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# ISOLATION AND SCREENING OF SALT TOLERANT AND PLANT GROWTH PROMOTING PROPERTIES OF BACTERIA FROM SALINE SOIL

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## ABSTRACT

Soil salinity is a limiting factor of agricultural production in arid and semi-arid areas that reduces yields and optimal crop production. This study aim of this work has to do with assessing the effect of bacteria isolated from the saline soil. The soil sample used for the research were sampled at DAMBATTA and KURA of Kano State using a randomized sampling method. The physicochemical properties and microbiological characteristics were determined using standard methods. The pH of DAMBATTA and KURA were (8.63 and 9.48) respectively, and were all weakly alkaline organic matter, phosphorus, and nitrogen, were all higher in Kura. The moisture content was higher in Kura (0.85%). Three (3) gram positive and four (4) gram negative bacteria isolates were isolated, seven (7) isolates that have high potential were identified as *Bacillus thuringiensis* (DBT 1), *Sphingobacterium spp* (DBT 2), *Myroides spp* (DBT 3), *Bacillus subtilis* (KK1), *Sphingobacterium psychroaquaticum* (KK5), *Bacillus cereus* (KK8), *Paenochrobactrum glaciei* (K4). The optimum salt tolerance showed that DBT 3 has the highest tolerance of 10 % whereas DBT2 recorded the least at 0.6% while isolates from Kura, KK1 has the highest salt tolerance of 5% and the least was recorded By K4 having 0.4%. For growth promoting properties, DBT3 and KK5 have the highest N-fixation rate, and the highest IAA was recorded in DBT1 and KK8. For P-solubilization potential, DBT3 and KK1 were highest. This study showed variation in bacterial isolates to salt concentration and their capacity to produce growth promotion properties.

**Keywords**: PGPR, Salt Tolerance, P-Solubilization, N-Fixation

## INTRODUCTION

Salt-affected cultivated land accounts for 19% of the world's 2.8 billion hectares of arable land. Salt-affected areas account for 20% of the world's irrigated farmland on average, although this figure rises to more than 30% in arid countries. According to Zare *et al.* (2014), there is a risk associated with the 10% annual increase in saline area around the planet. It was also stated that approximately half of the world's irrigated area has already been damaged to some extent by waterlogging and salinisation, and that much of the land expected to be irrigated in the future is highly vulnerable to similar damage. On a global scale, at least 200,000 to 300,000 ha of irrigated land are lost each year due to salinisation and waterlogging (Mattamana *et al.,* 2013).

Soil salinisation is a key soil degradation process that endangers the ecosystem and is acknowledged as one of the most pressing worldwide issues for crop production, food security, and sustainable agriculture (Lombardi *et al.,* 2022). Halophytes and halo-tolerant plants adapt to natural saline environments via physiological mechanisms such as cellular ion homeostasis and osmotic pressure regulation, reactive oxygen species detoxification, and changes in membrane composition and secondary metabolite production (Lombardi *et al.,* 2022). To deal with salt stress, significant research has concentrated on developing stress-tolerant plant lines through genetic engineering and plant breeding. One interesting strategy could be to introduce salt-tolerant microorganisms to promote plant growth in salinity-stressed locations. Both plant-associated fungi and bacteria have the potential to improve plant nutrition, yield biomass, and stress tolerance. However, the role of symbiotic bacteria in salt adaptation is not fully understood (Khalmuratova *et al.,* 2020). Therefore, it would be necessary to combine data from genomic, transcriptomic, proteomic, and metabolomic studies in order to identify important pathways and processes has regulated by microbial factors, which would lead to plant tolerance to salinity. Researching the composition and activities of microbial communities in saline soil may help clarify their crucial role in the biological mechanisms of controlling nutrient cycles in saline soil (Zhang *et al.,* 2019). Plant growth-promoting rhizobacteria (PGPR) and halophytes are believed to have co-evolved to allow these plants to thrive in salty environments (Da Silva *et al*., 2013; Pan *et al*., 2020).

Zhang *et al.*, (2018) reported on the variety of salt-tolerant bacteria that were isolated from the paddy rhizosphere in Taoyuan, China. From the 305 bacterial strains they identified, 162 were examined for salt tolerance at concentrations of up to 150 g/l NaCl. According to Zhang *et al.,* (2018), phylogenetic examination of 74 of these salt-tolerant strains revealed that they are members of the following orders: *Bacillales* (72%), *Actinomycetales* (22%), *Rhizobiales* (1%), and *Oceanospirillales* (4%). Most of the isolates also demonstrated the capacity to enhance rice's growth, yield, and tolerance to salt under salt-stress circumstances. Chrysanthemum plants cultivated in China's saline-alkaline soil exhibited enhanced salt tolerance thanks to the ST-PGPR strain Bacillus licheniformis SA03 (Zhou *et al.,* 2017). Sharma *et al.,* (2015) have identified the variety of salt-tolerant bacilli in the soil of India's eastern Indo-Gangetic plains. Of the 95 bacterial strains they isolated, 55 exhibited traits that promoted plant development and tolerance to salts above 4% NaCl. The diversity of ST-PGPR in coastal regions is also reported by a number of researchers. For instance, 121 bacterial strains were isolated from tsunami-affected areas of India's Andaman and Nicobar Islands, and 23 of them demonstrated salt tolerance up to 10% NaCl with PGP traits like siderophore, extracellular enzymes, phosphate solubilization, and the production of indole acetic acid (IAA)

(Amaresan *et al.,* 2016). With a broad range of pH adaptability and PGPR characteristics, including as phosphate solubilisation, IAA biosynthesis, acetoin and 2,3-butanediol synthesis, siderophore generation, and N2 fixation, D5A demonstrated the presence of salt tolerance genes (Liu *et al.,* 2016). By decreasing the levels of salicylic acid (SA) and abscisic acid (ABA), decreasing the efficiency of the photosystem, increasing the accumulation of IAA in the leaf, and preventing the accumulation of root chloride and proline during salt stress, Pseudomonas putida and *Novosphingobium* sp. have been shown to lessen the damage caused by salt stress in citrus plants (Vives-Peris et al., 2018). A Pseudomonas strain isolated from halophyte *Distichlis spicata* also improved the growth of different crops with salt stress (Palacio-Rodríguez *et al.,* 2017).

## MATERIAL AND METHODS

### Description of Study Area

The Kano River Irrigation Project I (KRIP) is located between latitudes 11°32'N to 11°51'N and longitudes 8°20'E to 8°40'E within the Sudan savannah zone of Nigeria (Abubakar *et al.,* 2004). Two selected towns from the Kano river Irrigation site will be selected as the sampling sites. The sites are Kura Local Government Kano State Nigeria which is along A2 highway of Kano to Zaria. It is located at the central part of Kano between latitudes $11^0$46'.17''N and longitudes 08° 25' 49" E and 206 $km^2$ (Nura *et al.,* 2024). The second location is Thomas dambatta which is located in the northern fringe of Kano state and bordered to the north and east by Kazaure and Babura local government of Jigawa state respectively. It lies approximately at latitudes $12^0$25'.59''N and longitudes 08° 30' 00" E (Mamman *et al.,* 2022).

### Sample Collection

A total of 40 soil sample, 20 cores from each sampling area was randomly collected at depth of 15cm using sterile soil auger. The soil samples collected was bulk to make a three (3) composite samples from each of the sampling area to have a total of six (6) samples. The samples were put into newly polyethene bags and transported to microbiology and soil science research laboratory of Bayero University, Kano for microbiological and physicochemical analysis respectively.

### Assessment of Some Soil Physicochemical Parameter

#### *Soil pH in $H_20$ (1:2.5)*

Ten grams (10g) of air-dried soil (2-mm Sieve) was weigh into a 50-ml beaker and 25ml of distilled water was added. The suspension was stirred several times for 30 minutes with a glass rod. The soil suspension was allowed to stands for about 30mins which allowed most of the suspension clay to settle out from the suspension. Electrodes of the pH meter (Janway, P757s) was inserted into the partly settles suspension to measure the pH (Eno *et al.,* 2009).

#### *Organic Carbon*

One (1g) of soil sample was weigh into 500ml conical flask. Ten milliliter (10ml) of 1N $K_2Cr_20_7$ solution was pipette accurately into each flask and swirled gently to disperse the soil. Twenty milliliter (20ml) of concentrated $H_2S0_4$ was added gently and immediately swirled. The flask is then swirl more vigorously for one minute and allowed to stand for 30mins for oxidation to complete. After 30mins, 100ml of distilled water was added and 4 drops of 1-10 phenanthroline indicator was added and titrated against standard 0.5N ferrous sulphate solution to obtain an end point. A blank was prepared with same treatment but without sample (Eno *et al.,* 2009). The results were calculated as follow:

$$\% \text{ organic C} = \frac{(Blank\ titre - actual\ titer)\ x\ 0.3\ x\ N\ x\ F}{\text{g of air-dry soil}}$$

Where;
F = Correction factor = 1.33
N= Normality of solution used

#### *Moisture Content*

An empty moisture can was weighed (W1) and five (5) grams of soil sample was added to the moisture can with tight fitting lid and re-weigh as (W2). The moisture can contain the sample was then dried in an oven at 105°C for 24hrs after which the set up was removed and put in desiccator to cool. After cooling, the sample was reweighed again (W3) (Eno *et al.,* 2009).

$$\text{Moisture Content (\%)} = \frac{W2 - W3}{W3 - W1} \times 100$$

#### *Electrical Conductivity (EC)*

Soil EC was determined by suspending the air dried sample in water at the ratio of 1:5 and after shaking the mixture, it was allowed to stayed overnight, filter and dip electrode into the filtrate in the beaker. Electrical Conductivity at 25°C temperature was recorded by the use of electrical conductivity meter (Janway, 4520) (Danbarati, 2016).

#### *Determination of Total Nitrogen*

Kjeldhal method adopted by Eno *et al.* (2009) was used to determined total N in the soil sample. One gram of soil sample was weighed into digestion tube. Then 2grams of a catalyst (mixture of $K_2SO_4$, $CuSO_4$ and selenium powder in 100:10:1 proportion) and 15 mL of $H_2SO_4$ was added in each tube including blank and heated gently until frothing cease. After the formation of clear solution in each tube, the digestion was heated continuously for 30 minutes. After cooling, the mixtures will then transfer to a kjeldhal distillation flask. Ten millilitres (10ml) of boric acid solution and 4 drops of indicator (Bromocresol green and methyl red) solution was added and ammonia was distilled off into the boric acid and titrated against 0.025N HCl. The percent of nitrogen was calculated as follows:

$$\% \text{ of Nitrogen} = \frac{A - B \times N \times 0.014 \times VD}{\text{Ad} \times \text{W}} \times 100$$

Where:
A= Volume of sample required for titration of the sample,
B= Volume of sample required for titration of blank,
N = Normality of HCl,
0.014 = Milliequivalent weight of nitrogen.
Ad = Aliquot taken
W = Weight of sample
VD = Volume of Digest

#### *Determination of Phosphorous Content*

Olsen method was used to determine the available phosphorous in the soil sample (Olsen *et al.,* 1954). The 2.5gm of soil sample was weighed into graduation container. Then, 50 mL of Olsen reagent (sodium bicarbonate) was added to the container and shake with a shaker (Innova 4000) operated at 180 rpm for 30mins and blank was also prepared without soil sample. Furthermore, each mixture was filtered through Whatman No 42 filter paper and 5 ml of filtrate was transferred to a volumetric flask. To each reaction mixture 10 mL of distilled water and 4 mL of ascorbic acid was added and incubated for 30 minutes for blue colour formation. The absorbance of this solution was determined at 883nm and phosphorous content in soil was thus calculated as follows:

$$\text{P (mg/kg)} = \frac{(Abs)}{\text{Slope}} \times \frac{VF}{W}$$

Where:

VF = Volume of flask
W = Weight of sample
Abs = Absorbance

***Isolation and Identification of Bacterial Isolates***
Dilution technique was used to reduce the density of organisms present in the soil to a countable number by diluting the highly concentrated sample. Dilutions of each soil sample was prepared using seven folds dilution techniques. Ten grams (1g) of soil sample was suspended in 9 ml sterile distilled water; diluted logarithmically up to $10^{-9}$ level. Before soil particle settled down, 1 mL of suspension from each test tube was transferred to next test tube of each series. By repeating this step, 9-fold dilution of each soil sample was prepared (Olsen *et al.,* 1987). An aliquot of 0.5ml each of the soil suspension from dilution $10^{-1}$ to $10^{-9}$ pour plates was made in NaCl (4%) supplemented nutrient agar and incubate at 37°C for 72 h. The colony forming units (CFU) was then enumerated (Danbarati *et al.,* 2016).

***Screening of Bacteria for Salt Tolerance from Saline Soils***
Pure culture of bacteria was achieved by subsequent sub-culturing. Salt tolerance activity of the isolates the salt tolerance activity of the isolates was tested on nutrient broth supplemented with varying concentrations of NaCl (0.2, 0.4, 0.6, 0.8, 5 and 10%) and incubated at 25 ± 2 °C for 2 days; after which the optical density of the samples was read at 600 nm using a spectrophotometer.

**Plant Growth Promoting Bacterial Properties of the Isolates**
***Quantitative Determination of Indole Acetic Acid***
Indole-3-acetic acid (IAA) production by the selected isolates was determined spectro-photometrically. Two batches of pre-sterile Ashby's broth (4% NaCl, pH 8) tubes were made. One set comprises broth of Ashby with L- tryptophan (10µg/mL) and other without tryptophan. Label a selected isolate to tubes in both sets. To each their tubes, 10µl of the overnight culture of the cultures of the isolates was added and incubated at 30oC for 72 hrs and tested for IAA production by Salkowski's reaction. Following incubation, the broth from each tube was centrifuged at 10,000 rpm for 10 min to pelletize the biomass.Two (2 mL) of supernatant of the bacteria each culture was mixed with 2 drops of orthophosphoric acid and 4 mL of Salkowski's reagent (50 mL of 35 % perchloric acid and 1 mL 0.5 M $FeCl_3$ solution), it was incubate for 30 min and observed for appearance of pink colour. (Abbasi, 2015; Sachdev *et al.,* 2009).

**Phosphate Solubilization**
Overnight grown bacteria culture of the selected isolate was spotted and inoculated individually on modified Pikovskaya's agar containing 4% NaCl was incubated at 30°C for 5 to 7 days. After incubation, the isolates showed a zone of clearance around the colony was considered as phosphate solubilizer (Goswami *et al*., 2015)

**Ammonia Production**
Ammonia production by the selected bacterial isolates was examined in peptone water (4% NaCl). The 24 h old freshly grown cultures of each isolate was inoculated into 10 mL peptone water tube. All the tubes were further incubated for 7 days at 37°C. After incubation Nessler's reagent (0.5 mL) was added and the development of brown to yellow colour was considered as a positive test for the production of ammonia and the quantities of ammonium produced was determined by macro kjeldahl method (Emtiazi, *et al.,* 2007: Goswami *et al.,* 2014).

**Statistical Analysis**
Descriptive statistics and analysis of variance (ANOVA) was used due to different variables from the treatment was performed for the data generated using Gestat soft 17th edition which is the latest edition of the software, to reveal whether there were significant differences or not in the outcome of the analysis. Variability was considered only when the value is $P< 0.05$ (Vidhya and Thatheyus, 2013).

**RESULTS AND DISCUSSION**
**Soil Chemical Properties**
Table 1 present the soil chemical properties of the two studies with Kura and Dambatta having a neutral to slightly alkaline soil pH of 7.88 and 6.74 respectively. The electrical conductivity, organic matter, total nitrogen and phosphorus were all higher at Kura with 6.39dSm$^{-1}$, 2.8%, 0.30% and 24.52mgkg-1 respectively. The variation of the soil properties among the two locations may result from the soil texture and agricultural practices. Similar findings were reported by Jibril *et al.*, (2008) in his studies on Soil Fertility Status of the Kano River Irrigation Project Area in the Sudan Savanna of Nigeria and Maina *et al*., (2012) in his investigation of Soil salinity assessment of Kadawa irrigation of the Kano River Irrigation Project (KRIP) that the soil of those areas were capable of accumulating more soil salinity in near future if proper irrigation management are not observed.

**Table 1: Soil Chemical Properties**

| Sampling Site | pH ($H_2O$) | pH ($CaCl_2$) | EC (dS/m) | O.C (%) | O.M (%) | N (%) | P (mg/kg) | M.C (%) |
|---|---|---|---|---|---|---|---|---|
| Kura (KK) | 7.88 | 6.13 | 6.39 | 0.80 | 2.80 | 0.30 | 24.52 | 0.85 |
| Dambatta (DBT) | 6.74 | 5.89 | 4.54 | 0.32 | 1.82 | 0.14 | 10.81 | 0.59 |

**Cultural and Biochemical Characteristics of Bacterial Isolates**
The bacterial count of the saline soil from the two locations were shown in table 2, revealed that the soil sample of Kura has a total count of $1.16 \times 10^8$ cfug-1 while Dambatta has $1.96 \times 10^8$. The higher count was recorded at Dambatta may result from low soil salinity of the soil which makes the bacteria to have high tolerant. The morphology and biochemical test recorded that Kura has two grams positive and negative (bacteria *Bacillus subtilis, Bacillus cereus* and *Sphingobacterium psychroaguaticum, Paenochrobactrum glaciei)* respectively, while Dambatta soil has one gram positive and two negative bacteria (*Bacillus thuringinensis* and *Sphingobacterium spp, Myroides spp*) respectively as shown in table 2.

**Table 2: Result Showing the Bacteria Count of the Isolates**

| Replication | Sampling Sites | |
|---|---|---|
| | Kura | Dambatta |
| 1 | $1.20 \times 10^8$ | $2.06 \times 10^8$ |
| 2 | $1.18 \times 10^8$ | $1.87 \times 10^8$ |
| 3 | $1.11 \times 10^8$ | $1.94 \times 10^8$ |
| Average | $1.16 \times 10^8$ | $1.96 \times 10^8$ |

**Table 3: Morphology and Biochemical Test Result of Isolates**

| Test | DBT 1 | DBT 2 | DBT 3 | KK 1 | KK5 | KK8 | K 4 |
|---|---|---|---|---|---|---|---|
| Cell Shaped | Rods | Rods | Rods | Rods | Rods | | Rods |
| Spore formation | Non | Non | Non | Sub-Terminal | Non | Central | Non |
| Gram reaction | + | - | - | + | - | + | - |
| Methyl Red | + | - | - | - | - | - | - |
| Voges Proskauer | + | - | - | + | - | + | - |
| Citrate | + | + | - | + | - | + | + |
| Catalase | + | + | + | + | + | + | + |
| Oxidase | - | + | + | + | + | - | + |
| Urease | + | - | - | - | - | - | - |
| Indole Production | - | - | - | - | + | - | - |
| Nitrate Reduction | + | + | + | + | + | + | + |
| Expected Organisms | *Bacillus thuringinensis* | *Sphingobacterium spp* | *Myroides spp* | *Bacillus subtilis* | *Sphingobacterium psychroaguaticum* | *Bacillus cereus* | *Paenochrobactrum glaciei* |

Key: Negative (-), Positive (+)

**Screening of Bacteria Isolates for Salt Tolerance**

The bacteria isolates were screened for varied salt tolerance. The bacteria isolate DBT3 has the highest salt tolerance at 10% whereas DBT2 recorded lowest salt tolerance of 0.6% from the soil sample isolate of Dambatta. The isolates that has high salt tolerance was recorded from the soil sample of Kura with KK1 found to tolerate up to 5% sodium chloride while K4 showed lowest salt tolerance of 0.4% (Figure 1). The ability of the tolerance level of the bacteria from the two locations depends on the capacity of the bacteria to tolerate the different salt concentration from the two different sites where the soil of Kura showed high salinity than that of Dambatta. Muhammas and Hassan also reported that *Stenotrophomonas rhizophilia* was able to tolerate salt concentartion up to 20% NaCl, which is contrary to this investigation which only one isolate was able to tolerate salt concentration at 15% NaCl. This finding contradicts a study conducted by Sharma *et al.,* (2021) which reported that bacteria screened for salt tolerance can grow only up to 7.5% salt concentration. Similar research was conducted by Mahmood *et al*., (2019) isolated and screened a bacterium isolate *Mesembryanthemun cyrstallium* L. which tolerate salt concentration up to 10% . Li *et al.,* (2013) reported in their investigation of *Myroides odoratimimus* tolerance to salt and found that it has an optimum of 5% NaCl tolerance but can also thrive up to 10% NaCl.

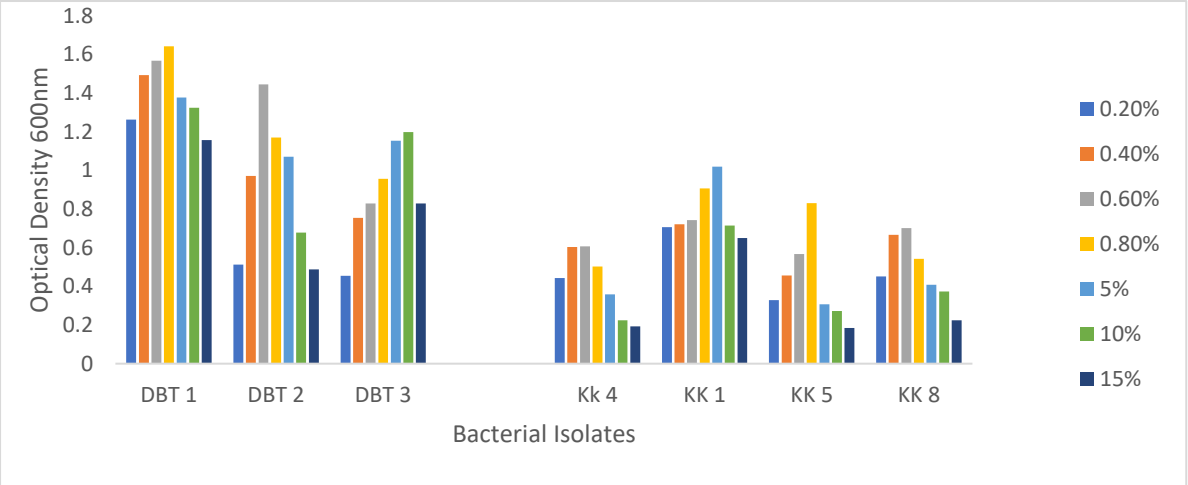


Figure 1: Screening of Bacteria Isolates for Salt Tolerance

**Screening of Bacterial Isolates for PGPR Properties**

The bacterial isolates with salt tolerance potential were further screened for PGPR properties such as Nitrogen fixation, Phosphate Solubilization, and Indole acetic acid production. All the bacteria isolates demonstrate the potential to produce these properties. N-fixation was recorded highest by DBT1 and lowest by KK1 (Fig. 2). Indole acetic acid production was recorded highest by DBT1 and lowest by KK5 (Fig.3), while in our investigation, DBT3 and KK8 (Fig. 4) were found to have the highest and lowest P-solubilization ability,

respectively. The accumulation of compatible solutes in their intracellular cell, such as trehalose, proline, and glycine betaine used to balance their osmotic pressure and harboring of genes responsible for the production of 2,3 butanediol and salicylic acid which may lead to more chances of producing growth promoting properties in salt stress. A similar study was reported by Komaresfla *et al.,* (2019), where they evaluated the PGPR on their potential for drought and salt tolerance. Islam *et al*., (2022) also reported some bacterial isolates that has the capacities to produce growth promoting properties in his research "Plant growth-promoting rhizobacteria: Salt stress alleviators to improve crop productivity for sustainable agriculture development". Furthermore, Panwar *et al.,* (2019) in his investigation "Salt-Tolerant Plant Growth Promoting Rhizobacteria for Enhancing Crop Productivity of Saline Soils" isolated bacterilas with plant growth promoting properties. The PGPR Mechanisms of Salt Stress Adaptation and Plant Growth Promotion was also reported by Hassan *et al.,* (2022).

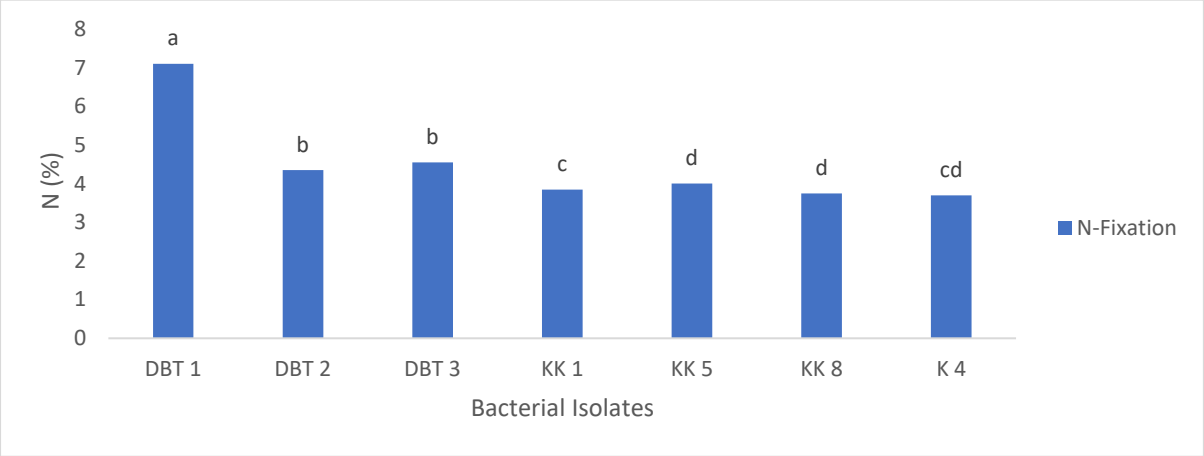


Figure 2: Nitrogen Fixation of the Bacteria Isolates

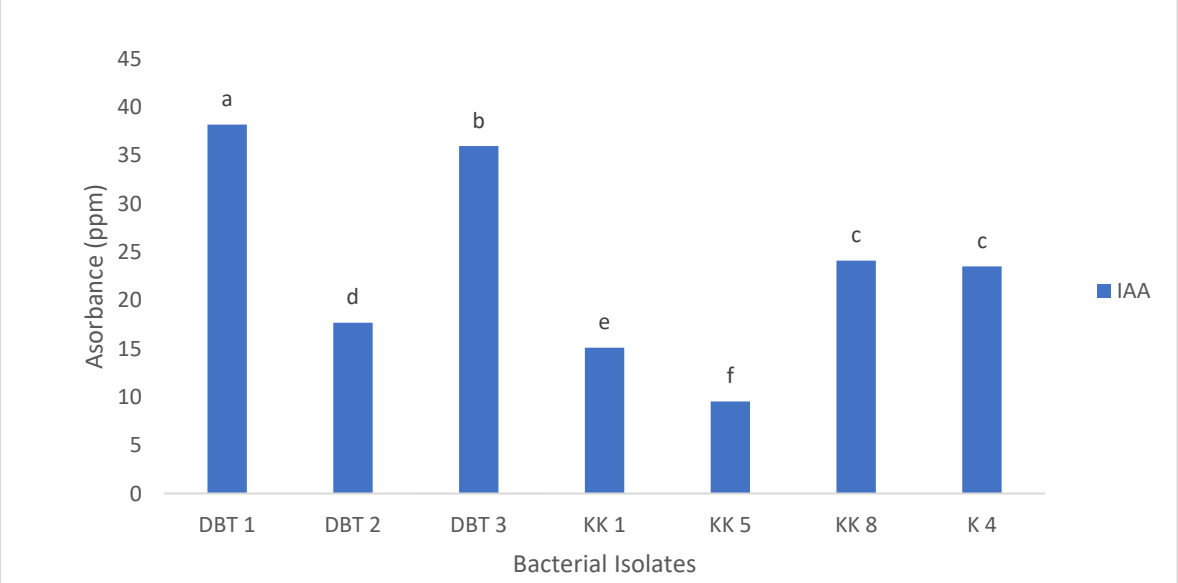


Figure 3: Indole Acetic Acid Production of the Bacteria Isolates

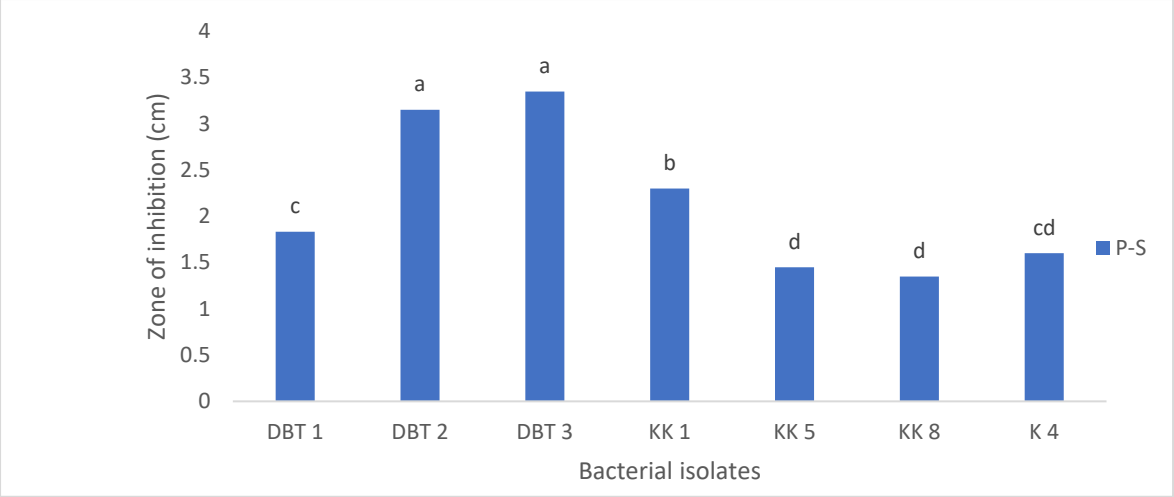


Figure 4: I Phosphate Solubilization of the Bacteria Isolates

## CONCLUSION

The soil samples from the two study sites were found to be all saline, and the soil sample from Kura has the highest salinity level. Three (3) gram positive and four (4) gram negative bacteria isolates were isolated from both the study site and showed varying salt tolerance with DBT3 (10%) recording the highest salt tolerance, while KK4 (0.4%) recorded the lowest salt tolerance. All the bacterial isolates demonstrated certain plant growth promotion activities, although the properties varied from one bacterial isolate to another.

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