

# Review of: "Assessment of Quality, Bacterial Population and Diversity of Irrigation Water in Selected Areas of Minna, Niger State, Nigeria"

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Potential competing interests: No potential competing interests to declare.

Note for authors:

1. Words needing omission are struck and replacements/additions are highlighted as bold.
2. Numbered comments are added within respective sections wherever it seems essential.
3. Spaces required between characters/words are underlined.

## Abstract

Comment 1: In the abstract, the first sentence should be a problem statement.

Comment 2: Since the study was conducted in 2016, a lot of change might have happened to the groundwater state and associated physicochemical and biological properties; then, what is the relevance of publishing such a study after so many years?

This research was carried out in the cropping season of 2016 to determine the quality, bacterial population, and diversity of irrigation water in selected areas of Minna, Niger state. The treatments comprised four locations where irrigation agriculture is practiced in Minna, namely Bali in Chanchaga, Fadikpe, Mechanic Village in Keterin Gwari, and Soje-A in Kpakungu, where water samples were collected at 3 points from the water sources and mixed together to represent the locations. The treatments were replicated three times and fitted to a Completely Randomized Design (CRD). The physical, chemical, and biological properties of the irrigation water were determined according to standard methods. Results showed that bacterial population and diversity were significantly affected by location and that all the physical and chemical properties of the irrigation water were significantly affected by location except for chemical oxygen demand (mg/l). Fadikpe had the highest bacterial population ( $2.5 \times 10^8$  CFU/ml) and was the least diverse. Other locations were equally diverse, with Soje-A having the lowest bacterial population ( $2.8 \times 10^7$  CFU/ml). Averagely, the bacterial population found in the irrigation water of Minna may not be biodegrading. *B. subtilis*, which has a higher potential for biodegradation, was unable to reduce the biochemical oxygen demand in this research. Water from Chanchaga ~~recorded the best quality~~ **was relatively best suitable** for irrigation, even though values of some of the physicochemical properties were higher than values recommended by the Food and Agriculture Organization standard. Further studies should therefore be carried out

to ~~establish~~ **identify** the potentials of *B.subtilis* in the bioremediation of Chanchaga water and to investigate the biodegrading potential of *Escherichia coli* ~~since it correlated negatively~~ **due to its negative correlation** with total dissolved solids, biochemical oxygen demand, chemical oxygen demand, and iron **content**.

## Introduction

Water covers 71% of the earth's surface (CIA, 2008). In developing countries, 90% of all wastewater goes untreated into local rivers and streams (UNEP, 2002). The most important use of water in agriculture is for irrigation in areas with little or no rainfall; it is a key component for producing enough food for the ever-growing population. Irrigation takes up to 90% of water in some developing countries (WBCSD, 2010). Some **(Please mention number)** years back, it was assumed that water was an infinite resource. ~~At this time, there were fewer people, with less than half of the present population. of the people on earth now, which~~ **Doubling of population in short time** has given rise to higher water demand for human consumption and use. This includes the growing of crops and raising of animals, and there is an increase in demand for water from industries and urbanization. In the future, more water will be needed to produce more food because the ~~e~~Earth's population is forecasted to be 9 billion by 2050 (United Nations press, 2007).

Water used to replace or supplement precipitation in crop production is called irrigation water (Hargreaves and Merkley, 1998). Irrigation is an essential part of crop production in areas with low annual rainfall. Therefore, a wide range of farmlands are irrigated worldwide for food production. Irrigation of crops is an important and long-used practice to increase agricultural and horticultural production.

Zia et al. (2013) reported that fresh water that can be used for irrigation and which is accessible to humans comprises less than 1% of the Earth's total water resources. In many cases, irrigation water is stored naturally or artificially prior to use.

The sources of irrigation water thus include rainwater, groundwater, surface water, and wastewater.

The use of wastewater for irrigation at different levels of crop production is a common practice throughout the world (McGrath and Lane, 1989). According to FAO (1997), using wastewater for urban and peri-urban food production is an important resource that can be used for meeting the challenges of rapidly growing cities in sub-Saharan Africa.

Most of the sources of water used for irrigation in Nigeria are water that contains contaminants of fecal origin from the sewage system and also contains heavy metals and other pollutants from urbanization, like human activities and ~~washing away~~ **run-off of through** soil from farmlands where fertilizers, pesticides, and other chemicals have been used.

In Minna, Nigeria, wastewater flows from different sources into the main drainage channels containing refuse, domestic and industrial waste, which may contaminate water used for irrigation or be a source of energy for heterotrophic nutrition and microbial growth and development.

The water used for irrigation contains microorganisms, which are living organisms that may be single-celled (Madigan and Martinko, 2006) or multicellular. Microorganisms are diverse, and they include bacteria, archaea, and most of the

protozoa;

the water also contains some species of fungi, algae, and some species of animals like rotifers.

Bacteria constitute a large domain of prokaryotic organisms; they live in symbiotic and parasitic relationships with plants, animals, and other hosts. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a milliliter of fresh water. There are approximately  $5 \times 10^{30}$  bacteria on Earth (Whitman et al. 1998), which forms a biomass that exceeds that of all plants and animals. Bacteria are vital in nutrient cycling, where many of the stages of nutrient cycling depend on these bacteria, such as nitrogen fixation from the atmosphere and putrefaction in the biological communities surrounding hydrothermal vents and cold seeps. Microbial activities, such as the ones mentioned earlier, affect water and soil health, hence **there is a** need to assess the extent of influence that the continuous use of wastewater for irrigation has on the immediate environment.

### Aim and Objectives

The aim of the study is to assess the quality, bacterial population, and **their** diversity in water used for irrigation in selected areas around Minna.

The objectives of this study are to:

1. Determine **the spatial variation in** the general bacteria population of water used for irrigation ~~as affected by location~~.
2. Determine the **spatial variation in** diversity of the bacteria population of water used for irrigation ~~as affected by location~~.
3. **Assess** ~~Correlation between~~ the physicochemical properties of irrigation water with the bacterial population.

### Materials and Methods

Comment3: Authors are suggested to add a study area map with sampling sites marked for more clarity.

#### Study Area

The study was carried out in four locations where irrigation schemes are used for growing vegetable crops around Minna. The locations fall under the southern ~~g~~Guinea ~~s~~Savanna ecological zone, and they include Soje-A in Kpakungu, which lies approximately on latitude 09°35' 46.6" N and longitude 06°32' 10.4" E; Mechanic village in Keterin Gwari, which lies approximately on latitude 09°36' 13.8" N and longitude 06°32' 15.8" E; Bali in Chanchaga, which lies approximately on latitude 09°32' 0.8" N and longitude 06°34' 53.9" N; and Fadikpe, which lies approximately on latitude 09°31' 53.3" N and longitude 06°35' 21.1" N. The average annual temperature is 27.0 °C (80.70 °F) with an average monthly temperature of 5.30 °C (9.50 °F). The total annual precipitation averages 492.9 litres/m<sup>2</sup>.

#### Soil, Water, and Vegetation Description

Minna is located in the southern Guinea savanna of Nigeria. The typical vegetation in the southern Guinea savanna consists of open savanna woodland. The areas used for the experiment are locations where irrigation agronomy is practiced for vegetable crop production.

#### Treatment and Experimental Design

Treatments are sewage water from locations practicing irrigation as follows: Soje-A in Kpakungu, Fadikpe, Mechanic village in Keterengwari, and Bari in Chanchaga, replicated three times and arranged in a Completely Randomized Design (CRD).

#### Water Sampling and Analysis

Sampling of the irrigation water was carried out during the early hours of the morning when farmers were irrigating their farmlands. Sterilized bottles were used to collect water samples from three different points and intervals along the drainage channel and also directly from hoses used by the farmers for conveying water to farms. Water samples collected were stored in an icebox and taken to the laboratory for microbial (bacteriological) analysis. The water samples were analyzed within 24 hours of collection. The physical, chemical, and microbial analyses were in accordance with the “Standard Methods for the Examination of Water and Wastewater,” as follows: Chloride content by Argentometric titration method, Total Hardness by 0.01 M EDTA titrimetric method, Alkalinity by 0.02M  $\text{H}_2\text{SO}_4$  titration method, Electrical conductivity using a conductivity meter (**mention model name and manufacturer**), sulphate by Nephelometric method, Total Dissolved Solids (TDS) by gravimetric method, Nitrate-nitrogen by UV spectrophotometric method, Biochemical Oxygen Demand (BOD) by the bottle incubation method, Chemical Oxygen Demand (COD) by titration with Ammonium sulphate solution, Heavy metals using inductive couple plasma mass 59 spectrometry (**Make & model info**) (ICPMS), pH using a potentiometric method.

#### Preparation of Agar

Twenty-eight grams of nutrient agar was weighed accurately into a 1000mL (1-litre) volumetric flask, and distilled water was added to dissolve the agar, after which the content of the volumetric flask was made to mark. The flask was corked tightly and placed in the autoclave for 15 minutes at a temperature of 121°C.

MacConkey Agar (MCA) and Salmonella Shigella Agar (SSA) were prepared according to the manufacturer's instructions, which involved weighing 52.55 grams of MCA into a 1000mL (1-liter) flask and then dissolving the agar and making up to mark using distilled water. The flask was corked tightly and placed in an autoclave for 15 minutes at 121°C.

63 grams of SSA was weighed into a 1000mL flask, and then the content was dissolved and made up to mark using distilled water. The agar was then boiled with frequent agitation to completely dissolve the agar, after which it was allowed to cool.

#### Culturing

1 mL of the  $10^6$  dilution was aspirated using a syringe and then distributed evenly in a petri dish, after which the warm media/agar was poured on the plate and swirled gently to mix the contents thoroughly. The content of the petri dish was allowed to gel and then transferred to an incubator set at a temperature of 37 °C for 24 hours. Colonies observed were counted and estimated as colony forming units per ml ( $\text{CFU}^{-1}\text{mL}$  of water).

#### Purification of Bacterial Isolates

Pure cultures were obtained by streaking different and distinct colonies onto sterile nutrient agar plates. The pure cultures obtained were then transferred onto agar slants in McCartney bottles and incubated at 37 °C for 24 hours. The bottles were then stored in the refrigerator for further analysis.

## Characterization and Identification of Bacterial Isolates

The characterization and identification of bacterial isolates were based on the colony morphology and biochemical tests carried out on pure cultures of isolates (Fawole and Oso, 2001; Dubey and Maheshwari, 2004; Garrity et al., 2004).

### Colony Morphology

The colony morphology of the microorganisms was based on the shape, size, elevation, edge, transparency, and pigmentation for the identification of colonies.

xxxxxx

### Gram Staining

A drop of distilled water was placed on a clean, grease-free slide, and a fresh culture of 24 hours was smeared on the slide with the aid of an inoculating wire loop. The smear was air-dried and heat-fixed by passing the underneath of the slide over the flame three times. The heat-fixed smear was then stained by flooding it with crystal violet solution (primary stain) for 30-60 seconds. The dye was quickly drained and washed with Gram's iodine (mordant) for 60 seconds. The iodine was drained off, and the slide was washed gently under the tap. Ninety-five percent ethanol (decolourizer) was then used to flush the violet stains for 10-15 seconds. The slide was again washed under the tap and counterstained with safranin for 30 seconds. The slide was drained, washed, and blot-dried. The slide was then observed first under the high-power lens and then under the oil immersion lens. Gram-positive bacteria appeared purple while Gram-negative bacteria appeared red (Fawole and Oso, 2001).

### Spore Staining

A smear of the isolate was prepared and passed over the flame several times to fix the smear. The slide was flooded with malachite green and heated over a beaker of boiling water for 10 minutes, making sure the stain did not dry up by adding more malachite green. The stain was washed under the tap and flooded with safranin for 20 seconds. The slide was washed under the tap, blot-dried, and observed first under high power and then under the oil immersion lens of a microscope. Spore formers had a green endospore resting in pink to red cells, while non-spore formers and vegetative cells appeared blue. Some spores were spherical to oval and **while others** were either smaller or larger than the diameter of the parent bacterium (Fawole and Oso, 2001).

### Motility Test

The hanging drop technique was used, in which a small drop of the bacterial broth culture was placed on the cover slip using a sterile inoculating loop. A thin film of petroleum jelly was applied around the edge of the depression of the cavity slide. The cavity slide was gently inverted over the cover slip and pressed down in order to make it airtight. The cavity slide was then observed under the  $\times 40\times$  objective lens of the microscope. The directional movement of bacterial cells indicated motility, while no movement of the bacterial cells indicated non-motility (Collins et al., 1989).

### Catalase Test

A 24-hour-old culture was used to carry out the test. A sterile wire loop was used to make a homogeneous suspension on

the slide. A drop of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was added to the suspension, and the occurrence of effervescence indicated a positive reaction, while its absence indicated a negative reaction (Fawole and Oso, 2001).

### Starch Hydrolysis

Nutrient agar with 1% starch solution was prepared and autoclaved at  $121^\circ\text{C}$  for 15 minutes. The medium was allowed to cool ~~at up to~~ **up to**  $42^\circ\text{C}$  and then poured into plates to set. The isolates were then streaked onto the plates and incubated at  $37^\circ\text{C}$  for 24 hours. After incubation, the plates were flooded with Gram's iodine, and the formation of a clear zone around the colonies showed a positive result, while no clear zone indicated a negative result (Brock et al. 1994).

### Indole Test

Peptone broth was used to carry out the test. The broth was prepared according to the manufacturer's prescription by adding 15 g of peptone powder to 1 litre of distilled water. Five ~~millilitres~~ **milliliters** of the broth were dispensed into McCartney bottles and autoclaved at  $121^\circ\text{C}$  for 15 minutes. The medium was seeded with the isolates and incubated at  $35^\circ\text{C}$  for 48 hours. After incubation, there was appreciable growth of the isolates. One ~~millilitre~~ **milliliter** of chloroform was added to the broth culture and shaken gently. Also, 2 ~~mL~~ of Kovac's reagent was added and shaken gently. The bottles were allowed to stand on the bench for 20 minutes to permit the reagent to rise to the top. A red colouration at the top layer indicated indole production, while a yellow colouration indicated a negative result (Fawole and Oso, 2001).

### Urease Test

Urea, a common organic nitrogen source for many microorganisms, can be hydrolyzed to ammonia and carbon (IV) oxide. Urease is an enzyme that catalyzes the breakdown of urea to ammonia and carbon (IV) oxide. Bijou bottles containing 3 ~~mL~~ of sterile modified Christensen's urea broth were prepared by slanting. The slants were inoculated with isolates and incubated at  $37^\circ\text{C}$  for 18-24 hours. The development of a pink colour indicated a positive result (Brock et al., 1994).

### Oxidase Test

This test was carried out using freshly prepared oxidase reagent. After placing a filter paper in a clean Petri dish, 2-3 drops of the oxidase reagent were placed on the filter paper. With the aid of a sterile glass rod, a colony of each of the isolates was picked and smeared on the filter paper in the Petri dish. The development of a blue-black colour within a few seconds on the filter paper indicated a positive result (APHA, 2002).

### Lactose Test

An inoculum from a pure culture was transferred aseptically to a sterile tube of phenol red lactose broth. The inoculated tube was incubated at  $35\text{--}37^\circ\text{C}$  for 24 hours, and the results were determined. A positive test was indicative of a color change from red to yellow as a result of a pH change to acidity. The pH indicator phenol red is red at neutral pH but turns yellow at  $\text{pH} < 6.8$ . It also changes to magenta or hot pink at  $\text{pH} > 8.4$ .

### $\text{H}_2\text{S}$ Test

Using the plate method for the  $\text{H}_2\text{S}$  Test, a well-isolated colony from a fresh culture of the test bacterium was picked using a sterile wire loop, and the culture was streaked over the NA agar plate to get well-isolated colonies. Thereafter, plates

were incubated aerobically at  $35\pm 22^{\circ}\text{C}$  for about 24 hours to observe the color of the developed colonies. Black colonies and/or colorless or colored colonies with a black center indicated a positive test.

### ~~0~~O<sub>2</sub> Relationship Test

Different requirements for molecular oxygen were observed by growing bacteria in thioglycolate test tube cultures. ~~A test-tube culture was started with~~ **Starting with** autoclaving **a test-tube culture of** thioglycolate medium containing a low percentage of agar to allow motile bacteria to move throughout the medium. Thioglycolate has strong reducing properties, and autoclaving flushed out most of the oxygen. Thereafter, the test tubes were inoculated with the bacterial cultures to be tested and incubated at  $37^{\circ}\text{C}$ . Over time, oxygen slowly diffused throughout the thioglycolate tube culture from the top, with bacterial density increasing in the area where the oxygen concentration was best suited for the growth of that particular organism. The growth of bacteria at the top of tube A indicated that they are obligate (strict) aerobes that cannot grow without an abundant supply of oxygen, whereas the growth in tube B, concentrated at the top of the tube and growth throughout the tube, typified facultative anaerobes that thrive in the presence of oxygen but also grow in its absence by relying on an electron acceptor other than oxygen. Other possibilities include growth at the bottom of the test tube, which is an indication that they are obligate anaerobes, which are killed by oxygen, and indifferent growth in the presence of oxygen in a tube, implying that the isolates are aerotolerant anaerobes. They do not use oxygen because they usually have a fermentative metabolism, but they are not harmed by the presence of oxygen as obligate anaerobes are. Another test tube can show a “Goldilocks” culture. The oxygen level has to be just right for growth, not too much and not too little. These microaerophiles are bacteria that require a minimum level of oxygen for growth, about 1%–10%, well below the 21% found in the atmosphere.

### Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS) version 9.0, and the mean was separated using the least significant difference (LSD) at the 5% level of probability. A correlation matrix was used to correlate the physical and chemical properties of irrigation water with the bacteria population and diversity.

### Results and Discussion

Bacterial spp. observed across the locations were slightly different in colony morphology (Table 1) ~~but as well as~~ quite different in assimilation of carbon sources and reactions to gram stain (Table 2). ~~Averagely~~ **Mostly**, they were round or circular in isolate shape, rod-shaped in cell shape, entire in margin, moist in texture, opaque in structure, and convex in elevation depending on the growth media (Table 1). The *Bacillus* spp. were quite more able to assimilate N and C sources than *E. coli* and *Salmonella* (Table 2). This, however, could not explain why *Salmonella*, with poor utilization of N and C sources, was averagely the most abundant in the irrigation water. The possible reason ~~why *Salmonella* was averagely the most abundant was~~ **due to** ~~is~~ its facultative aerobic nature, i.e., its ability to use other electron acceptors other than ~~0~~O<sub>2</sub>. This may explain why oil-contaminated water of the mechanic village sustained the highest populations of *E. coli* and *Salmonella* and the least population of aerobes (Table 3). Their ability to utilize the hydrocarbons ~~contained~~ **present** in water from the mechanic village or their ability to survive in oxygen-deprived water due to oil contamination conferred resilience and survival in the water.



Bacteria population and diversity of water vary with location. Results from Table 3 show that irrigation water obtained from Fadikpe had the highest bacteria population of  $2.5 \times 10^8$  CFU/mL of water sample, probably as a result of its near-neutral pH of 6.7 (Table 4), which is similar to the result obtained by Agbabiaka and Oyeyiola (2012), who worked on water and soil obtained from Foma River, Ilorin. The highest bacteria population (Table 3) was recorded at pH 6.5-7.0 (Table 4), which is within the recommended FAO (1995) standard for irrigation water of 6.5-8.5. *Bacillus megaterium* and *Salmonella spp.* counts were highest in irrigation water obtained from Fadikpe (Table 3), probably because some of the physical and chemical properties of water (Table 4) favored their proliferation. A lower BOD (342 mg/L) probably increased their population, coupled with the nitrate content of 0.4 mg/L (Olutiola et al., 2010), even though the BOD was above the recommended FAO (1995) standard of 100 mg/L. The presence of *Salmonella spp.* ( $2.1 \times 10^7$  CFU/mL) and *Escherichia coli* in the water obtained from Mechanic Village (Table 3) also suggested that the water contained more fecal contaminants than water obtained from other locations. The high Biochemical Oxygen Demand of (756.33 mg/L) (Table 4) obtained in Chanchaga water suggested low mineralization of organic matter and, consequently, a low nitrate content of 0.15 mg/L. This is consistent with the report of Olutiola et al. (2010), who ~~maintained~~ **mentioned** that high microbial decomposition of organic matter would result in high nitrate content. It was, however, observed that the BOD value was higher than the COD (Table 4).

Comment 4: In the Table 4, please check the value for TDS as it doesn't seem concurrent with EC values because it doesn't follow general relation between EC and TDS which is  $TDS = EC \times 0.5$ . I think you had mentioned values of TSS instead of TDS because TDS is well below the half of EC values in all the samples.

Comment 5: In Table 4 please mention any possible reason for such a low pH in case of Chanchaga treatment.

Comment 6: In Table 4 please check your conductivity value as well for Chanchaga treatment as if we consider charge balance, the sum of anions should be less than or equal to TDS but here the sulphate concentration is higher than the EC and TDS value.

The value in Chanchaga is probably due to the high concentration of ammonia in the water. According to Debnath et al. (2014), the COD value is usually higher than the BOD value, but in a case where the BOD value is higher than the COD value, it means the water contains a high amount of ammonia and a low amount of nitrate. The organic materials probably accumulated as a result of low mineralization, hence resulting in a low concentration of nitrate. High BOD and COD concentrations observed in the wastewater (Table 4) may be a result of using chemicals that contain high levels of organic contaminants (Salem et al., 2011).

Chloride and sulphate content of irrigation water from Soje-A were the highest (Table 4), suggesting that the water was contaminated with industrial and domestic inputs. Higher values of lead and copper in the irrigation water from Mechanic Village (Table 4) may be a result of oil contamination from petroleum products.

Results from the correlation matrix showed that, with the exception of BOD, lead, copper, and iron, all other physical and chemical properties correlated negatively with *Salmonella spp* (**Table 5**), which means the presence of *Salmonella spp* reduced all other physical and chemical properties except BOD and the heavy metals. While pH, BOD, lead, copper, and



iron correlated positively with *Bacillus megaterium*, all the physical and chemical properties of the irrigation water obtained correlated negatively with *Bacillus subtilis* except total hardness and BOD (Table 5). This means *Bacillus subtilis* has the potential for reducing the chemical oxygen demand (COD) of polluted water; this is supported by the result obtained by Hamza et al. (2009), who observed that *Bacillus subtilis* isolated from the Kaduna refinery reduced the COD by 82.9%. *Escherichia coli* correlated negatively with TDS, BOD, COD, iron, sulphate, and nitrate, but correlated positively with electrical conductivity, total hardness, total alkalinity, chloride, lead, copper, and pH (Table 5), suggesting that *E.coli* may have potential for bioremediation. This has, however, not been confirmed.

Comment 7: For clear understanding of irrigation suitability, authors should have provided a comparison with global standard limits of physicochemical parameters, and additional parameters ( $\text{Na}^+$ ,  $\text{K}^+$ ) should be assessed for calculating the irrigation suitability index.

## Conclusion

Comment 8: As a part of best practice, it is suggested to authors that they please refrain from discussing results, citing tables in the conclusion section, and rather try mentioning the inferences from the study.

In conclusion, the highest bacteria population was recorded at Fadikpe, and it was significantly different from the bacteria population at other locations (Table 3). The location with the highest bacteria diversity was Chanchaga, followed by Mechanic village and Soje-A (Table 3). Chanchaga irrigation water was the most suitable, recording the lowest values of water properties measured, apart from the Biochemical Oxygen Demand (BOD) (Table 3). Some of the values of water properties were, however, higher than the FAO recommended standard for irrigation water. Further studies should therefore be carried out to establish the potentials of *Bacillus subtilis* in the bioremediation of Chanchaga water. Farmers using water from other locations should be encouraged to harvest water during the rainy seasons in wells that can be resupplied during the dry seasons. This strategy is hoped to **might** alleviate the sufferings of the poor rural farmers sourcing for suitable irrigation water in Minna.