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Investigation and Correlation of Soil Biotic and Abiotic Factors Affecting Agricultural Productivity in Semi-Arid Regions of North Gujarat, India.

Bakhtiyar Alam Syed

Ph.D Research scholar,
Dept. of Biotechnology,
Hemchandracharya North Gujarat University,
Patan
sami.syed40@gmail.com

Beena Patel

R & D Department,
Abellon Group of Companies. Sydney House,
Premchand Nagar Road,
Ahmedabad,INDIA
beena.patel@abelloncleanenergy.com
www.abelloncleanenergy.com

Abstract: *Climatic changes influence various abiotic and biotic factors affecting agricultural productivity. Current report is first investigation for Aravali district of North Gujarat, India considered semi-arid region having extreme climatic conditions. Physico-chemical, micro and macro nutritional parameters of 20 soil samples were analyzed by biochemical methods and Inductively Coupled Plasma-Mass Spectrophotometry (ICP-MS). Screening of potassium, phosphorus and zinc solubilizing bacteria was performed and 8 bacterial isolates displayed mineral solubilization activity. Mineral solubilizing bacteria were identified by 16s rDNA technology. SPSS software was used to perform statistical analysis.*

Significant variations were noted between the 20 samples in particle size distribution, bulk density, WHC, moisture content, pH, electrical conductivity, organic carbon, organic matter, nitrogen, potassium, phosphorus, magnesium, sulfur, calcium and chloride ($p<0.02$), The bacterial count was 100 fold lower than reported healthy soil. Eight mineral solubilizing bacteria from Bacillus, Pseudomonas and Enterobacter genera were identified through 16s rDNA technology.

The study concludes that inefficient conversion of soil minerals into plant available form through mineralization process is due to low density of microbes. Incorporation of best agronomic practices that retain moisture, improve C:N ratio, application of mineralizing bacteria and augmentation of essential minerals are suggested to improve agricultural productivity.

Keywords: *Soil, microflora, plant growth promoting bacteria, soil fertility, 16s rDNA microbial identification, mineral solubilization.*

1. INTRODUCTION

Climate change influences the basic components of agricultural systems like crop and livestock production, balances of hydrological systems, input supplies etc. However, these biophysical effects and the human responses to them are complex and uncertain (Adams *et al.* 1998).

In light of recent ongoing changes in the global climate, it is important to study the fundamental characteristic features of the soil to understand the dynamics of geochemical, biophysical and biochemical interactions on the earth's surface as the nature of a habitat in which a community of organisms reside is decided by the complex interaction of geology, climate and vegetation type (Varghese *et al.* 2012).

Crop yields are directly affected by climatic factors such as temperature and precipitation and the frequency and severity of extreme events like droughts, floods and wind storms. Agricultural systems are complex ecosystems, a change in single component could trigger cascade of changes in other components of the ecosystem. Climate change may also affect pest types, intensities of infection and frequencies of attack and availability of irrigation facilities (Adams *et al.* 1998).

It is necessary to understand and estimate the effects of climate change on agricultural production and food supply. The changes in food prices, crop and livestock yields, resource availability and

technological changes make the agriculture activity vulnerable to lots of economical fluctuations. Humans adapt to the effects of climate change on agriculture by either making short-term changes in consumption and production practices or long-term technological changes. Failing to consider these human adaptations would overestimate the potential damage from climate change and underestimate the potential benefits of human adaptations (Adams *et al.* 1998).

The current study focuses on agricultural potential of Aravali district of North Gujarat, as this region is vulnerable to agricultural uncertainties due to its critical geographical location, rocky terrain, lack of sufficient resources to combat the effects of climate changes and anthropogenic interventions in the area.

This study is first of its kind in this region with primary objectives to investigate; (1) the availability of nutrients in the form of micro and macro nutrients in the soil (2) presence of plant growth promoting microorganisms in the soil and (3) to understand correlation between abiotic factors like climate, micronutrients, micronutrients and biotic factors like microorganisms to explore potential agro-economic benefits of the region.

2. EXPERIMENTAL METHODS AND MATERIALS

2.1. Soil Sample Collection

Soil samples were collected in sterile container by auger from 20 sites of Aravalli district of Gujarat in June 2013. Samples were preserved at 4°C for isolation of bacteria.

2.2. Physical and Biochemical Analysis of Soil Samples

All 20 soil samples were properly mixed and divided for analysis. pH and conductivity were performed using pH meter (Auto pH system PM300, Eutech Instruments) and conductivity meter (HI-2300 EC/TDS/NaCl meter, Hanna Instruments) respectively. Analysis of nitrogen content was performed by kjeldahl method (Bremner J. M. 1965), phosphorus by Olsen method (Olsen *et al*.1954), organic matter and organic carbon by gravimetric method (Sharma *et al*.2010). Sodium, potassium and calcium were measured by Stanford and English method (1949) using flame photometry (Flame photometer 128, Systronics). Micro-elements were analysed by ICP-MS (Perkin Elmer, Optima-2100 DV) after acid digestion by aqua regia (Samuel Melaku, 2000).

2.3. Screening of Bacteria and Obtaining Pure Culture

Colony forming unit (CFU) assay was performed using one gram of the soil for each sample and spreading 0.5 ml aliquots from each dilution on the surface of nutrient agar plates (McLean, 2001). Serially diluted 10^{-3} and 10^{-4} soil sample were used for isolation and screening of soil bacteria. The diluted samples were spread on nutrient agar medium for the growth of non-fastidious bacteria, eosin metylene blue agar medium to isolate gram negative bacteria, benzoate medium was used for the growth of bacteria from the genus pseudomonas. The plates were incubated at 37°C for 48 hours. The pure cultures were obtained based on the morphological characteristics of colony. Total 25 individual colonies of bacteria based on the variations in morphological characterisitics were selected for the mineral solubilization assay.

2.4. Mineral Solubilization

All selected 25 distinct bacterial isolates were tested for phosphate solubilization, potassium solubilization and zinc solubilization activity by previously reported following methods.

Pikowaskya media (1948) containing $Ca_3(PO)_4$ as insoluble form of phosphate as reported by Nautiyala (1999) was use to test phosphate solubilization; Tris minimal salt media (Fasim. *et al*. 2002) containing ZnO as insoluble form of zinc to test zinc solubilization. Alexandrov agar medium containing 0.5% potassium aluminum silicate (Sugumaran and Janartham, 2007) as insoluble form of potassium was used to assay the potassium solubilizing bacteria.

All isolates were inoculated in the wells in specific media plates as mentioned above. After 48 hours of incubation at 37ºC, clear zone of utilization were checked on the plates containing insoluble zone of clearance of respective minerals in mineralization specific plates.

2.5. Morphological Identification and Gram's Staining

All the isolates showing mineral solubilization were labelled A-H and the bacterial isolates were identified on the basis of classification schemes published in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984). Gram staining of all the colonies having mineral solubilization activity was performed.

2.6. Genomic Identification

Colony PCR was performed by Each colony was suspended in 2 ml sterile double distilled water, incubated at 55-60°C for 30 minutes. Three micro-liters of suspension was used as the template DNA for PCR. 12.5 µL 2X PCR mastermix (Genei), 1 µL of universal forward (8F-AGAGTTTGATCCTGGCTCAG) and reverse primers (1492R-GGTTACCTTGTTACGACTT) each, 0.5 µL DMSO was used in 25 µL PCR reaction with PCR amplification cycle of initial denaturation at 94°C for 7 minutes, 35 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 45 sec and extension at 72°C for 45 sec and final extension at 72°C for 8 minutes. The PCR products were checked on 1.5% agarose gel (Genei) and was compared with the DNA ladder (Zipruler-1, Fermentas). The PCR products and DNA ladder were run in 1X TAE buffer at 110 volts for 15 mins and the gel profile was viewed by gel documentation system (Biorad-XR+ System) using Quantity One software. The gel profile gave clear ~1500 bp band. The amplified PCR products were used for genetic identification. The PCR products obtained after the colony PCR were used as the template for cycle sequencing using Sanger's Chain termination method. Suitable amount of amplified PCR products was used for seperate forward and reverse reactions. Big Dye Terminator (ABI) was used for flourocently tagging the dideoxynucleotide. The purified products were loaded onto the Genetic analyzer 3730xl for detection and analysis. The data was obtained in the form of notepad sequence and chromatogram files. Chromas Lite software was used to view the chromatograms. The forward and reverse sequences obtained for each PCR product were aligned to form a consensus sequence by MEGA 4.0 software. These consensus sequences were then analysed by BLAST to identify the micro-organisms. The evolutionary history was inferred using the Neighbor-Joining method (Saitou *et al.*1987). The bootstrap consensus tree inferred from 500 replicates was taken to represent the evolutionary history of the taxa analyzed (Felsenstein. 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura. 1980). Phylogenetic analyses were conducted in MEGA 4.0 (Tamura *et al.*2007). Distance matrix and phylogenetic tree for each of the identified bacterial isolates were prepared by MEGA 4.0.

2.7. Statistical Analysis

All soil samples were statistically analyzed using SPSS statistical software version-20 (IBM). One sample t-test was performed between all soil samples' physio-chemical, micro and macro biochemical parameters to understand variation between all 20 samples' characteristics. Pearson's and Spearman's correlation analysis was perform to analyze correlation between soil particle size with water holding capacity and moisture content with water holding capacity. Statistical significance was consider when p value was <0.05.

3. RESULTS

3.1. Physical and Biochemical Characteristics of Soil

The physico-chemical analysis showed that the soil has high pore size and low waterholding capacity and moisture content. The results of physico-chemical analysis are shown in Table-1.

Table -1. *Physico-chemical analysis of soil samples*

Sr.No.	Parameter	Mean (n=20)	P-value*	Normal Range
1	Color	Brown	--	Brown
2	Soil Texture	Loamy Sand	--	Loamy
3	Particle size distribution-Sand %	80.65±5.7	0.000	40.00%
4	Particle size distribution-Silt %	4.03±0.24	0.000	40.00%
5	Particle size distribution-Clay %	16.17±5.3	0.029	20.00%

6	Bulk Density (gm/cm^3)	1.08±0.06	0.000	1.2-1.6
7	Pore size %	42.21±8.18	0.004	--
8	Water Holding capacity (ml)	27.21±7.58	0.016	--
9	Moisture Content %	8.12±2.2	0.007	18-20
10	pH	7.32±0.19	0.000	6.5-7.5
11	Electrical conductivity (mS/cm)	1.11±0.11	0.000	--

**one sample T-test*

The biochemical analysis showed normal values of organic matter and organic carbon. The microbial population in the soil samples was lower than the previously reported results for agricultural soil samples (Leisack *et al*. 2001). The element concentrations in the soil varied significantly as compared to the normal range. P, Na and Mn concentration in the soil were in the normal range but concentrations of N, Cu, Fe, Zn and S were below normal range whereas the concentrations of K, Mg, Cl and Ca were higher than the normal range. It was noteworthy that boron was not detected in any of the 20 samples studied. Table-2 shows biochemical and macroelement analyses while microelement analysis of the soil samples results are shown in Table-3.

Table-2. *Bio-chemical and Macro-elements analysis of soil samples*

Sr.No.	Parameter	Mean (n=20)	P-value*	Normal Range
1	Organic Carbon (%)	0.72±0.1	0.002	0.5-0.75
2	Organic Matter (%)	1.08±0.17	0.002	0.85-1.3
3	Nitrogen (%)	0.16±0.14	0.020	0.05-0.1
4	C:N Ratio	4.5±0.4	0.005	15-20
5	Phosphorus (ppm)	29.86±8.17	0.015	20-40
6	Potassium (ppm)	1961.95±450.33	0.007	150-250
7	Magnesium (ppm)	2302±673.82	0.019	60-180
8	Calcium (ppm)	5060±813.66	0.002	1000-2000
9	Sulfur (ppm)	5.6±0.13	0.351	10 - 20
10	CFU/gm of test soil samples	2.8 X 10^4	0.003	2-6 X 10^6

**one sample T-test*

Table -3: *Microelement analysis of soil samples*

Sr. No.	Parameter (ppm)	Mean (n=20)	P-value*	Normal Value
1	Sodium	15.6±1.4	0.339	15
2	Boron	0±0	0.000	20
3	Copper	1.3±0.72	0.137	6
4	Iron	53.53±40.36	0.242	100
5	Manganese	51.58±38.65	0.242	50
6	Zinc	5.58±5.58	0.363	20
7	Chloride	165.66±62.61	0.046	100

**one sample T-test*

Negative correlation between water holding capacity and percentage pore size was observed while positive correlation between water holding capacity and moisture was noted using spearman's and pearson's coefficient analysis (Table-4).

Table-4: *Effect of pore size on water holding capacity of soil*

Sr. No.	Tests parameters (n=20)	Correlation	R value	p-value
1	Pore size Vs. Water holding capacity	Pearson's	- 0.5	0.31
		Spearman's	- 0.54	0.27
2	Water Holding capacity Vs. Moisture	Pearson's	0.6	0.2
		Spearman's	0.49	0.32

3.2. Bacterial Colonies Isolation From Soil Suspension:

Bacterial population per gram of soil of the test soil samples was significantly lower compared to the results of previous studies. The results are included in Table-2.

3.3. Mineral Solubilization

Total 8 bacterial isolates showed ~1-6 mm zone of utilization mineral solubilizing activity from the 25 bacterial isolates. Table-5 shows the mineral solubilization activity of each of the 8 bacterial isolates.

Table-5. *Mineral Solubilization activity of Bacterial Isolates*

Sr. No.	Bacterial colony ID	Phosphate Solubilization	Potassium Solubilization	Zinc Solubilization
1	A	+	+	+
2	B	+	-	-
3	C	+	+	-
4	D	+	-	-
5	E	+	+	-
6	F	+	+	-
7	G	+	-	-
8	H	+	+	-

All eight bacterial isolates i.e. A, B, C, D, E, F, G and H showed P-solubilization indicated by zone of utilization in the plates containing Pikowaskya media with $Ca_3 (PO)_4$ as insoluble form of phosporus. The formation of zone of utilization proves the conversion of insoluble form of $Ca_3 (PO)_4$ to the soluble form due to the enzymatic activities by the bacteria. The zone of utilization of phosphate is shown in Figure-1.

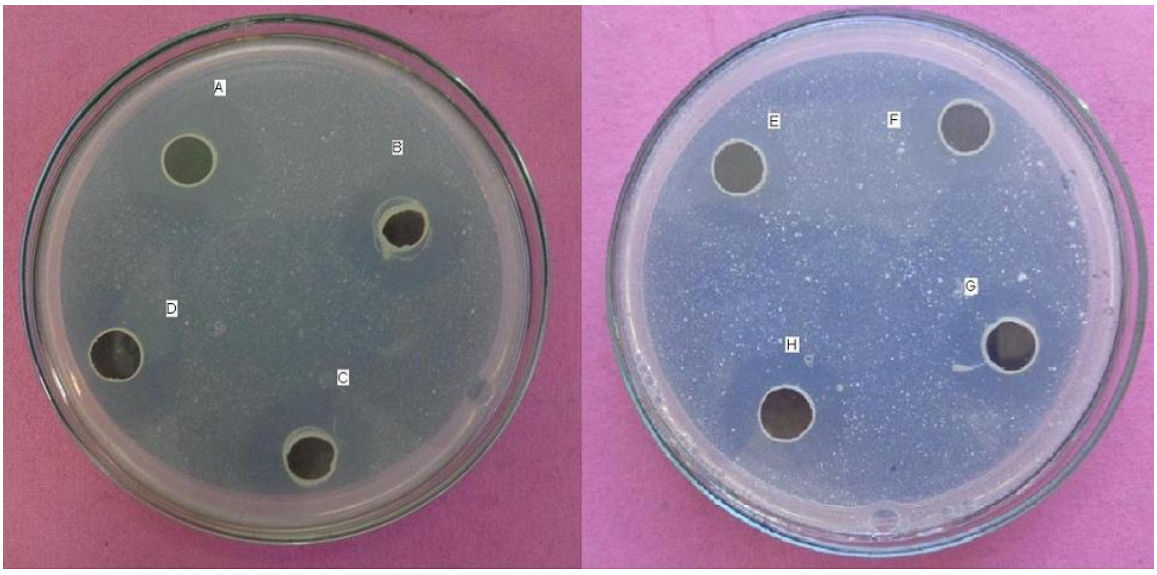

Figure-1: *Phosphorus solubilization Assay*

A= *Pseudomonas putida* **B**= *Enterobacter spp AN2* **C**= *B subtilis* **D**= *B. licheniformis*

E= *B. megaterium* **F**= *B. cereus* **G**= *E.cancerogenous* **H**= *B. Firmus*

Potassium solubilizing activity was observed for 5 bacterial isolates i.e. A, C, E, F and H in Alexandrov medium enriched with potassium aluminum silicate as an insoluble pottassium. The zone of utilization of potassium is shown in Figure-2

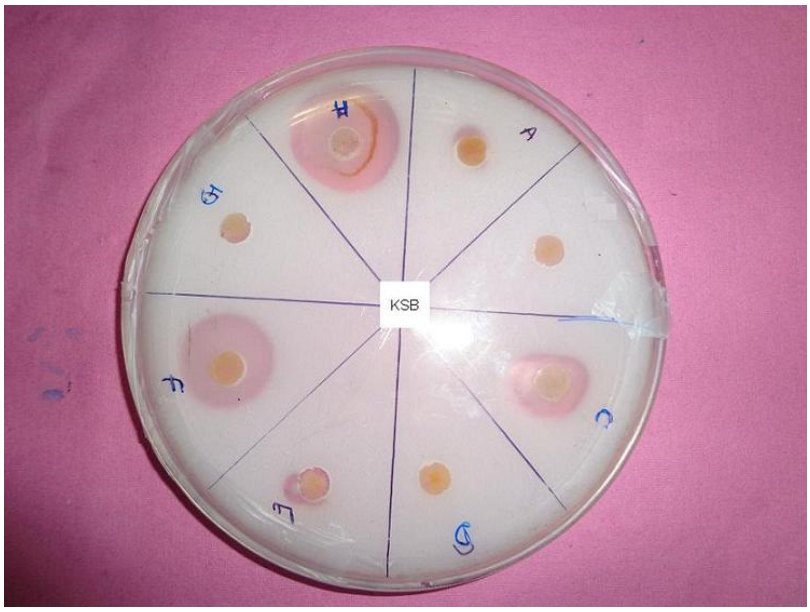

Figure-2: *Potassium solubilization Assay*

A= *Pseudomonas putida* **B**= *Enterobacter spp AN2* **C**= *B subtilis* **D**= *B. licheniformis*

E= *B. megaterium* **F**= *B. cereus* **G**= *E.cancerogenous* **H**= *B. firmus*

Zinc solubilization activity was observed in the plate containing ZnO as insoluble form of zinc in Tris minimal medium for one bacterial isolate A. The zone of utilization of zinc is shown in Figure-3. Bacterial isolate A solubilized all three minerals whereas isolates B, D and G solubilize only phosphorus.

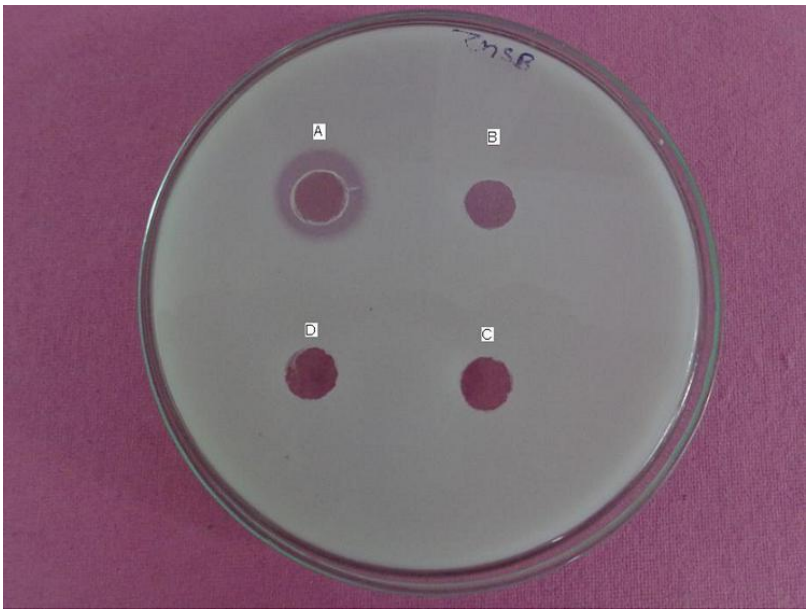

Figure-3: *Zinc solubilization Assay*

A= *Pseudomonas putida* **B**= *Enterobacter spp AN2* **C**= *B subtilis* **D**= *B. licheniformis*

3.4. Morphological Identification and Gram's Staining

The bacterial isolates having the mineral solubilization were identified by Gram's staining and 16s rDNA technology. The colonial characteristics were also studied for these bacterial isolates (Table-6). The gram's staining of mineral solubilizing bacteria showed three gram's negative rods (A, B and G) and five gram's positive (C, D, E, F, and H) bacillus colonies (Fig-4)**.** The colonial characteristics are described in Table-6.

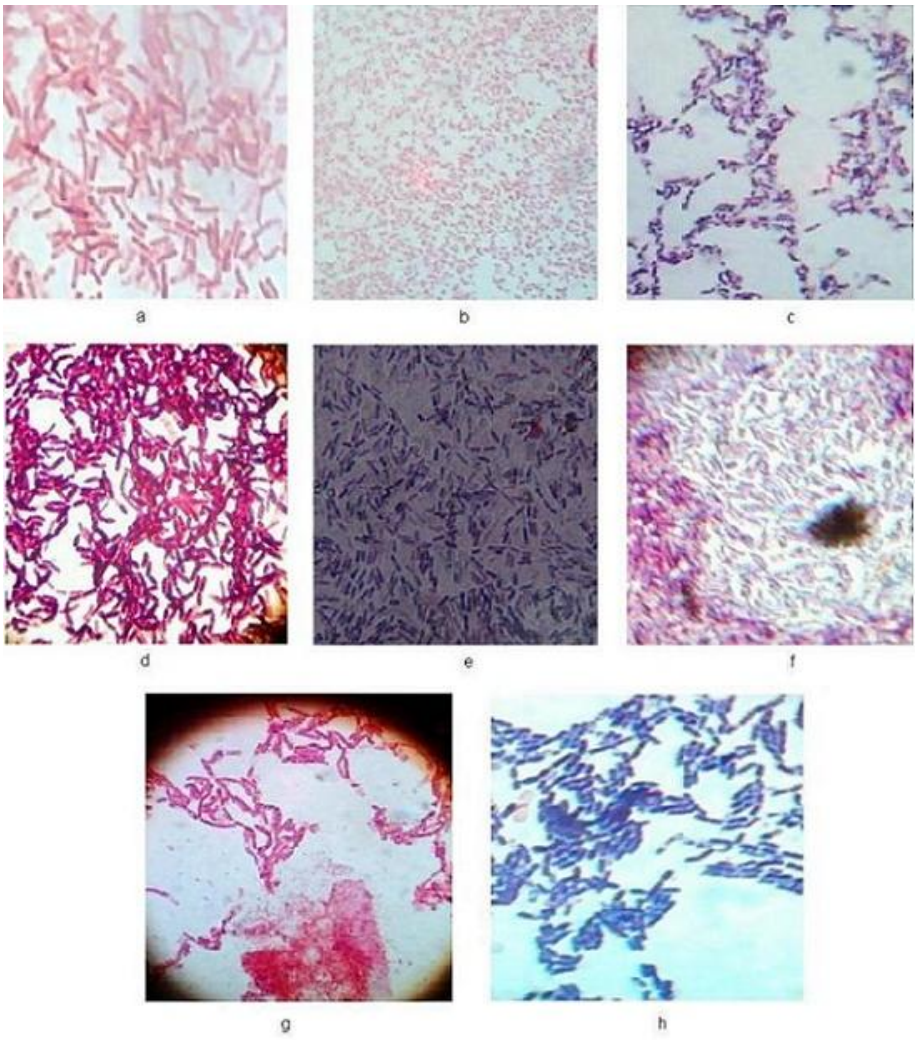

Figure-4: *Gram staining images of pure bacterial cultures.*

A= *Pseudomonas putida* **B**= *Enterobacter spp AN2* **C**= *B subtilis* **D**= *B. licheniformis*

E= *B. megaterium* **F**= *B. cereus* **G**= *E.cancerogenous* **H**= *B. firmus*

Table-6: *Colonial Characteristics of Bacterial Isolates*

Colony ID	Size (mm)	Shape	Elevation	Margin	Colour	Surface	Motility	Morphology	Gram reaction
A	2-4	Irregular	Flat	Undulate	Fluoroscent	Glossy	Yes	Rod	-ve
B	2-4	Circular	Convex	Entire	Cream	Slimy	Yes	Rod	-ve
C	2	Irregular	Flat	Lobrate	Cream	Smooth	Yes	Bacillus	+ve
D	2-4	Irregular	Convex	Undulate	White	Smooth	Yes	Bacillus	+ve
E	2-4	Irregular	Raised	Undulate	Yellow	Slimy	Yes	Bacillus	+ve
F	2-7	Irregular	Flat	Undulate	Cream	Dry	Yes	Bacillus	+ve
G	2-4	Circular	Convex	Entire	White	Slimy	Yes	Rod	-ve
H	3	Circular	Raised	Regular	Orange	Glossy	Yes	Bacillus	+ve

3.5. 16s rDNA Identification Results

The amplified PCR products (Figure-5) of ~1500 bp were used to identify the bacteria on ABI 3730xl genetic analyzer. The results of 16s rDNA identification are shown in Table-7. Out of total 8 bacterial cultures, two bacteria belonged to *Enterobacter* genus, one from *Pseudomonas* genus and five were from *Bacillus* genus. Phylogenetic tree analyses were performed using MEGA 4.0.

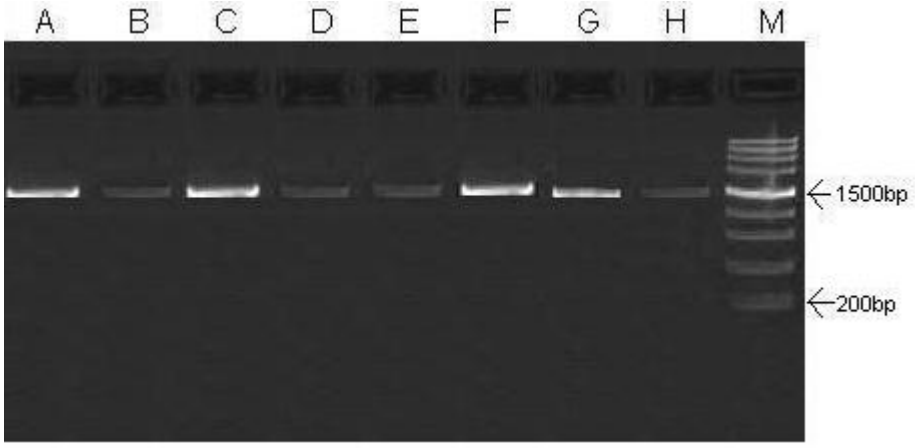

Figure-5. *PCR products (~1500bp) amplified with primers 8f and 1492r*

A= *Pseudomonas putida* **B**= *Enterobacter spp AN2* **C**= *B subtilis* **D**= *B. licheniformis*

E= *B. megaterium* **F**= *B. cereus* **G**= *E.cancerogenous* **H**= *B. firmus* M=DNA marker

Table-7. *16s rDNA Identification of Bacterial Isolates*

Sr. No.	Bacterial colony ID	Identification Result	GenBank Accession Number
1	A	*Pseudomonas putida* isolate BCNU106	DQ229315.1
2	B	*Enterobacter sp.* AN2	GQ451698.1
3	C	*Bacillus subtilis subsp. subtilis strain* MPA 1035	HQ231914.1
4	D	*Bacillus licheniformis strain* Y822	HQ005269.1
5	E	*Bacillus megaterium strain* ZH28	HM103348.1
6	F	*Bacillus cereus strain* YYW-8	HM362783.1
7	G	*Enterobacter cancerogenus strain* H3	FJ009375.1
8	H	*Bacillus firmus strain* PAN MC15	HQ285922.1

4. DISCUSSION

Agricultural output is strongly associated with soil nutritive and physical quality (Tiwari. e*t.al* 2008) as well as microbial interaction with soil and plant (Gianinazzi, *et al.*1994). However over a period of time many land pockets are damaged due to climatic changes and human interventions (Gore *et al.* 2011). The current paper addresses such issue at *Aravali* district of north Gujarat which has history of rock erosions and extreme climate. However there are no reports available for this region that addresses factors of soil quality and microbial activities responsible to maximize agricultural productivity.

The soil texture of the region covered under this study is rocky with low moisture and water holding capacity with higher pore size of the soil. The results are in accordance with similar studies carried out by Jeff Ball (2001) showing positive correlation between the soil texture and water holding capacity while negative correlation between pore size and water holding potential. Soil texture and physical parameters affect plant growth by affecting the growth of root zones. Many studies have established statistical relationships to predict the success of germination and early development from soil factors like soil water temperature, soil structure and mechanical impedance (Lindstrom *et al*., 1976; Schneider and Gupta, 1985). Soil pH significantly affects the availability of macronutrient as well as micronutrients. Availability of molybdenum and macronutrients except phosphorus increases with slightly alkaline pH and availability of micronutrients except molybdenum is facilitated at slightly acidic pH. Availability of nitrogen is less sensitive to pH than availability of available phosphorus. Soil pH in the range of 6.0-7.5 is necessary for optimum phosphorus uptake by plants as phosphorus forms insoluble complex with iron and aluminium at pH below 6.0 and with calcium at pH above 7.5 (Brady & Weil 2008)

The bulk density of the soil is high and this restricts the growth of roots and could also represses the growth of microorganisms that could benefit the plants by establishing symbiotic relationships

with the plants. High atmospheric temperature and less humidity in this region is responsible for lower moisture content, adversely affecting growth of plants by limiting the root growth and minerals uptake. Less moisture in soil also leads to poor uptake of nitrogen by plants (Dijkstra *et al*. 2008).

Organic matter and organic carbon are well within the normal range. Nitrogen concentration in the soil is less than the normal range. Less nitrogen in the soil affects the plants directly as it promotes rapid growth, increases leaf size and quality and promotes fruit and seed development. It is a constituent of amino acids and chlorophyll. It plays a role in almost all plant metabolic processes. (Tucker 1999). Thus, insufficient nitrogen in the soil could lead to improper plant growth. Nitrogen content also indirectly affects the plant growth and plant-microbes symbiotic relationships as optimum nitrogen content is essential for normal C:N ratio. Less c:n ratio results in the utilization of N by microorganisms and its immobilization thus plants face further N-deficiency. The microbial population of the soil was very less than the other previous reports (Liesack 2000, Reichardt. 2001), may be due to high bulk density of soil, less soil moisture, high atmospheric temperature, low atmospheric humidity and lack of sufficient vegetation in the region.

Phosphorus concentration was within range whereas K was almost 8 times higher than the normal range, Mg around 12 times higher and Ca 2-3 times higher than the normal range. K, Ca and Mg are essential macronutrient having important roles in enzyme activation, protein synthesis, stomatal activity, chlorophyll synthesis, photosynthesis, transfer of sugars and water and nutrient transport (Tucker 1999). The abnormal levels of these nutrients in the soil could adversely affect the plant growth (Tucker 1999). Surprisingly, these elements were not available to plants in plant uptake forms, which require mineral solubilization process by bacterial population. Limited bacterial population or bacterial count resulted into accumulation of these minerals in soil without its uptake by plants. As discussed, the microbial population of the soil was almost 100 times less than the previously reported results due to preiously mentioned biotic and abiotic factors.

Na and Mn concentrations are in the normal range but Cu, Zn, and Fe concentrations were less than the normal which are essential micronutrients having important roles in various metabolic pathways in plants as well as microbes. These nutrients are required for transcriptional activities in microbes (Weinberg. 1989). Thus, fewer microbial population could results of deficiency in essential nutrients besides lower soil moisture, high bulk density and C:N ratio. Boron was not detected in any of the 20 samples studied. B has important role in maintaining the integrity of cell wall, transport of sugars, cell division and many other metabolic pathways. The function of B interrelate with those of N, P, K and Ca in plants and absence of B in the soil might interrupt the metabolic activities retarding over all plant growth. Chlorine is essential mineral for higher plants (Marschner, 1995). Cl is a major osmotically active solute in the vacuole and is involved in both turgor- and osmoregulation. It regulates the activities of enzymes and maintains membrane potential (White. 2001) thus high level of Cl could create osmotic imbalance in the plant system.

The biotic factors like soil organisms like symbiotic bacteria and fungi, earthworms can enhance the agricultural production by secretion of essential plant hormones, aiding aeration of soil, percolation, nitrogen fixation directly to plants and soil and decomposition of plant materials to form humus thus, increasing fertility. Birds and insects are essential for pollination of plants. Whereas the pests, parasites and nematodes adversely affect the agricultural producion by inhibiting the optimal growth of plants, pests directly or indirectly induce diseases to plants, weeds compete with crops for space, water, nutrients and sunlight (Shennan 2008).

The agricultural productivity in this region is low even though the nutrients and minerals are present in the soil. To validate hypothesis of bacterial mineralization of available minerals, biotic factors were also studied in this report. The nutrients present in the soil are not available to the plants due to their complex and insoluble forms. It has been reported that microorganisms producing gluconic acid have a major role in the solubilization of insoluble minerals (Illmer et. al. 1992) and converting minerals into soluble forms for uptake by plants. Keeping this in mind, mineral solubilization activity assay of 25 bacterial isolates was performed for P, K and Zn to detect the presence of the bacteria that have the potential to convert complex insoluble minerals to

soluble plant available forms. The assay results showed presence of 8 bacterial isolates from *Pseudomonas*, *Bacillus* and *Enterobacter* genera having mineral solubilization potential indicated by the formation ~1-6 mm zone of utilization. Thus, mineral solubilizing bacteria are present in the test soil samples but their population is too less to be able to mineralize available nutrients/minerals fully into soluble forms in sufficient amounts to be used by plants.

5. CONCLUSION

The study is one of the first reports from the *Aravali* district of Gujarat, India reporting impact of abiotic and biotic factors like presence of useful mineralizing bacteria in very inferior amount that restricted over all agricultural output in spite of having sufficient macro and micro elements and nutrients useful for plant and microbial metabolism. The study suggests incorporating plant useful mineralization bacteria with required minerals along with best agronomical practices to retain moisture that helps in overall agricultural output in the study region.

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REFERENCES

[1] Aparna Misra and Germund Tyler. 1999, Influence of Soil Moisture on Soil Solution Chemistry and Concentrations of Minerals in the Calcicoles Phleum phleoides and Veronica spicata Grown on a Limestone Soil, Annals of Botany 84: 401±410, 1999 Article No. Anbo.1999.0941

[2] Brady N. C. and Weil R. R. 2008. The Nature and Properties of Soils. 14 ed. Pearson-Prentice Hall, Upper Saddle River, NJ. 990 pp.

[3] Brajesh Sharma, M.R. Dalwadi, D.B. Panchal, J.C. Patel and H.D. Panchal. 2010, Critical levels of phosphorus for maize in alkaline soils of Anand and Kheda districts, An Asian Journal of Soil Science Vol. 5 No. 2 (December, 2010) : 287-290

[4] Bremner J.M. 1965, Nitrogen availability indexes IN "Methods of soil analysis". Part 2. (C.A. Black ed.). Magronomy 9, Am. Soc. of Agron., Medison, Wisconsin p. 1324-1345.

[5] E. Epstein. 1965, "Mineral solubilzation" pp438-466 in Plant Biochemistry (J. Bonner & J.E. Vamer, eds) Academic Press, London. 1965

[6] Eugene D. Weinberg. 1989, Roles of trace in transcriptional control of microbial secondary metabolism; Biology of metals, Springer-Verlag 1990 Biol Metals (1990) 2:191-196

[7] Feike A. Dijkstra, Weixin Cheng. 2008, Increased soil moisture content increases plant N uptake and the abundance of 15N in plant biomass. Plant Soil (2008) 302:263–271 DOI 10.1007/s11104-007-9477-0

[8] Felsenstein J. 1985, Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

[9] Functions of boron in plant nutrition: Agronomy Note: 20 Mule team Borax: Part of Rio Tinto (http://www.borax.com/docs/agronomy-notes/functionsofboroninplantnutrition-final-feb2012.pdf?sfvrsn=2)

[10] Gianinazzi, S. and Schuepp, H. 1994, Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkh€auser Verlag, Basel. p. 226.

[11] Illmer P, Schinner F. 1992, Solubilization of inorganic phosphates by microorganisms isolated from forest soil. Soil Biol Biochem 1992;24:389–95.

[12] Jeff Ball: Soil and water relationships. 2001, The Samuel Roberts Noble Foundation (http://www.noble.org/ag/soils/soilwaterrelationships/redirect/)

[13] Kimura M. 1980, A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111-120.

[14] Kjeldahl, Johan Z. 1883, "A new method for the determination of nitrogen in organic bodies." Analytical Chemistry 22 (1883): 366.
[15] Krieg, N. R. and Holt, J. G. 1984, Bergey's Manual of Systematic Bacteriology, Vol. 1, eds: Williams and Wilkins. Baltimore.
[16] Krishna R. Tiwari, Bishal K. Sitaula, Roshan M. Bajracharya, Trond Børresen. 2008, Effects of soil and crop management practices on yields, income and nutrients losses from upland farming systems in the Middle Mountains region of Nepal: Nutrient Cycle Agroecosystem DOI 10.1007/s10705-009-9289-0
[17] Liesack W, Schnell S, Resbech NP. 2000, Microbiology of flooded rice paddies. FEMS Microbiol. Rev. 24: 625-645.
[18] Lindstrom, M.J., Papendick, R.I. and Koehler, F.E. 1976, A model to predict winter wheat emergence as affected by soil temperature, water potential and depth of planting. Agronomy J. 68: 137-140.
[19] M. Ray Tucker. 1999, Essential Plant Nutrients: their presence in North Carolina soils and role in plant nutrition, October 1999
[20] Marschner H. 1995, Mineral nutrition of higher plants, 2nd edn. London: Academic Press.
[21] McLean, K.L. 2001, Biological control of onion white rot using Trichoderma harzianum PhD thesis, Lincoln University, Canterbury.
[22] Olsen, S.R., C.V. Cole, F.S. Watanabe, and L.A. Dean. 1954, Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dep. of Agric. Circ. 939.
[23] P. G. Gore, B. A Roy, H. R. Hatwar. 2011, Impact of climate change on land degradation over India: NCC research report, Indian Meteorological Department, May 2011.
[24] Philip J. White and Martin R. Broadley. 2001, Chloride in Soils and its Uptake and Movement within the Plant: A Review. Annals of Botany 88: 967-988, 2001 doi: 10.1006/ anbo.2001.1540
[25] R.P. Singh & S.K Mishra. 2012, Department of Agricultural Chemistry and Soil Science. Available macronutrients (N, P, K and S) in the soil of Chiraigaon block of district Varanasi (U.P.) in relation to soil characteristics.
[26] Reichardt W, Briones A, de Jesus R, Padre B. 2001, Microbial population shifts in experimental rice systems. Appl. Soil Ecol. 17:151-163.
[27] Richard M. Adams, Brian H. Hurd, Stephanie Lenhar, Neil Lear, 1998. Effects of global climate change on agriculture: an interpretative review: Clim ResVol. 11: 19–30.
[28] Rinoy Varghese, Mohamed Hatha AA, 2002. Significance of soil microorganisms with special reference to climate change, Indian J. Edu. Inf. Manage., Vol. 1, No. 3
[29] Saitou N & Nei M. 1987, The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.
[30] Samuel Melaku, Richard Dams, Luc Moens. 2000, Determination of trace elements in agricultural soil samples by inductively coupled plasma-mass spectrometry: Microwave acid digestion versus aqua regia extraction
[31] Schneider, E.C. and Gupta, S.C. 1985, Corn emergence as influenced by soil temperature, matric potential and aggregate size. Soil Sci.Soc.Am.J. 49:415-422.
[32] Shennan Carol. 2008. Biotic interactions, ecological knowledge and agriculture. Phil. Trans. R. Soc. B . 363: 717–739
[33] Soil Nutrient Management for Maui County. College of Tropical Agriculture and Human Resources (CTAHR), University of Hawai'i (http://www.ctahr.hawaii.edu/mauisoil/a_ comp03.aspx)
[34] Soil Testing in India, Department of Agricultural Cooperation, Ministry of Agriculture, Govt of India, New Delhi Jan 2011
[35] Stanford, S., and L. English. 1949, A use of flame photometer in rapid soil tests of potassium and calcium. Agron. J., 41:446-447.
[36] Tamura K, Dudley J, Nei M & Kumar S. 2007, MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24:1596-1599.

AUTHORS' BIOGRAPHY

Mr.BakhtiyarAlam Syed holds Master degree in Biotechnology from KadiSarva Vishvavidhyalaya. He worked as Research Associate at Xcelrislabs for 1.5 years and experienced in handling clients samples of prokaryotes to eukaryotes DNA on various sequencing platforms at genome service industry.

Mr.BakhtiyarAlam Syed is currently pursuing Doctor of Philosophy from Hemchandracharya North Gujarat University, Gujarat, under the guidance of Dr.Beena Patel for subject entitled "Identification of Drought and Saline resistant Bamboo varieties as energy crop and wasteland reclamation". He has gained expertized in molecular biotechnology in DNA sequencing, gene cloning, microbial identification, soil sciences, tissue culture, drought and saline stress response studies in plants with respect to morphological, biochemical and genetic variables and application of statistical tools

Mr.BakhtiyarAlam Syed has interacted with farmers for their farm soil and crop health issues through surveys and Focus Group Discussion in Gujarat, India. He has synchronized regional problem of farmers through social science to apply his research finding to improve marginal and susceptible land of Gujarat.

Dr BeenaPatel is Senior General Manager and leading research at AbellonCleanEnergy Ltd, Ahmedabad. Her research focus is to characterize potential waste biomass to explore its application into downstream technology for harnessing valuable products like fuel, feed, organic chemicals, and fertilizers through zero discharge process engineering. She develops Life Cycle Analysis module for her research projects and leads LCA analysis for lignocellulosic bioethanol production at Indo-USA project.

She has worked on waste land salvage process by soil fertility improvement through inorganic ion solubilizing bacteria and mobilization process to convert NPK into plant available form as well to improve water holding capacity of land. On the agriculture front, Dr. Patel has developed formulations of organic bio-fertilizer at Abellon to cater large scale organic farming.