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

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## Abstract

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

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## 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). Around 50 years ago, it was assumed that water was an infinite resource, with less than half of the present population. 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 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 Merkle, 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. Transporting water to the fields requires various methods such as pipes, sprinklers, liquid-fuel engine-driven systems, or watering cans (Bandiane *et al.*, 2018).

The sources of irrigation water include rainwater, groundwater, surface water, and wastewater. 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. Currently, Nigeria possesses an estimated total arable land area of approximately 34.6 million hectares, of which only 40% is utilized for cultivation, and less than 5% is allocated for irrigation (Adelodun, 2018). Research by Akinbile *et al.* (2016) has revealed that irrigated agriculture heavily relies on artificial water application, accounting for approximately 80% of global water consumption and 86% in developing countries as of 1995. In many cases, irrigation water is stored naturally or artificially prior to use.

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, other human activities and run-off through soil from farmlands where fertilizers, pesticides, and other chemicals have been used (Amneera *et al.*, 2013; Liu *et al.*, 2020).

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. Extensive research has demonstrated that the quality of irrigation water (IWQ) significantly influences both crop production and soil performance. IWQ can be classified into several categories: salinity hazard (total soluble salt content), sodium hazard (proportional relationship between sodium and calcium/magnesium), pH level (acidic or alkaline), alkalinity (carbonate and bicarbonate levels), and specific ions (chloride, sulfate, boron, and nitrate). Additionally, consideration should be given to microbial pathogens, which are

another aspect of irrigation water quality that can impact the suitability of cropping systems (i.e., the types of crops grown and the practices employed to cultivate them) (Bauder *et al.*, 2014).

These microorganisms which may be single-celled (Madigan and Martinko, 2006) or multi-cellular are diverse, and they include bacteria, archaea, and most of the protozoa, 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.
2. Determine the spatial variation in diversity of the bacteria population of water used for irrigation.
3. Assess correlation between the physicochemical properties of irrigation water with the bacterial population.

**Standard for Irrigation Water**

Parameters (units)	World standard
Electrical conductivity (ds/m <sup>3</sup> )	0.7-3.0
Nitrate (mg/l <sup>3</sup> )	5-30
Total dissolved solids (mg/l <sup>3</sup> )	450-2000
Chloride (mg/l <sup>3</sup> )	0-30
Biochemical oxygen demand (mg/l)	100
Chemical oxygen demand (mg/l)	250
pH	6.5-8.5
Copper (mg/l)	0.2
Iron (mg/l)	5.0
Lead (mg/l)	5.0
Alkalinity (mg/l)	200
Total hardness (mg/l)	350
Sulphate (mg/l)	20

FAO (1995)

## Materials and Methods

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 Guinea sSavanna 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 Fadikwe, 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 35.0 °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, Fadikwe, Mechanic village in Keteren gwari, 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 slope positions 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 H<sub>2</sub>SO<sub>4</sub> titration method, Electrical conductivity using a conductivity meter (Mettler Toledo), 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 (PlasmaQuant MS) (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<sup>0</sup>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<sup>0</sup>C. Sixty three 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

One milliliter of the 10<sup>6</sup> 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<sup>0</sup>C for 24 hours. Colonies observed were counted and estimated as colony forming units per ml (CFU<sup>-1</sup>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<sup>0</sup>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.

### 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 x 40x 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 ( $H_2O_2$ ) 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  $121^{\circ}C$  for 15 minutes. The medium was allowed to cool up to  $42^{\circ}C$  and then poured into plates to set. The isolates were then streaked onto the plates and incubated at  $37^{\circ}C$  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 milliliters of the broth were dispensed into McCartney bottles and autoclaved at  $121^{\circ}C$  for 15 minutes. The medium was seeded with the isolates and incubated at  $35^{\circ}C$  for 48 hours. After incubation, there was appreciable growth of the isolates. One 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  $37^{\circ}C$  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 35-37°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 pH <6.8. It also changes to magenta or hot pink at pH >8.4.

## H<sub>2</sub>S Test

Using the plate method for the H<sub>2</sub>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±22 °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.

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

Different requirements for molecular oxygen were observed by growing bacteria in thioglycolate test tube cultures., 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°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) as well as quite



different in assimilation of carbon sources and reactions to gram stain (Table 2). 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 probably is its facultative aerobic nature, i.e., its ability to use other electron acceptors other than O<sub>2</sub> (Bergey's, 2015). 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 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 (Lokesh Sharma *et al.*, 2022).

**Table 1.** Colony Morphology of bacteria spp in the Macroculture

ISOLATE DESCRIPTION	SHAPE OF COLONY	MARGIN	COLOR	TEXTURE	STRUCTURE	ELEVATION	INFERRED ORGANISM
Chan1, Soj3, Mech1	Round	Entire	White shiny	Moist	Opaque	Convex	<i>Bacillus subtilis</i>
Chan2, Fad1, Soj2	Round	Entire to undulate	Milk white that turned yellow to brown or black	Mucoid	Opaque	Concave	<i>Bacillus Megaterium</i>
Mech2	Circular	Entire	Gray on blood agar	Moist	Opaque or Translucent	Concave on Mac Conkey, Low Convex on NA	<i>Escherichia coli</i>
Chan3, Mech3, Soj1, Fad 2	Circular	Entire	Smooth and white colony or greyish white in blood Agar	Moist	Translucent	Convex	<i>Salmonella</i>

Chan= Chanchaga isolate, Fad= Fadikwe isolate, Mech= Mechanic village isolate, Soj = Soje A isolate.

**Table 2.** Biochemical Test for bacteria spp in the Macroculture

ISOLATE DESCRIPTION	G.R	S.S	Mort	Oxid	Ure	Cata	S.H	Indole	Lact	H <sub>2</sub> S	O <sub>2</sub> r/ship	INFERRED ORGANISM
Chan1, Soj3, Mech1	+	+	+	+	+	+	+	-	-	+	Aerobic	<i>B.megaterium</i>
Chan2, Fad1, Soj2	+	+	+	+	+	+	-	-	-	+	Aerobic	<i>B.subtilis</i>
Mech2	-	-	+	-	-	+	-	+	AG	-	FA	<i>E.coli</i>
Chan3, Mech3, Soj1, Fad 2	-	-	+	-	-	-	+	-	-	+	FA	<i>Salmonella spp</i>

Growth (+), No Growth (--), gram reaction (G.R), spore stain (S.S), mortality (Mort), oxidase (oxid), urease (ure), catalase (cata), starch hydrolysis (S.H), lactose (lac), acid with gas (AG), facultative anaerobe (FA)

Bacteria population and diversity of water varied with location. Results from Table 3 shows that irrigation water obtained from Fadikwe 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 Fadikwe (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 (Langholz and Michele, 2013, Vogt *et al.*, 2019). 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 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).

**Table 3.** Effect of Location on Bacteria Population and Diversity

Location	Bacteria population ( $\times 10^6$ CFU/ml)	Diversity			
		<i>Bacillus megaterium</i> ( $\times 10^6$ CFU/ml)	<i>Bacillus subtilis</i> ( $\times 10^6$ CFU/ml)	<i>Escherichia coli</i> ( $\times 10^6$ CFU/ml)	<i>Salmonella spp</i> ( $\times 10^6$ CFU/ml)
Chanchaga	108.33 <sup>b</sup>	20.33 <sup>b</sup>	41.67 <sup>a</sup>	0.00 <sup>b</sup>	31.67 <sup>b</sup>
Fadikwe	245.33 <sup>a</sup>	88.33 <sup>a</sup>	0.00 <sup>d</sup>	0.00 <sup>b</sup>	61.67 <sup>a</sup>
Mechanic village	56.67 <sup>c</sup>	0.00 <sup>d</sup>	11.00 <sup>b</sup>	22.00 <sup>a</sup>	20.67 <sup>c</sup>
Soje-A	28.33 <sup>d</sup>	8.67 <sup>c</sup>	8.00 <sup>c</sup>	0.00 <sup>b</sup>	9.67 <sup>d</sup>
SE $\pm$	2.42	1.35	0.65	0.29	1.40
Sig,	**	**	**	**	**

\*\* = highly significant.

Means with the same letter(s) in a column are significantly different at  $P \leq 0.05$

CFU= colony forming units. Sig = Significance

**Table 4.** Effect of Location on Physicochemical Properties of Irrigation Water

Treatment	EC (µmho/cm)	pH	(TDS) (mg/l)	Total hardness (mg/l)	Total alkalinity(mg/l)	Chloride(mg/l)	SO <sub>4</sub> <sup>2-</sup> (mg/l)	NO <sub>3</sub> (mg/l)	BOD (mg/l)	COD (mg/l)	Pb (mg/l)	Cu (mg/l)	Fe (mg/l)
Chanchaga	0.5 <sup>b</sup>	3.60 <sup>d</sup>	318.7 <sup>b</sup>	231.00 <sup>c</sup>	17.00 <sup>d</sup>	29.80 <sup>d</sup>	288.72 <sup>d</sup>	0.15 <sup>d</sup>	756.3 <sup>a</sup>	574.0 <sup>d</sup>	0.03 <sup>b</sup>	0.04 <sup>d</sup>	2.7 <sup>b</sup>
Soje A	1.2 <sup>a</sup>	7.11 <sup>b</sup>	764.9 <sup>a</sup>	260.00 <sup>b</sup>	421.00 <sup>b</sup>	285.07 <sup>a</sup>	478.94 <sup>a</sup>	0.85 <sup>a</sup>	360.0 <sup>b</sup>	764.0 <sup>a</sup>	0.07 <sup>b</sup>	0.17 <sup>ba</sup>	2.9 <sup>b</sup>
Fadikwe	0.72 <sup>b</sup>	6.7 <sup>c</sup>	458.3 <sup>b</sup>	184.00 <sup>d</sup>	132.50 <sup>c</sup>	74.48 <sup>c</sup>	383.35 <sup>b</sup>	0.40 <sup>b</sup>	342.0 <sup>b</sup>	584.0 <sup>b</sup>	0.14 <sup>a</sup>	0.37 <sup>a</sup>	6.2 <sup>a</sup>
Mechanic village	0.97 <sup>a</sup>	7.11 <sup>a</sup>	622.7 <sup>a</sup>	328.00 <sup>a</sup>	470.00 <sup>a</sup>	260.25 <sup>b</sup>	362.10 <sup>c</sup>	0.28 <sup>c</sup>	261.0 <sup>b</sup>	624.0 <sup>b</sup>	0.15 <sup>b</sup>	0.42 <sup>a</sup>	1.6 <sup>c</sup>
SE±	0.093	0.02	3.54	4.04	3.11	0.86	2.67	0.01	85.47	39.55	0.01	0.07	0.3
Sig.	**	**	**	**	**	**	**	**	*	NS	**	*	**

\*\* = highly significant, \* = significant, NS = not significant

Means with the same letter(s) in a column are significantly different at  $P \leq 0.05$

NB; EC= Electrical Conductivity, TDS= Total Dissolved Solids, SO<sub>4</sub><sup>2-</sup> = Sulphate, NO<sub>3</sub>= Nitrate, BOD= Biochemical Oxygen Demand, COD= Chemical Oxygen Demand, Pb= Lead, Cu= Copper, Fe= Iron

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. In spite of that, the pH of water was lowest and not reflective of the actual pH probably due to the presence of localized contaminants. 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). Ratio of COD to BOD were low and ranged from 0.76 to 2.12 with the lowest obtained at Chanchaga and the low obtained at mechanic village. Low COD: BOD ratios of less than 10 recorded across sites means that the compounds were relatively degradable (<https://slideplayer.com/amp/10219336/>). 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. Chlorine is used in many household cleaners as a disinfectant, bleaching agent, automatic dishwasher detergents, toilet bowl cleaners, laundry soaps (www.webmd.com). Similarly, sulphate mineral salt can be found in consumer products like laundry and dish detergents, toothpaste, shampoo, liquid hand soaps (www.webmd.com). Higher values of lead and copper in the irrigation water from Mechanic Village (Table 4) may be as a result of oil contamination from petroleum products. Aigbenua (2023) observed lead and copper contamination in soils around oil well clusters.

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 volume and concentrations of all other physical and chemical properties except BOD and these heavy metals. This also implies that *Salmonella spp* could serve as a bio-indicator of the toxicity of these 3 heavy metals and can therefore be used to monitor their toxicity (Khatri and Tyagi, 2015). Similarly, pH, BOD, lead copper, and iron correlated

positively with *Bacillus megaterium*, implying that *B. megaterium* is bio-indicating the toxicity of Pb, Cu and Fe and can therefore be used to bio monitor the toxicity of these heavy metals (Jain *et al.*, 2010.). All the physical and chemical properties of the irrigation water including COD correlated negatively with *Bacillus subtilis* except total hardness and BOD (Table 5). This means *Bacillus subtilis* has potential for reducing 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 Kaduna refinery reduced the COD by 82.9%. This also implies that *Bacillus subtilis* have the potential to bio remediate the heavy metals under-studied (Sharma *et al.*, 2021). *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 bio remediating iron, sulphate and nitrate (Kouret *et al.*, 2021).

**Table 5.** Correlation Coefficients for the Relationships between Water Physical, Chemical and Bacteriological Properties.

	EC	PH	TDS	T.H	T.A	Cl	SO <sub>4</sub>	NO <sub>3</sub>	BOD	COD	Pb	Cu	Fe
<b>BAC</b>	-0.6142	-0.1037	-0.515	-0.7925	-0.6467	-0.7208	-0.2872	-0.3387	0.0630	-0.5215	0.2924	0.1992	0.8840
<b>SAL</b>	-0.6078	-0.1319	-0.5766	-0.7446	-0.6488	-0.7335	-0.3556	-0.4113	0.0583	-0.5958	0.3207	0.1871	0.8355
<b>BM</b>	-0.5488	0.03237	-0.2995	-0.8483	-0.5574	-0.6031	-0.0516	-0.0978	0.0114	-0.3116	0.2559	0.2405	0.9543
<b>BS</b>	-0.5338	-0.9287	-0.2433	0.0283	-0.5412	-0.4898	-0.7196	-0.5553	0.7345	-0.2702	-0.7108	-0.6123	-0.4442
<b>ECO</b>	0.7150	0.4011	-0.3967	0.8491	0.6350	0.5053	-0.1366	-0.309	-0.4155	-0.0732	0.6195	0.5081	-0.5642

*BAC* = Bacteria, *SAL* = salmonella, *BM* = *Bacillus megaterium*, *BS* = *Bacillus subtilis*, *ECO* = *Escherichia coli*, *EC* = Electrical conductivity, *TDS* = Total dissolve solids, *T.H* = Total hardness, *TA* = Total alkalinity, *Cl* = chloride, *SO<sub>4</sub>* = SulphaSincete, *NO<sub>3</sub>* = Nitrate, *BOD* = biochemical oxygen demand, *COD* = chemical oxygen demand, *Pb* = Lead, *Cu* = copper, *Fe* = Iron.

## Conclusion

Since only culturable and aerobic or facultative aerobic organisms can be obtained using the method described here, there will be a need for a targeted metagenomic approach to be followed to determine the full diversity in the different locations bearing in mind that a lot of bioremediation processes depend on anaerobic conditions. Chanchaga irrigation water was the most suitable water recording the lowest value of water properties measured apart from the Biochemical Oxygen Demand (BOD). Its pH of water value was also lower than the FAO recommended standard for irrigation water and this should also be investigated. Further studies should 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 might alleviate the sufferings of the poor rural farmers sourcing for suitable irrigation water in Minna.

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