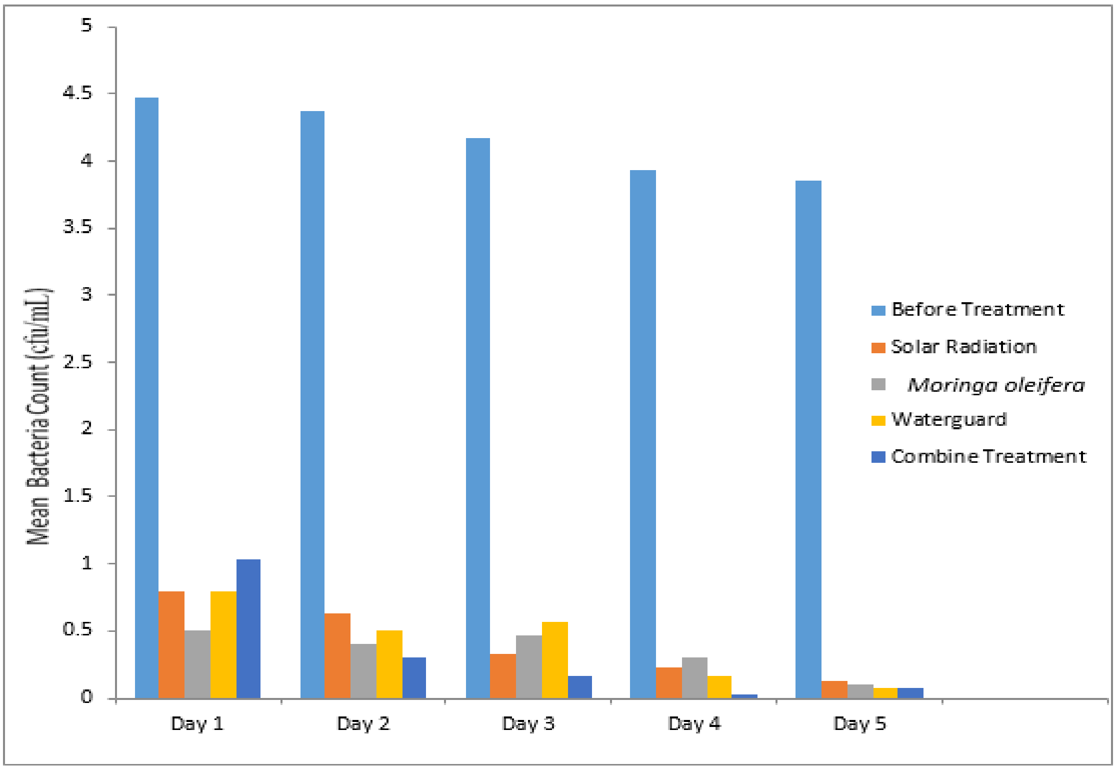

Fig. 2: Daily effect of treatment on bacterial loads of water samples from Umur stream.

[Each of the mean value was a product of three determinations. Means that do not share a letter in a row are significantly different ($p < 0.05$).]

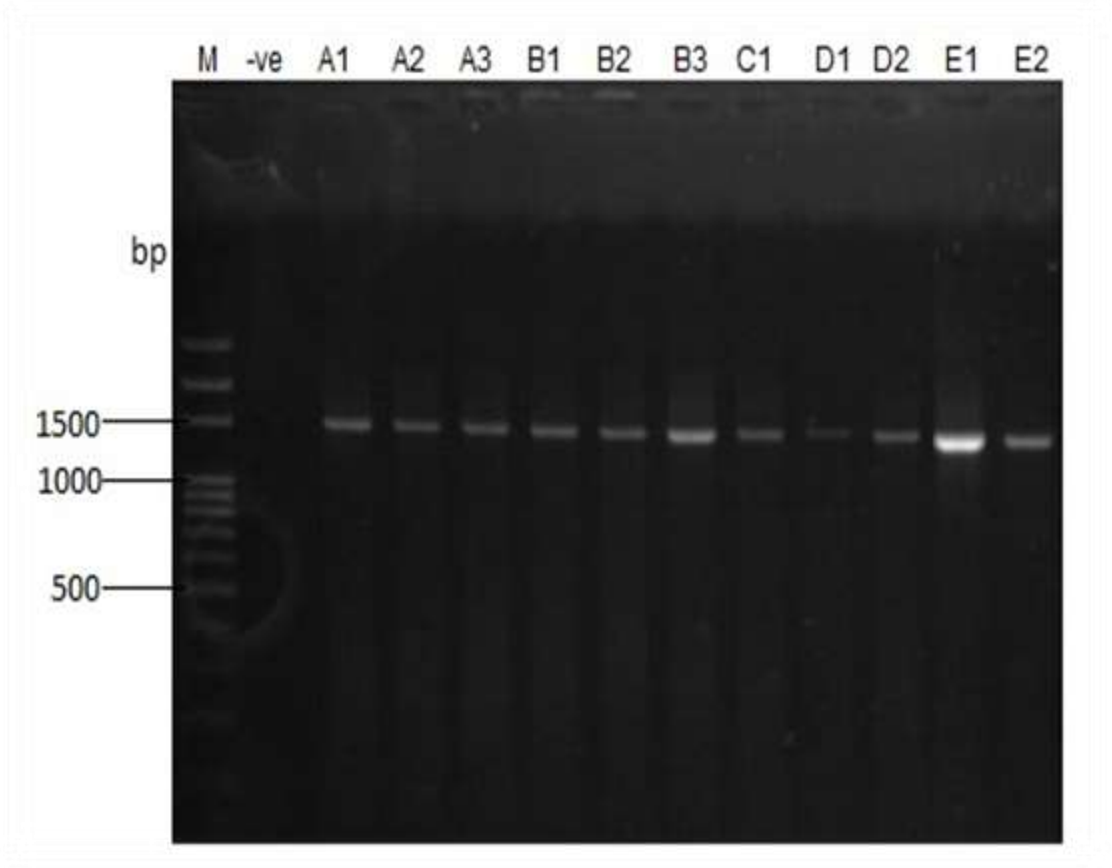

Fig. 3: DNA electrophoresis profiles of bacterial 16S rRNA gene fragments amplified from the isolates.
Key: A1 – E2 are bacterial isolates, M – DNA Marker used (1500, 1000, 500 bp)

Table 2 shows that there was dramatic fall in the mean pH values following treatment. In Umur stream, the mean value of pH obtained before treatment were significantly different from the mean values obtained for all the treatment methods used ($p < 0.05$). In Umur stream, solar treatment shows no significant difference with water guard and combine treatment ($p > 0.05$) but they differs significantly with *Moringa oleifera* treatment (Table 2). This shows that the treatment impacted significantly on the pH of the water samples. pH was not affected significantly in water samples from Bele stream since there was no significant differences observed in the mean values obtained ($p > 0.05$). The ranges of EC recorded in the studied water samples were within the stated limit set by WHO (2011). There were significant differences among the mean values recorded for EC ($p < 0.05$) except for treatment with water guard which do not significantly affect the EC of water samples from Bele stream. This shows that the treatment methods especially solar and *Moringa oleifera* significantly alters the EC of the water samples. Mean DO_2 values measured from the different location were within the allowable limit prescribed by WHO (2011). In Bele stream, treatment with solar and *Moringa oleifera* do not show significant variation from control water sample ($p > 0.05$) while treatment with water guard and the combine effect were significantly different from the control water sample at 95.0 % confidence level. However, it is observed that, solar treatment caused BOD values for all the water samples to be significantly higher than the mean values obtained from the control water samples ($p < 0.05$). The results shows that COD increase significantly with solar treatment and *Moringa oleifera* there was no significant difference among them ($p > 0.05$). High turbidity was observed in water samples from Bele and Umur streams. These values were high compared to the declared WHO (2011) guideline for turbidity provided for safe drinking water. The treatment used impacted significant differences ($p < 0.05$) on turbidity in water samples collected. The mean values of TDS found from the water samples under study were within the set standard. The lowest mean TDS values of 29.00 mg/L and the highest TDS values of **577.67** mg/L corresponding to water samples treated with *Moringa oleifera* from Umur stream. Solar treatment had no significant effect on water samples from Bele and Umur stream ($p > 0.05$). Treatment with *Moringa oleifera*, water guard and combine significantly ($p < 0.05$) increase the TDS of water samples from both locations. Sulphate found in Umur streams were within the permissible level prescribed by WHO (2011). However, treatment methods significantly reduced sulphate level in water sample from Umur streams ($p < 0.05$).

Table 1: Effect of Treatments on Physicochemical Parameters of WaterSample from Bele Stream

Parameter	Before treatment (control)	Solar radiation	*M. oleifera*	Water Guard	Combine treatment	SEM	p-value
pH	6.13^{a}	6.13^{a}	6.13^{a}	6.27^{a}	6.20^{a}	0.06	0.45
EC (µS/cm)	952.00^{a}	87.33^{d}	432.00^{c}	953.33^{a}	479.00^{b}	2.00	0.00
DO_2	3.10^{b}	3.07^{b}	3.00^{b}	2.23^{c}	3.40^{a}	0.03	0.00
BOD	1.17^{b}	1.97^{a}	0.70^{c}	0.53^{c}	1.00^{b}	0.07	0.00
COD	2.33^{b}	3.93^{a}	1.40^{c}	1.07^{c}	2.00^{b}	0.14	0.00
Turbidity (NTU)	115.00^{c}	125.33^{b}	60.30^{d}	126.00^{b}	133.67^{a}	0.81	0.00
Colour (PtCo)	549.67^{a}	536.00^{a}	276.00^{b}	533.30^{a}	269.67^{b}	9.12	0.00
TDS	47.67^{d}	51.67^{cd}	261.67^{b}	53.67^{c}	295.00^{a}	1.42	0.00
SO_4^{3-}	51.33^{d}	57.00^{c}	68.00^{a}	65.00^{b}	51.00^{d}	0.65	0.00
NO_3^-	12.97^{d}	20.20^{b}	18.03^{c}	21.03^{a}	18.40^{c}	0.14	0.00
PO_4^{3-}	0.65^{d}	0.28^{e}	1.04^{b}	0.73^{c}	1.35^{a}	0.01	0.00

Key: TDS - Total dissolved solids, DO_2 - Dissolved Oxygen, BOD - Biochemical Oxygen Demand, SO_4^{3-} - sulphate, NO_3^- - Nitrate, PO_4^{3-} - phosphate, COD - Chemical Oxygen Demand, µS/cm- microSiemens/centimeter, NTU - Nephelometric Turbidity Unit, PtCo - Platinum Cobalt, EC - Electrical Conductivity and SEM - Standard errors of the means. All parameters are in mg/L except otherwise stated. Each of the mean value was a product of three determinations. Means that do not share a letter in a row are significantly different ($p < 0.05$).

Table 2: Effect of Treatments on Physicochemical Parameters of Water Sample from Umur Stream

Para Meter	Before treatment (control)	Solar radiation	*M. oleifera*	Water guard	Combined treatment	SEM	p-value
pH	6.93^{a}	6.67^{b}	6.20^{c}	6.53^{b}	6.60^{b}	0.07	0.00
EC (µS/cm)	554.67^{c}	442.67^{d}	343.00^{e}	1473.33^{a}	625.67^{b}	2.06	0.00
DO_2	4.60^{a}	3.17^{b}	0.50^{e}	2.20^{d}	2.47^{c}	0.04	0.00
BOD	0.93^{d}	2.23^{a}	2.03^{b}	1.70^{c}	1.90^{b}	0.05	0.00
COD	1.87^{e}	4.47^{a}	4.20^{b}	3.40^{d}	3.80^{c}	0.10	0.00
Turbidity (NTU)	16.08^{e}	39.23^{d}	86.50^{a}	81.28^{b}	77.67^{c}	0.47	0.00
Colour (PtCo)	550.00^{a}	104.00^{d}	550.33^{a}	442.33^{b}	281.67^{c}	1.00	0.00
TDS	272.00^{d}	273.67^{d}	577.67^{a}	283.00^{c}	367.00^{b}	1.00	0.00
SO_4^{3-}	27.67^{a}	20.67^{b}	17.67^{c}	18.67^{c}	21.67^{b}	0.62	0.00
NO_3^-	4.03^{e}	7.10^{b}	8.17^{a}	6.77^{c}	6.63^{d}	0.04	0.00
PO_4^{3-}	0.38^{d}	0.28^{e}	1.88^{b}	0.47^{c}	2.75^{a}	0.04	0.00

Key: TDS - Total dissolved solids, DO_2 - Dissolved Oxygen, BOD - Biochemical Oxygen Demand, SO_4^{3-} - sulphate, NO_3^- - Nitrate, PO_4^{3-} - phosphate, COD - Chemical Oxygen Demand, µS/cm- microSiemens/centimeter, NTU - Nephelometric Turbidity Unit, PtCo - Platinum Cobalt, EC- Electrical Conductivity and SEM - Standard errors of the means. All parameters are in mg/L except otherwise stated. Each of the mean value was a product of three determinations. Means that do not share a letter in a row are significantly different ($p < 0.05$).

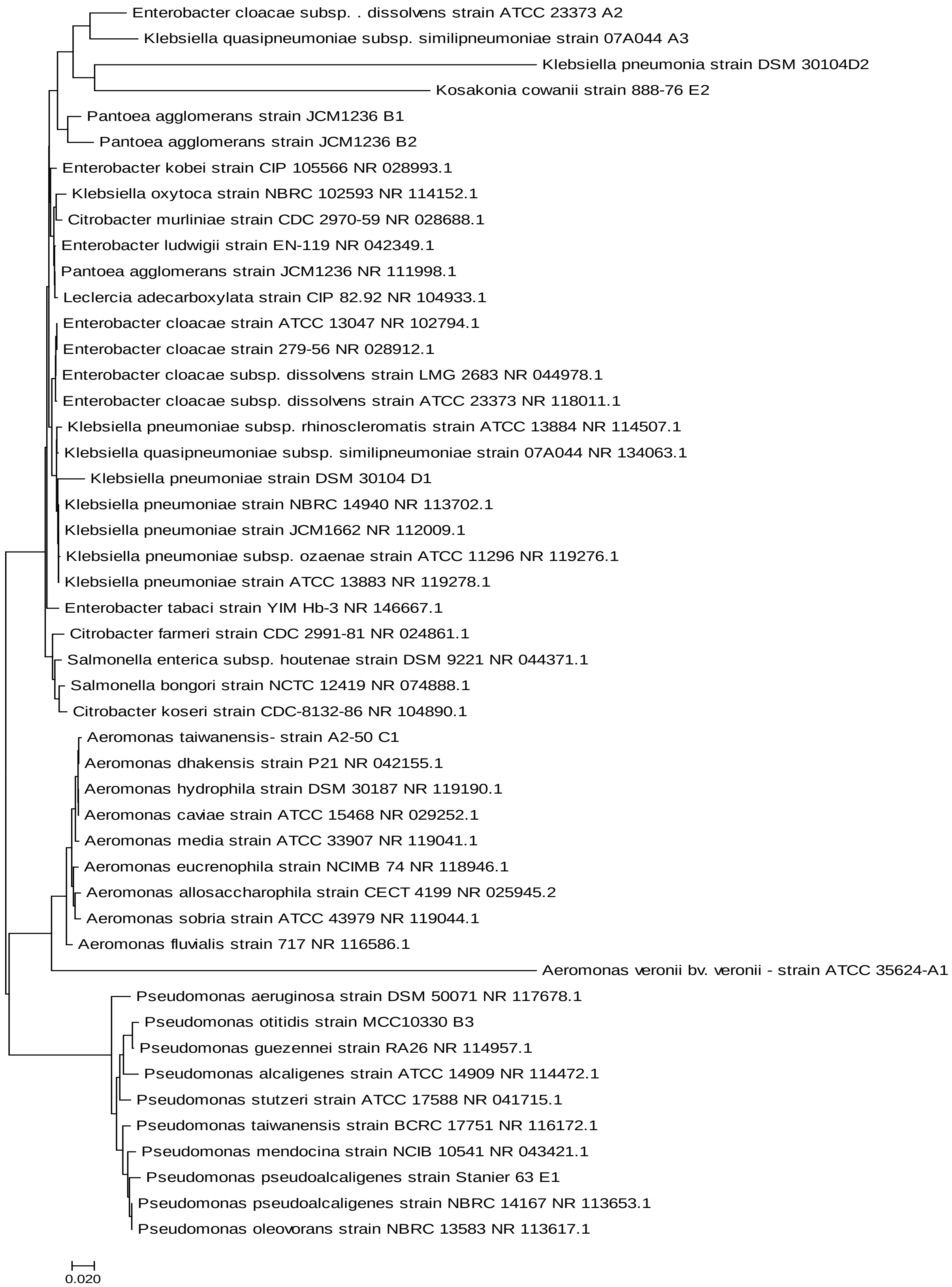

Fig. 4: Phylogenetic tree of the bacteria strains derived from 16S rRNA sequence data obtained.

Treatment with solar, *Moringa oleifera* and water guard significantly increased the sulphate content of water samples from Bele stream ($p < 0.05$) while their combine effect gave no significant difference and all values recorded for this stream were fairly higher than the WHO (2011) permissible limit. The values of nitrate measured were generally low compared to the WHO permissible limit in drinking water. Following treatment with water samples from Bele stream, there was significant difference among treatment with solar, wate rguard while no significant difference with *Moringa oleifera* treatment and combine effect on nitrate level. The nitrate content of the water samples recorded following *Moringa oleifera* treatment was not supported by work of Bengtsson (2003) where nitrate values recorded were not affected by treatment with *Moringa oleifera.* The range of phosphate recorded was between 0.17 to 2.75 mg/L. Following analysis, significant differences were observed on phosphate content of all the water samples ($p < 0.05$). The values were within allowable limit given by WHO (2011).

Conclusion

It was concluded from the analyses of this study that water bodies in the study area are contaminated with bacterial strains which are capable of causing infectious diseases as a result, human health is continuously being threatened due to lack of potable water. Synergistic method of water treatment is more effective; though single methods of water treatment can also be employed in reduction of bacteria population.

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