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PHYLOGENETICAL CHARACTERIZATION OF CYANOBACTERIA FROM PADDY FIELD OF CHHATTISGARH (PART I)

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ABSTRACT: With respect to Chhattisgarh, rice is the principal crop of the state. India Covers 66% of cultivable land and mostly grown under kharif cropping season. To increase sustained productivity without decreasing soil quality, algal bio fertilizers are used widely now days in the state. Use of local isolates as algal inoculants is being stressed due to their competitiveness in the field for establishment of better ecological adaptability for developing composite starter. Culture of algal bio fertilizer programme on a regional basis, survey, isolation and screening of stress-tolerant cyanobacteria have been started at various parts of India. The soils of Chhattisgarh state comprise mostly of iron- rich red soil, laterite soil, and red and yellow soil and brown forest soil. So the above study comprises the characterization of physical and chemical properties collected from 4 districts of Chhattisgarh state.

INTRODUCTION: Cyanobacteria also referred as blue-green algae are the pioneer oxygenic phototrophs on earth whose distribution around the world is suppressed only by bacteria. Fossil evidence points to their presence in geographically diverse regions during the pre-cambrian (2 to more than 3.5 billions years ago). They are the large morphologically diverse group of phototrophic prokaryote, which occur in almost every habitat on earth. This versatility may explain the remarkable lack of morphological (and presumably physiological) change seen in 3.5 billion years old fossilized cyanobacteria and their modern day counterparts.

Their long evolution history has been marked by key geographical and biotic transitions including the creation of oxygenic photosynthesis, a prerequisite for the development of proliferation of metabolically complex microbial and higher eukaryotic life forms [1, 2]. They are very close to bacteria than higher plants. Cells are generally blue-green to violet but sometimes red or green.

The green of the chlorophyll is masked by the blue accessory pigment phycocyanin. These pigments lie in phycobilisoms, which lie on thylakoid. Their cell walls are made of amino sugars and amino acids. Flagella are completely absent and the moment in the members is accomplished by gliding motion [3, 4].

Their evolutionary antiquity and ability to fix CO_2 using water as an electron donor provided them with a decisive edge over other microbes to dominate the biosphere in the past and inhabit extreme environmental niches. Majority of the extinct species of cyanobacteria are obligate photoautotrophs, though a small fraction is mixotroph.

The light dark cycle is perhaps the most significant and persistent selection agent that has shaped the physiology of photoautotrophs all through their origin and evolution. This clearly points to a dominant role of diuranal rhythm on regulation of the mode of carbon utilization in cyanobacteria [5-8].

The role of cyanobacteria as biological inputs in agriculture has been well documented and substantiated has reported that algal inoculation was equally effective for the high yielding dwarf rice (*Oriza sativa* L.) varieties under high fertility. Cyanobacteria also occupy a variety of terrestrial environments. Soil is one of the most potential habitats for algal growth particularly in moist or waterlogged conditions. They play a significant role in maintaining soil fertility and in soil reclamation [8-12].

Agronomic benefits of Cyanobacteria: The major ill effects of intensive agriculture during last three decades are manifest in the erosion of soil fertilizing, in terms of depletion of soil nutrients and organic matter. Only the integrated use of organic manures, biofertilizers and chemical fertilizers has been found to be promising option. In this context cyanobacteria like all living organism act as some and sinks of various compounds by bio-geochemical cycling and being ubiquitous, can play a significant role in integrated nutrient management and sustenance of soil fertility in agriculture.

The significance of these phototrophic prokayotes in agriculture was first recognized by De, who attributed the self maintenance of the N-structure of tropical rice field soils to the growth of N_2 fixing BGA. It is well known that besides bringing about an improvement in field of rice (ranging from 5 to 25%), they also, produce indirect or direct beneficial changes in the physical, chemical and biological properties of soil and soil-water interface in rice fields, which are of agronomic importance.

Cyanobacteria among other groups of algae are known to take up P in excess of their immediate needs which may subsequently be released in the form of available P. They are also known to mobilize insoluble rock phosphate fertilizers and aid in efficient utilization of low cost supplements. The photosynthetic liberation of O_2 by cyanobacteria creates an oxygenic environment in the rice fields, especially in stand still water, which is highly beneficial to the paddy ecosystem, because it prevents the accumulation of high amounts of harmful oxidizable organic matter and toxic iron and sulphide compounds.

Algal inoculation is also known to provide beneficial effects on a number of other crops, including barley, oats, cotton, banana, sugarcane, maize and vegetable including tomato and radish.

The influence of cyanobacteria on aggregation of a rice field and salt affected soils has been shown to be 51.7 to 293.3%. Such improvements in soil aggregating result in better hadraulic conductivity infiltration and permeability. It ultimately leads to uniform distribution and increased available of plant nutrients.

MATERIAL AND METHOD: Characterization of cyanobacterial strains can be done using morphological, biochemical or physiological attributes in recent times Macromolecules such as Proteins and DNA have been used for profiling the strains these are more reliable and discriminatory besides economical in terms of time and costs.

This section embodies the common procedures applied frequently throughout the course of present investigation. All investigations in the soils of paddy fields were performed in the four divided Blocks of Chhattisgarh regions. Soil samples of paddy fields were collected randomly from Bilaspur, Champa, Raipur, and Durg, districts of Chhattisgarh. Soil samples from paddy fields were collected in the month of August and September. During this period the age of paddy crops was 2-3 months. The soils were contained fertilizers which were used by the farmers.

The paddy fields of Chhattisgarh were divided in to 4 Blocks:

- Block 1 : Bilaspur, Bilha
- Block 2 : Champa, Baradwar
- Block 3 : Raipur, Bhatapara
- Block 4 : Durg, Rajnandgaon

Physico-chemical Analysis of Soil: The soil is a porous mixture of inorganic particles, organic matter, air and water. This mixture also contains a large variety of living organisms. The inorganic particles are organic matter make up the soil solids, white and soil pore space is occupied by air and water.

The soil environment is influenced by several factors including geological, physico-chemical and biological properties of the soil, climate and human activities prevailing in that area. Soil analysis includes soil sampling and analysis for different physico-chemical properties of the soil.

Soil texture and structure are two important physical properties of the soil that influence soil aeration, water retention and water movement. Sandy soils are well aerated but have low water holding capacity. Granulated clayey soils are not only well aerated and well drained but they can retain sufficient water and nutrients for plant use.

A number of physicochemical properties such as pH, electrical conductivity and organic carbon were analyzed from different Blocks of Paddy fields.

Determination of pH: pH of the soil samples were determined by pH meter which operates on the principle of determination of (H^+) ion concentration in logarithmic variation in linear relation to voltage generated at the glass membrane according to Nernst equation. The above study has been done using the procedure as folloows10 gram of soil was dissolved in 25 ml of distilled water. Suspension was shaken for 30 minutes, pH meter was calibrated by using buffer solutions of pH 4.0 and 7.0.The electrode was dipped in soil-water suspension. The reading was measured in triplicate [13].

Determination of Electrical Conductivity: Electrical conductivity was measured by Equiptronics No. E.Q. 665 (**Fig. 5-8**). The conductivity values for different soil samples from block no. 1-4 were in the range of 0.21 to 0.85 m mho/cm. The value is within the permissible limits. The conductivity values of E helped to improve cyanobacterial growth. Where electrical conductivity grows above 2 m mhos/cm than soil became salty in nature due to the presence of ions, like metals, which allows the electric current to pass through them. The electrical conductivity have been calculate by using the procedure 1:2 soil water soil water- suspension was prepared by dissolving 10 gram of soil in 20 ml distilled water. Suspension was shaken for 30 minutes. The conductivity cell was dipped in soil water suspension. The galvanometer of conductivity meter was balanced and the conductance of soil solution was measured [13].

Determination of Organic Carbon: One of the most widely used rapid soil test for the assessment of available N_2 is based upon the estimation of readily oxidisable organic carbon which roughly represents 58% of the soil organic matter, the seat of nitrogen in soil, the most easily Oxidisable carbon determination method is modified walkey-black method. 5.0 g. of soil was transferred in 500 ml of conical flask and it was pipette in 10 ml of 1N $K_2Cr_2O_7$. 20 ml con. H_2SO_4 was added and it was shaken for 5 minutes. The solution was kept to stand for 30 minutes. The color of the content was changed to yellowish green. Then 10 ml of more $K_2Cr_2O_7$ was added and it was left for 30 minutes. The mixture was diluted with 200 ml water and 10 ml of H_3PO_4 was added 1 ml diphenylamine indicator was added. Deep violet color was appeared. It was titrated with 0.5 N ferrous ammonium sulphate till the color was changed first to purple and finally to green and end point were noted for individual soil sample. Blank experiment was carried out without using soil aliquots [14-15].

RESULT AND DISCUSSION:

Soil pH: The pH of Block No. 4 and 01 are the same. (**Fig. 5**) where as acidic pH range i.e. 6.7, 6.7 have been shown by soil samples from block 2 & 3 and alkaline pH (7.3, 7.6 and 7.9) were shown by block no. 03, 01 and 04. Sharma *et al.* (1980) showed that micronutrient availability is pH dependent (**Fig. 1-4**). Cyanobacteria are infrequent below pH 6.0 in most temperate soils, they were entirely absent below pH 5.4 in several samples from Ireland. Cyanobacteria may also influence the pH due to their metabolic activity. The pH determines the solubility of CO_2 and minerals in the medium and directly or indirectly influences the metabolism of algae.

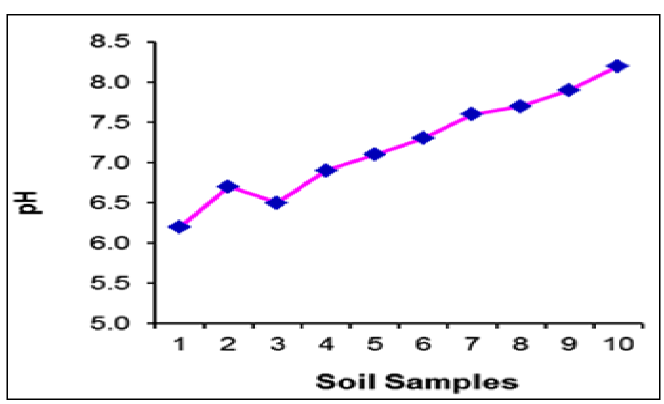

FIG.1: pH OF SOIL SAMPLES FROM BLOCK 1

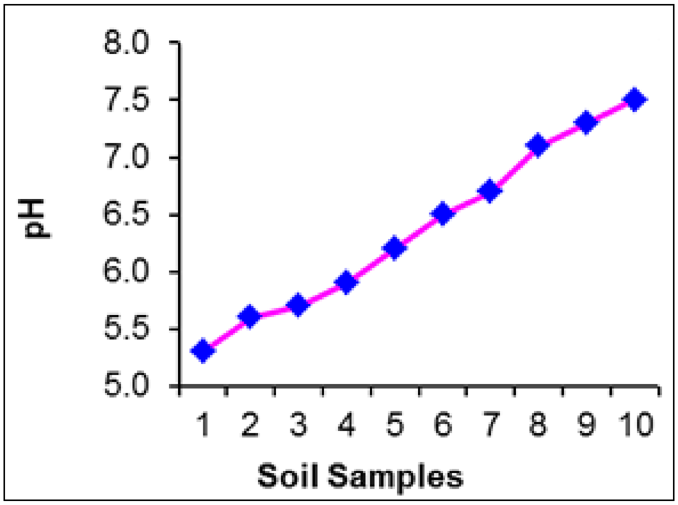

FIG. 2: pH OF SOIL SAMPLES FROM BLOCK 2

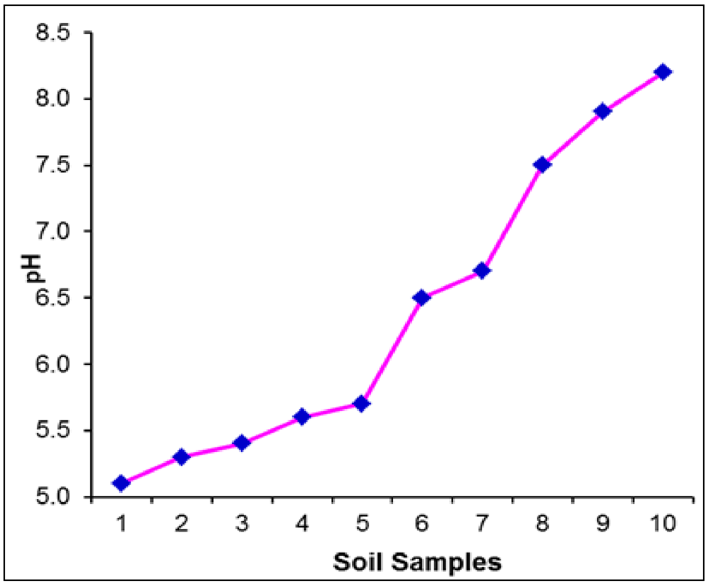

FIG. 3: pH OF SOIL SAMPLES FROM BLOCK 3

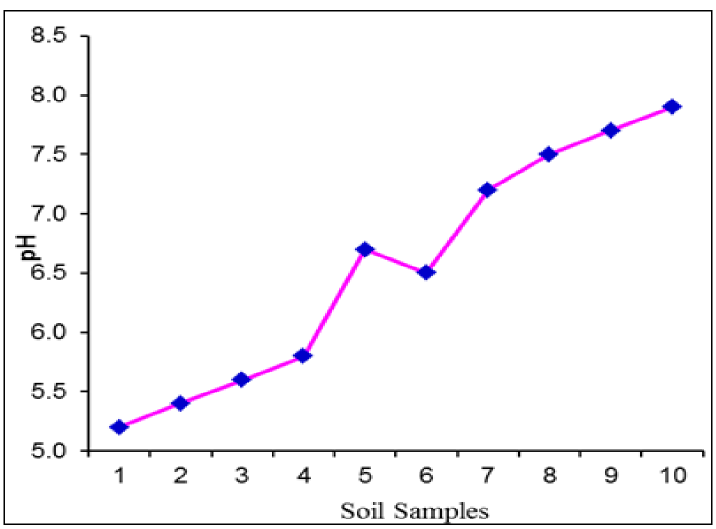

FIG. 4: pH OF SOIL SAMPLES FROM BLOCK 4

Determination of Electrical Conductivity: The conductivity values for different soil samples from block no. 1-4 were in the range of 0.21 to 0.85 m mho/cm. The value is within the permissible limits. The conductivity values of E helped to improve cyano-bacterial growth. Where electrical conductivity grows above 2 m mhos/cm than soil became salty in nature due to the presence of ions, like metals, which allows the electric current to pass through them.

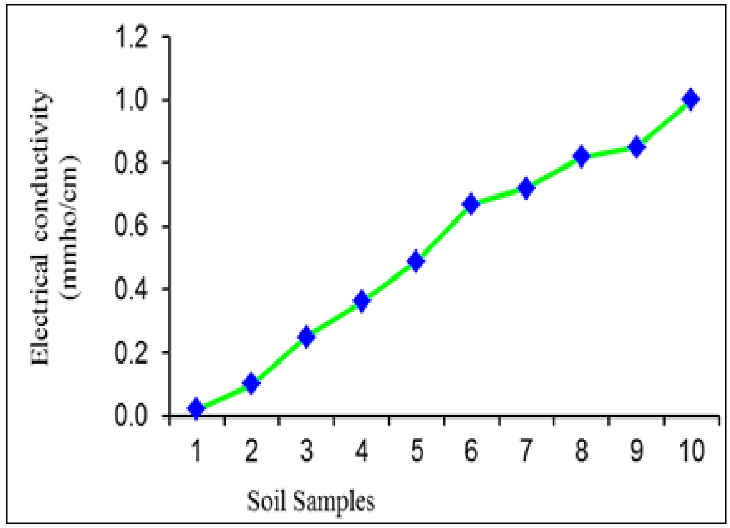

FIG. 5: ELECTRICAL CONDUCTIVITY OF SOIL SAMPLES FROM BLOCK 1

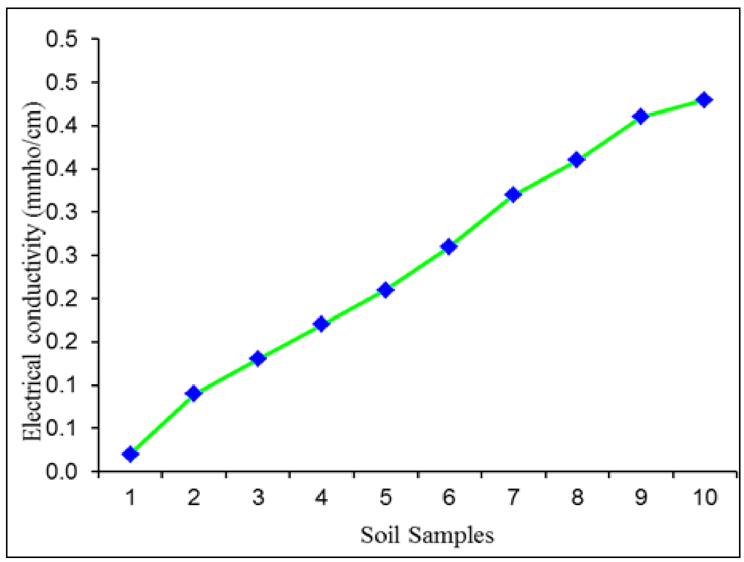

FIG. 6: ELECTRICAL CONDUCTIVITY OF SOIL SAMPLES FROM BLOCK 2

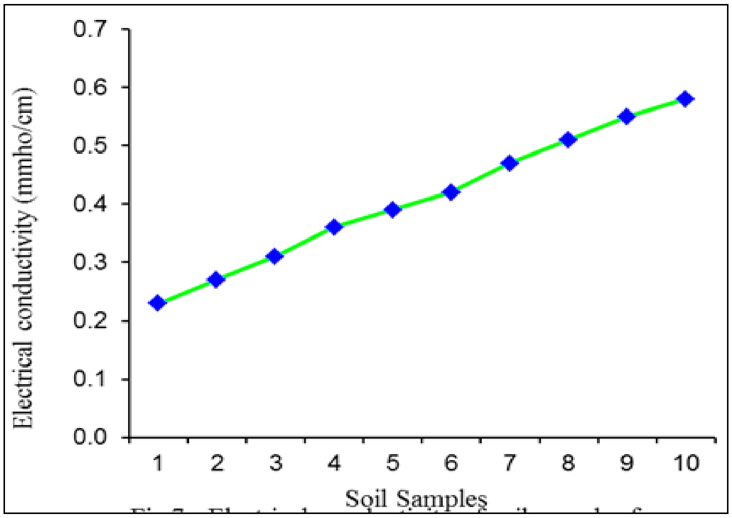

FIG. 7: ELECTRICAL CONDUCTIVITY OF SOIL SAMPLES FROM BLOCK 3

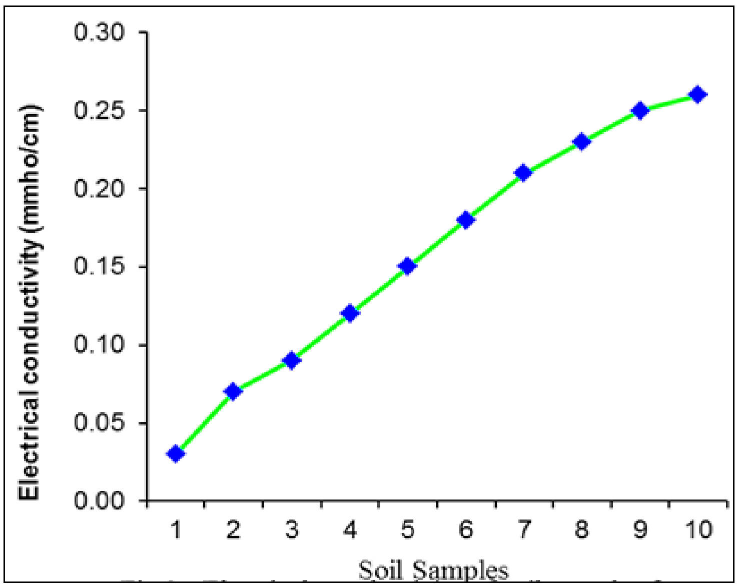

FIG. 8: ELECTRICAL CONDUCTIVITY OF SOIL SAMPLES FROM BLOCK 4

Determination of Organic Carbon: Soil organic matter represents an index of fertility status of soil besides evaluating the N_2 and other nutrients supplying capacity of soil, percent organic carbon of soil samples from different selected sampling blocks are shown in **Fig. 9-12. Fig. 13** showed the occurrence of organic carbon found in different selected soil samples from various blocks the maximum organic carbon content (0.819%) was shown by block 02 followed by block 03, the least organic carbon was evident in block 01 i.e. 0.395%. The major part (>90%) of soil nitrogen exists as complex combination in the organic matter fraction. It became available to crops and other soil microorganism after breakdown to simple forms followed by mineralization.

Hence, easily oxidizable organic carbon and mineralizable nitrogen are considered to be quite satisfactory as an index of N_2 availability in soils. The correction factors for organic carbon due to incomplete oxidation, but this factor is not valid across different soils and varies from soil to soil. This can easily be improved by making provision of external heat under reflex which results in almost complete decomposition of organic material and give organic carbon values equivalent to there obtained by wet or dry combustion.

The acid dichromate digestion solution used in this method decomposes at temp above 150°C, limiting the temperature that can be employed in any release method. The reduction of dichromate is affected by the pressure of other redo active components of the soil, due to which variation in % organic carbon is exhibited by different soil samples. The pressure of Fe^{2+}, Mn^{2+} and Li^{+} causes an over estimation of soil organic carbon, since all these are oxidized during the digestion.

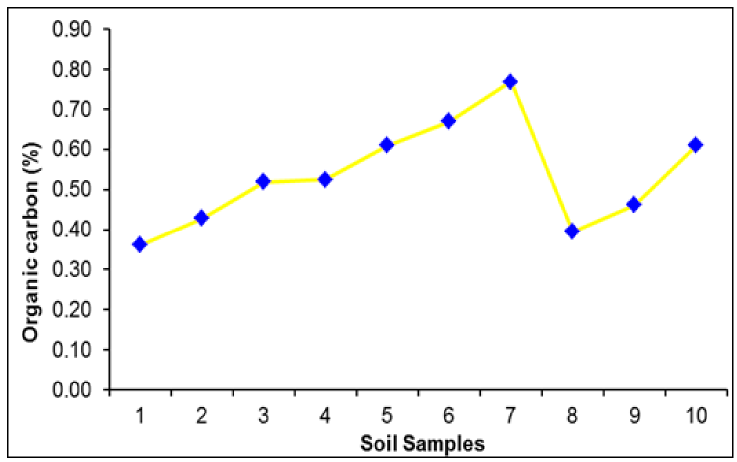

FIG. 9: ORGANIC CARBON OF SOIL SAMPLES FROM BLOCK 1

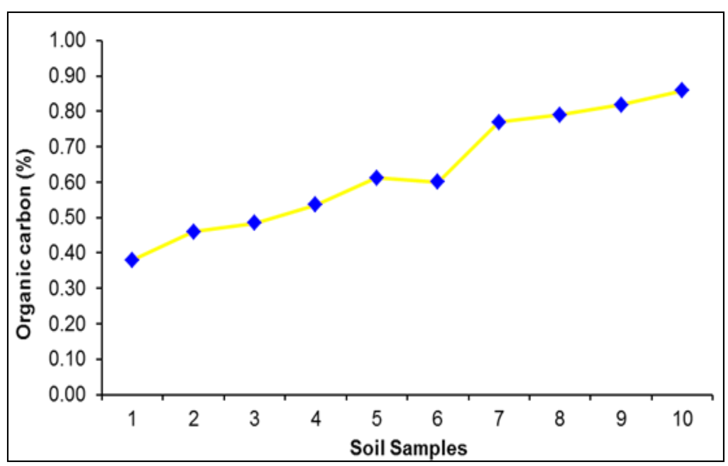

FIG. 10: ORGANIC CARBON OF SOIL SAMPLES FROM BLOCK 2

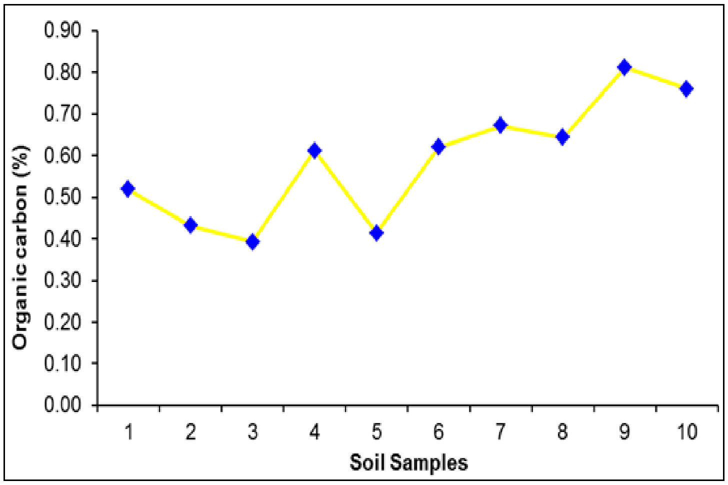

FIG. 11: ORGANIC CARBON OF SOIL SAMPLES FROM BLOCK 3

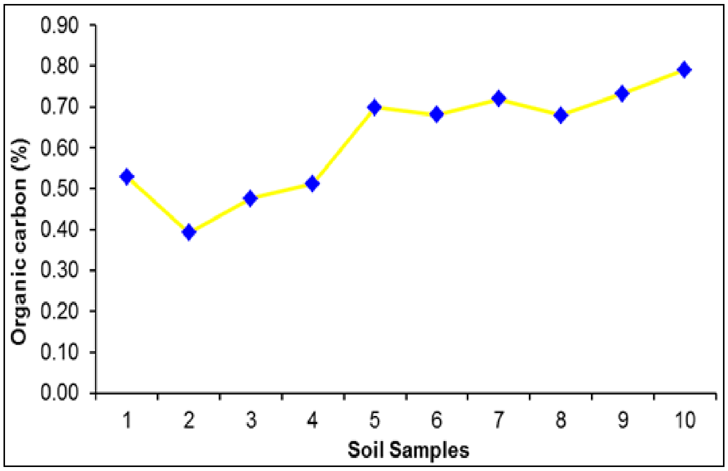

FIG. 12: ORGANIC CARBON OF SOIL SAMPLES FROM BLOCK 4

CONCLUSION: The physico chemical analysis of soils from 04 different selected blocks of Chhattisgarh state has shown that pH of block no. 2 & 3 are alkaline and in others acidic. It has evidenced that the micronutrient availability is pH dependent. The electrical conductivity helped to improve cyanobacterial growth. The maximum organic carbon content (0.819%) was shown by block 02 sample. In cyanobacteria taxonomy based on morphological, nutritional or developmental feature does not always correlate with the evolutionary relationships and morphological parameters are considerably altered due to release culturing condition. Analysis of photosynthetic pigments, isoenzyme variations or differentiated cell culture may also be misleading because of the variable expression of cyanobacterial gene product. Therefore, there is a need to improve taxonomical criteria by supplementing the information on the basis of molecular characterization.

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