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# Role Of Cyanobacteria And Azolla In Inorganic Carbon Sequestration And Nutrients Enrichment In Soil

Sangeetha B. M., Aarthi. S, Niranjana. R, Vidhya Lakshmi. R

*Department of Environmental Engineering*

*Park College Of Technology*

*Coimbatore, Tamil Nadhu*

*India*

***Abstract*—Reducing carbon dioxide emissions in order to stabilize atmospheric Carbon levels is crucial. Currently, the geological storage of carbon dioxide is considered to be the most economical method of carbon sequestration. The current drawback of carbon mineral sequestration is its relatively high energy requirement and cost. Therefore, our study focuses on the tailored synthesis of high-purity precipitated calcium carbonate and other nutrient particles. We chose to carry out "inorganic mineral carbon sequestration" using *Azolla microphylla* and *Nostoc muscorum*. In our project, we study the calcite formation resulting from the single species and as a conglomerate using various "treatments", namely T1, T2, T3, T4 and T5. The treatments contain *Nostoc muscorum, Azolla microphylla*., Gypsum and the soil sample taken. The cyanobacteria, Anabaena maintains a mutually beneficial symbiotic relationship with the *Azolla*. Cyanobacteria possess the ability to fix nitrogen and enrich the soil nutrients, hence is being proposed for its employment in the Green Belt because of the potential benefits involved.**

***Index Terms*—Inorganic mineral carbon sequestration, *Azolla microphylla,, Nostoc muscorum, Anabaena,*** Cyanobacteria ,**Green Belt**

## I. INTRODUCTION

### *A. Carbon Sequestration*

Atmospheric gas composition has considerably and quickly changed in last decades due to the industrial emissions, fuel combustion, deforestation and land use changes. All these activities have increased the natural background of atmospheric level of greenhouse gases[1]. $CO_2$ is the most abundant green house gas. Therefore, $CO_2$ mitigation is mandatory to alleviate the serious repercussions that this gas is causing on global climate.

Carbon capture and sequestration [2] is the process of capturing waste $CO_2$ from large point sources, transporting it to a storage site, and depositing it underground so that it will not re-enter the atmosphere. Sequestration can take many forms. Mineral carbonation, in which the $CO_2$ is reacted with minerals to form solid carbonates, is truly a permanent method, because the mineral carbonates are stable over geologic time periods.

Employment of cyanobacteria in biomineralization of carbon dioxide by $CaCO_3$ precipitation offers novel and self-sustaining strategies for point-source carbon capture and sequestration [3]. Carbon sequestration involves the capturing and storing of carbon and its subsequent removal from the global carbon cycle.

### *B. Azolla SP.*

*Azolla* has been deemed a "super-plant" as it can draw down as much as a tonne of nitrogen per acre per year (0.25 kg/m²/yr); this is matched by 6 tonnes per acre of carbon drawdown (1.5 kg/m²/yr) [4]. Its ability to use atmospheric nitrogen for growth means that the main limit to its growth is usually the availability of phosphorus: carbon, nitrogen and sulphur being three of the key elements of proteins, and phosphorus being required for DNA, RNA and in energy metabolism. Close examination of an *Azolla* leaf reveals that the upper lobe has an ovoid central cavity, the "living quarters" for filaments of *Anabaena* which is a cyanobacteria [5].

### *C. Nostoc sp.*

*Nostoc muscorum* is a free-living microorganism which inhabits both terrestrial and freshwater aquatic environments.

*N. muscorum* are important for the nutrient cycling of carbon and nitrogen within the soil ecosystems in which they are found. The process of fixing atmospheric nitrogen contributes plant-available nitrogen to the soil, improving plant growth [6].

The ideal environment for *N. muscorum* is one with pH in the range of 7.0 to 8.5, with a lower pH limit of 5.7. *N. muscorum* grows best when light intensity is less than that of direct sunlight, but can continue to grow and fix nitrogen in the presence of glucose and absence of sunlight [7].

*Nostoc muscorum* is a phototroph, requiring $CO_2$ for growth. It can also utilize glucose and sucrose for growth. *N. muscorum* has heterocysts, which are specialized nitrogen-fixation cells. Heterocysts, (5-10% of cells) appear when *N. muscorum* is transferred to nitrogen free media. Appearance of heterocysts is concurrent with an increase in nitrogenase activity, which reduces $N_2$ to $NH_3$. Magnesium is also required for *N. muscorum* to carry out nitrogen fixation.

*1) Impacts on Soil Fertility:N. muscorum* is known to have major impacts on soil structure, chemistry, and

biology [8]. It improves the stability of soil aggregates, making soil environments less susceptible to disturbances such as floods, desiccation, and freeze-thaw cycles. This is important particularly in arctic soils which are subject to yearly freezing and thawing, in desert soils, which are dry and erode easily, and in rice paddies, which are constantly flooded [9].

*N. muscorum* is an ecosystem engineer, creating environments within soil that are suitable for microbial and plant growth. Both physical and chemical characteristics of *N. muscorum* enable the formation of stable soil aggregates. Its filamentous structure binds soil particles together

Its ability to obtain carbon and nitrogen from the air allows this microbe to avoid competition for these nutrients with other microbes and plants. It also benefits microbes and plants in its vicinity by increasing soil organic matter in the form of carbohydrates and adds nitrogen that can be assimilated by plants.

### *D. Soil Inorganic Carbon (SIC) Sequestration*

The SIC pool is important in soils of arid and semi-arid climates and mostly comprises of carbonates, including calcite, dolomite, aragonite and siderite. The soils in the Semi-arid tropics area have both pedogenic and non-pedogenic calcium carbonates.

Formation of secondary or pedogenic carbonates is an important mechanism of soil Carbon sequestration. When the formation of secondary carbonates is facilitated by biological processes, such carbonates are also called biogenic carbonates.

The role of SIC sequestration on soil carbon dynamics in relation to the climate change is less understood than that of SOC sequestration. There is a strong need to assess the formation of secondary carbonates, the magnitude of leaching and the impact of land use and management on overall SIC dynamics [10].

## II. METHODOLOGY

The experiment was conducted in the controlled environment using pots. It was done to show the role of Nostoc sp. and Azolla sp. in carbon sequestration. Besides this, the micro and macro nutrient content including the soil pH and Electrical Conductivity were also studied.

### *A. Soil Analysis*

The soil analysis was carried out in the laboratories of "Soil Survey and Land Use Organization", Government of Tamil Nadu, Coimbatore. The TABLE 1 gives the details on the soil analysis and its appropriate method by which it was done.

### *B. Soil characteristic*

From the results obtained from the soil analysis, the initial test soil was characterized. The soil chosen for the experiment was found to be of the textural class of "Clay Loam", having an available Nitrogen, available Phosphorus and available Potassium of 60kg/ha, 9.5kg/ha, 230kg/ha respectively. Its pH was 7.4. EC was identified as 0.92 $dSm^{-1}$. Organic Carbon was about 1.1%.

### *C. Treatments*

The details of treatments utilised in the experiment are given below:

T0 : Initial Soil Sample
T1 : Control (Soil)
T2 : Soil + Gypsum
T3 : Soil + Gypsum + *Azolla microphylla*
T4 : Soil + Gypsum + *Nostoc muscorum*
T5 : Mixture (Soil + Gypsum + *Azolla microphylla* + *Noctoc muscorum*)

TABLE 1. Methods employed for Soil Analysis

| S.No | Soil Analysis | Method |
|---|---|---|
| **I** | **Mechanical Analysis** | Robinson's International Pipette Method |
| II | **Chemical analysis** | |
| | i. Available Nitrogen | Alkaline Permanganate Method |
| | ii. Available Phosphorus | Molybdenum Blue Method |
| | iii. Available Potassium | Flame Photometric Method |
| | iv. Organic Carbon | Walkley Method |
| | v. Micro Nutrients (Fe, Mn, Zn and Cu) | Atomic Absorption Spectrophotometer |
| | vi. Exchangeable Calcium and Magnesium ions | Complexometric Titration using EDTA |
| | vii. pH | Using glass electrode in the ELICO pH meter. |
| | viii. Electrical Conductivity | Using ELICO conductivity bridge. |

### *D. Pot Preparation*

The soil sample was collected from the field which was thoroughly puddled. It was taken in equal quantities in 10 separate pots of same size, of which 2 pots were assigned per treatment.

2 pots were kept aside for T1 (Control). These were thoroughly mixed with water without any lumps in the soil. They were left for curing process.

The rest of pots assigned for T2, T3, T4 and T5 were mixed with 50gms of Gypsum each and water was added till it was completely mixed with no lump formation. The pot was filled with 2.5cm of water above the soil surface for the "curing process". This condition was maintained for 2 days. The setup was kept still, without any disturbance in the shade net house (50% sunlight).

### *E. Collection of Nostoc muscorum*

The *Nostoc sp.* was collected from the rice fields during mid-day, when the specimen blooms. It was washed thoroughly with water to remove all the mud sticking to it. The culture was identified as *Nostoc muscorum* in Tamil Nadu Agricultural University, Coimbatore. It was then weighed to 15gms. They were manually ground using a "Mortar and pestle". These were taken in 4 conical flasks.

### *F. Collection of Azolla microphylla*

**T**he *Azolla microphylla* was collected from "Azolla Culture Centre" in Tamil Nadu Agricultural University, Coimbatore. 15gms of Azolla was collected each for T3 and T5.

### *G. Application of Nostoc muscorum and Azolla microphylla:*

The collected *Nostoc muscorum* was added to the Treatment T4 and T5 (2 pots each). The set up was slightly stirred. The collected *Azolla microphylla* was laid to the Treatment T3 and T5.


Fig 1. Growth of Azolla microphylla after 30 days

Fig 2. Formation of the matte of Nostoc muscorum after 30 days of study period

Fig 3. Formation of the matte of Nosctoc muscorum and the growth of Azolla microphylla in the T5 (Mixture) after 30days

All the treatments were slightly stirred every 2 days and a water level of 2.5cm above the soil surface was maintained equally in all pots. Otherwise, the set up kept under the shade net house was never disturbed for a period of 30 days.

### *H. Microscopic analysis of all the treatment sample*

The treatment samples were obtained from the pot cultures. The sample specimens were transferred to the "sample collection kit" and taken to the laboratory and examined under Scanning Electron Microscope (SEM) to check the formation of calcite crystals. The results of SEM were confirmed by EDAX. Both SEM and EDAX were performed in Chemistry Department, IIT Madras.

*1) Scanning Electron Microscope (SEM):* The samples taken to the laboratory were transferred to microscopic slides of size 2cmX 2cm and it is dried at room temperature for a day. Once dried, the samples are stuck to the carbon tape, which is in turn stuck to the magnetic plate. The sample surface is ionized to get a clear image on the SEM.

*2) EDAX (Energy Dispersed Analysis of X-rays):* Acceleration voltage between 2-10kV were applied. The elemental composition of the crystals was determined qualitatively with EDAX EDS detector.

## III. RESULT

The treatment samples were taken for soil analysis and microscopic analysis to check the calcite precipitation, elemental constituents and to observe the presence of various soil nutrients. It was also done to indicate its characteristic and behavioural pattern.



### *A. Microscopic analysis*

Microscopic analysis was performed by SEM and they were confirmed by EDAX.

*1) Microscopic Analysis of Control (T1):* The SEM result of the control sample (T1) does not show any formation of calcite crystals. The EDAX result further confirms that there is no calcium content in the control sample (T1) given for analysis.

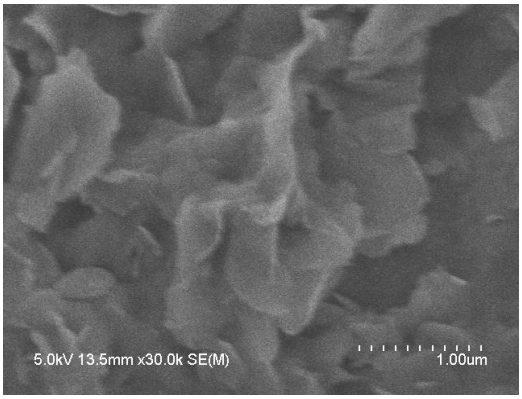


Fig 4. SEM image of control sample

TABLE 2. EDAX results of control

*2) Microscopic Analysis of T2 (Soil + Gypsum):* The SEM image does not indicate the presence of the calcite crystals. But the EDAX result shows meagre amount of Ca as there is noted addition of Gypsum, whereas the calcium content is nil in the control sample.

| Element | App Conc. | Intensity Corrn. | Weight % | Weight% Sigma | Atomic % |
|---|---|---|---|---|---|
| C K | 0.00 | 0.3346 | 0.00 | 0.00 | 0.00 |
| O K | 1.36 | 0.9924 | 57.66 | 2.43 | 71.99 |
| Si K | 0.76 | 1.0033 | 31.84 | 1.97 | 22.65 |
| K K | 0.25 | 1.0115 | 10.50 | 1.53 | 5.36 |
| Totals | | | 100.00 | | |

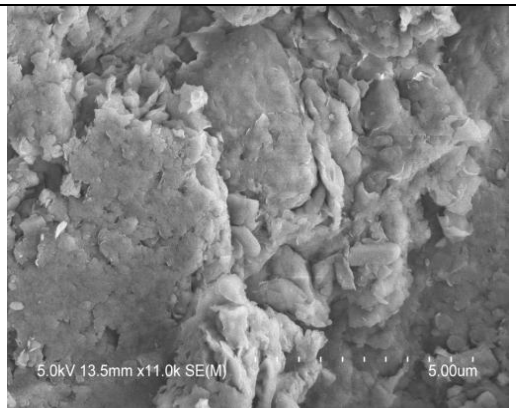


Fig 5. SEM image for T2 (Soil + Gypsum)

TABLE 3. EDAX result for T2 (Soil + Gypsum)

*3) Microscopic Analysis of T3 (Soil + Gypsum + Azolla microphylla):* The SEM image of T3 sample shows the calcite precipitation on Azolla microphylla. The EDAX result confirms the increase in the calcium content. There is notable increase in other nutrients like Mg, Al, Cl, S etc.

| Element | App Conc. | Intensity Corrn. | Weight % | Weight % Sigma | Atomic % |
|---|---|---|---|---|---|
| C K | 0.28 | 1.5148 | 64.19 | 1.73 | 70.94 |
| O K | 1.86 | 0.5629 | 34.49 | 1.73 | 28.62 |
| Ca K | 0.12 | 0.9525 | 1.32 | 0.35 | 0.44 |
| Totals | | | 100.0 | | |

| Element | App Conc. | Intensity Corrn. | Weight % | Weight % Sigma | Atomic % |
|---|---|---|---|---|---|
| C K | 2.22 | 0.5418 | 33.68 | 2.46 | 45.05 |
| O K | 3.47 | 0.7025 | 41.17 | 1.89 | 41.35 |
| Mg K | 0.13 | 0.8920 | 1.17 | 0.26 | 0.78 |
| Al K | 0.54 | 0.9432 | 4.74 | 0.37 | 2.82 |
| Si K | 1.52 | 0.9372 | 13.41 | 0.68 | 7.67 |
| Ca K | 0.68 | 0.9589 | 5.83 | 0.49 | 2.34 |
| Totals | | | 100.0 | | |

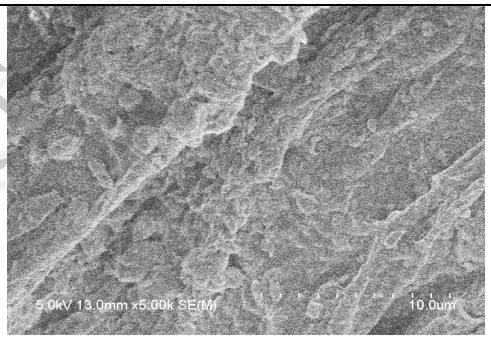


Fig 6. SEM image forT3 (Soil + Gypsum + Azolla microphylla)

TABLE 4. EDAX result for T3 (Soil + Gypsum + Azolla microphylla)

*4) Microscopic Analysis of T4 (Soil + Gypsum + Nostoc muscorum):* The SEM image of T4 sample shows the formation of calcite crystals on *Nostoc muscorum*. The EDAX result confirms the increase in the calcium content, which is more than that of the precipitation on *Azolla microphylla*. An increase in the nutrients like Mg, Al etc was also noted. The EDAX result of T4 show the increase in the calcium content which confirms that *Nostoc muscorum* has sequester carbon in the form of calcite.

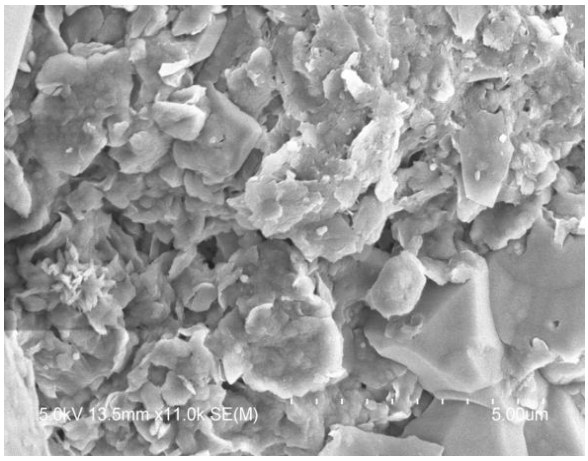


Fig 7. SEM image for T4 (Soil + Gypsum + Nostoc muscorum)

TABLE 5. EDAX for T4 (Soil + Gypsum + Nostoc muscorum)

| Element | App Conc. | Intensity Corrn. | Weight % | Weight % Sigma | Atomic % |
|---|---|---|---|---|---|
| C K | 4.06 | 0.7776 | 37.62 | 1.95 | 47.43 |
| O K | 4.88 | 0.7205 | 48.76 | 1.72 | 46.15 |
| Mg K | 0.13 | 0.8394 | 1.10 | 0.22 | 0.69 |
| Al K | 0.17 | 0.9001 | 1.38 | 0.21 | 0.77 |
| Si K | 0.50 | 0.9386 | 3.81 | 0.29 | 2.06 |
| S K | 0.11 | 0.9427 | 0.81 | 0.20 | 0.38 |
| Cl K | 0.12 | 0.8105 | 1.11 | 0.22 | 0.47 |
| Ca K | 0.72 | 0.9609 | 5.42 | 0.39 | 2.05 |
| Totals | | | 100 | | |

*5) Microscopic Analysis of T5 (Mixture):* The SEM image of T5 sample shows the formation of calcite crystals on the combined culture of *Azolla microphylla* and *Nostoc muscorum*. The EDAX result confirms the increase in the calcium content and this is also found to contain the maximum amount of Ca, when compared with the other treatment samples.

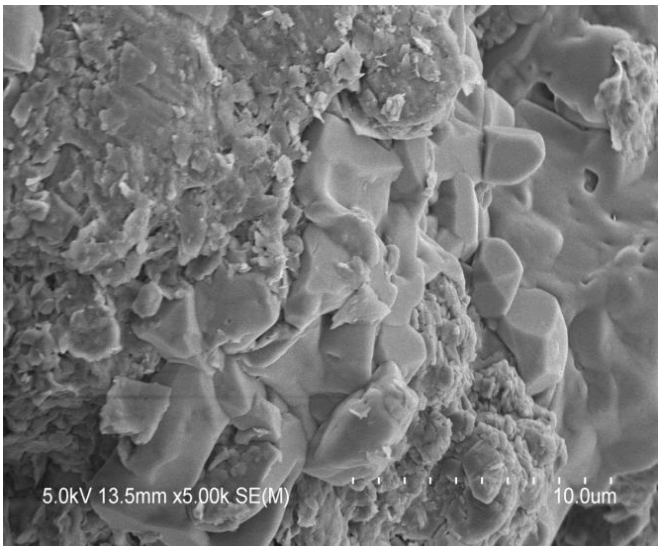


Fig 8. SEM image for T5 (Mixture)

TABLE 6. EDAX result of T5 (Mixture)

| Element | App Conc. | Intensity Corrn. | Weight % | Weight % Sigma | Atomic % |
|---|---|---|---|---|---|
| O K | 8.29 | 1.2725 | 57.85 | 1.14 | 74.33 |
| Mg K | 0.15 | 0.8451 | 1.68 | 0.39 | 1.35 |
| Al K | 0.61 | 0.9100 | 6.24 | 0.43 | 4.52 |
| Si K | 2.36 | 0.9075 | 24.31 | 0.75 | 16.92 |
| Ca K | 0.34 | 0.9654 | 6.32 | 0.43 | 1.62 |
| Fe K | 0.31 | 0.8035 | 3.59 | 1.02 | 1.26 |
| Totals | | | 100.0 | | |

As the SEM results suggest that the calcite precipitation increases, so does the EDAX results. This depicts that the elemental weight % of Ca goes up along the treatment order. It thus proves that the introduction of *Nostoc sp.* and *Azolla sp.* as individual specimen and as a consortium induces calcite precipitation and enhances the mineral content in the study soil.

## *B. Soil analysis*

It was done to analyze the macro and micro nutrient content in the treatment soil samples. It also shows the pH, Electrical Conductivity and Organic Carbon as unique variables. This was also done to indicate the presence of $Ca^{2+}$ and $Mg^{2+}$ ions.

*1) Detailed Soil Analysis Report:* The soil analysis report shows the results obtained for pH, EC and Organic Carbon.

TABLE 7. Soil Analysis Report of pH, EC and OC

| S.No | Treatments | pH | Electrical Conductivity (dSm-1) | Organic Carbon (%) |
|---|---|---|---|---|
| 1 | Initial (T0) | 7.4 | 0.92 | 1.1 |
| 2 | Control (T1) | 8.07 | 0.90 | 1.12 |
| 3 | Soil + Gypsum (T2) | 8.0 | 1.1 | 1.14 |
| 4 | Soil + Gypsum + Azolla sp. (T3) | 8.0 | 0.8 | 1.18 |
| 5 | Soil + Gypsum +Nostoc sp. (T4) | 8.14 | 0.78 | 1.2 |
| 6 | Mixture (T5) | 8.02 | 0.71 | 1.22 |

The pH of the treatment soil samples remains unaffected by the introduction of *Nostoc muscorum* and *Azolla microphylla*. The nature of the soil remained basic throughout and improved the soil characteristics.

*2) Content of Macro and Micro Nutrient:*

TABLE 8. Macro Nutrient Content

TABLE 9. Micro Nutrient Content

| S.No | Treatments | Availale Nitrogen (kgha-1) | Available Phosphorus (kg ha-1) | Available Potassium (kg ha-1) |
|---|---|---|---|---|
| 1 | Initial (T0) | 60 | 7.5 | 230 |
| 2 | Control (T1) | 64 | 7 | 330 |
| 3 | Soil + Gypsum (T2) | 70 | 8 | 340 |
| 4 | Soil + Gypsum + Azolla sp. (T3) | 64 | 7.5 | 305 |
| 5 | Soil + Gypsum +Nostoc sp. (T4) | 62 | 8 | 305 |
| 6 | Mixture (T5) | 81 | 8.5 | 360 |

| S.No | Treatments | Fe (ppm) | Mn (ppm) | Zn (ppm) | Cu (ppm) |
|---|---|---|---|---|---|
| 1 | Initial (T0) | 8.89 | 5.88 | 1.39 | 1.83 |
| 2 | Control (T1) | 8.67 | 4.16 | 1.73 | 1.36 |
| 3 | Soil + Gypsum (T2) | 4.89 | 5.07 | 1.41 | 0.72 |
| 4 | Soil + Gypsum + Azolla sp. (T3) | 3.78 | 8.32 | 1.27 | 2.04 |
| 5 | Soil + Gypsum +Nostoc sp. (T4) | 7.11 | 7.44 | 2.32 | 1.83 |
| 6 | Mixture (T5) | 11.78 | 12.36 | 2.30 | 2.43 |

*3) Content of Na and the presence of Ca2+ and Mg2+ ions:* The soil analysis report shows the content of Na and the presence of Ca, Mg ions.

TABLE 10. Content of Na and the presence of $Ca^{2+}$ and $Mg^{2+}$ ions

| S.No | Treatments | Na (m.eq/l) | Ca2+ (m.eq/100 gm soil) | Mg2+ (m.eq/100 gm soil) |
|---|---|---|---|---|
| 1 | Initial (T0) | 1.740 | 5.00 | 1.50 |
| 2 | Control (T1) | 2.390 | 5.50 | 3.00 |
| 3 | Soil + Gypsum (T2) | 4.020 | 6.50 | 3.90 |
| 4 | Soil + Gypsum + Azolla sp. (T3) | 4.130 | 10.00 | 8.00 |
| 5 | Soil + Gypsum +Nostoc sp. (T4) | 4.670 | 9.20 | 12.00 |
| 6 | Mixture (T5) | 4.890 | 12.85 | 17.00 |

The Na content obtained from the soil analysis of various treatments proves to increase gradually from T0 to T5. This shows the improved enrichment of sodium in the soil by the *Nostoc sp.* and *Azolla sp.*

### *C. Comparative study of the soil analysis report*

The comparative study of the soil analysis report is being done by the graphical representation of the obtained results. Various parameters are being discussed and the results are manipulated.

*1) Comparison of Electrical Conductivity:* The Electrical Conductivity of the soil treatments tends to decrease along the treatment order. It has shown an increase in T2, in which only Gypsum was added and there was no addition of cyanobacteria. The decrease in EC of the soil proves that the salinity of the soil goes down, which improves the soil fertility. This also shows that growth of *Nostoc sp.* and *Azolla sp.* further reduces the EC in the soil.

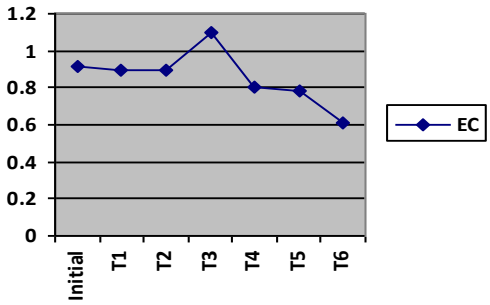


Fig 9. Comparison of Electrical Conductivity

*2) Comparison of Organic Carbon:* The Organic Carbon content of the soil treatments tends to increase along the treatment order. It has shown a gradual increase. The increase in OC in the soil enhances mineralization. This proves to be a source of energy for the growth of the microbes inhabiting the soil and also *Nostoc sp.* and *Azolla sp.* that were introduced.

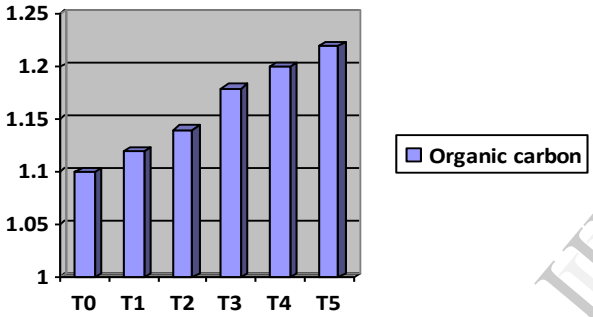


Fig 10. Comparison of Organic Carbon

*3) Comparison of Macro Nutrients:* The macro nutrients viz., available Nitrogen, available Phosphorus and the available Potassium in the soil treatments have shown fluctuations. The presence of macro nutrients ultimately has shown progressive increase in the final treatment T5, which contains the mixture of soil, gypsum, *Nostoc sp.* and *Azolla sp.* This increase in the macro nutrients of the soil proves to improve the soil fertility. This also shows that growth of *Nostoc sp.* and *Azolla sp.* enriches in the soil.

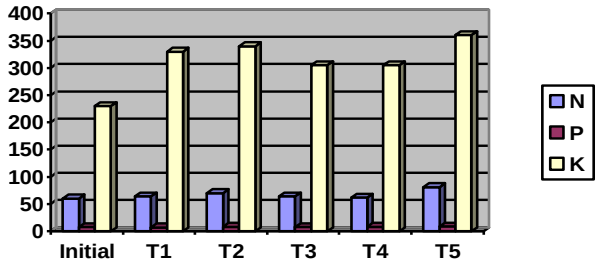


Fig 11. Comparison of Macro Nutrients

*4) Comparison of Macro Nutrients:* The micro nutrients viz., Fe, Mn, Cu and Zn in the soil treatments have shown fluctuations. The presence of the micro nutrients ultimately has shown progressive increase in the final treatment T5, which contains the mixture of soil, gypsum, *Nostoc sp.* and *Azolla sp.* This increase in the micro nutrients of the soil, further proves to improve the soil fertility. This also shows that growth of *Nostoc sp.* and *Azolla sp.* enriches in the soil.

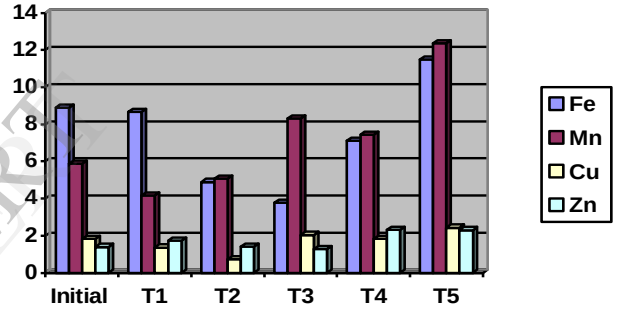


Fig 12. Comparison of Micro Nutrients

*5) Comparison of Ca2+ and Mg2+ ions:* The $Ca^{2+}$ and $Mg^{2+}$ ions of the soil treatments tend to gradually increase along the treatment order. The $Mg^{2+}$ ions have shown a steady increase which is depicted in the graph. The $Ca^{2+}$ ions have shown reasonable increase in T3 (*Azolla microphylla*) than T4 (*Nostoc muscorum*). Finally it tends to go up in T5, which contains the mixture of soil, gypsum, *Nostoc sp.* and *Azolla sp.* This also shows a potential increase in the presence of the $Ca^{2+}$ and $Mg^{2+}$ ions. It enriches the soil.

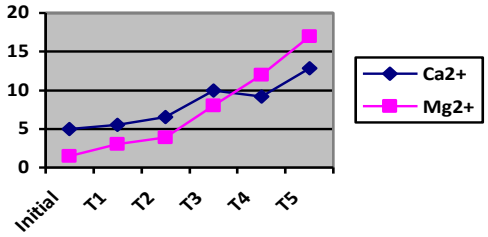


Fig 13. Comparison of Ca2+ and Mg2+ ions

## IV. DISCUSSION

### *A. Role of cyanobacterial system on carbon sequestration/Calcite precipitation*

The cultivation of photosynthetic microorganisms such as cyanobacteria has been proposed as an alternative for $CO_2$ bioremediation. These are attractive organisms for $CO_2$ bioremediation since they have a very high productivity when compared to other photosynthetic organisms. These cultures also have several characteristics that argue for potentially higher productivities than higher plants. In the present investigation the high amount of calcite precipitation was reported in the pot cultures treated with *Nostoc sp.* and *Azolla sp.* A mean increase in the micro and macro nutrients was also recorded.

Soil temperature is the prime factor influencing the calcite precipitation by cyanobacteria and calcite precipitation is high when the soil temperature is 29ºC±32ºC. It was also reported that temperature ranging from 22±32ºC exerted positive effect on bacterial precipitation of carbonate, increasing both the ability to form crystals and the extent of calcite precipitation by each strain. In the present study, similar results were obtained. Soil temperatures ranging from 22±36ºC favoured calcite precipitation. In the current investigation it was noticed that when Cyanobaterial cultures were applied as a consortium, the mean amount of calcite precipitation was more. Higher the biomass generation rate higher the calcite precipitation.

### *B. Confirmation of Nostoc sp. And Azolla sp. included Calcite precipitation by scanning electron microscope:*

Much of the carbon that is represented in the global carbon cycle is sequestered primarily as calcium and calcium-magnesium carbonates. In many cases, the carbonates are biogenic origin, some precipitated by bacteria, cyanobacteria and fungi. The microalgae are capable of using free $CO_2$ and bicarbonate ions as a source of inorganic carbon during photosynthesis, transporting them across the fine plasmatic membrane where they accumulate in the cell as an organic carbon reservoir for photosynthesis.

In the present study the SEM images confirmed the calcite crystals formation by Cyanobatcerial strains and the size and shape of the crystals varied depending on the Cyanobacterial species and the amount of calcium was comparatively high in the treatment that contained the consortium.

### *C. Influence of Nostoc sp. And Azolla sp. on plant nutrients in soil*

In the present study, the high mean amount of Nitrogen, Phosphorus and Potassium content were recorded in the sample of T5 (Mixture) obtained from the pot culture, which was the combination of *Nostoc sp.*, *Azolla sp.* and gypsum as compared to other pots treated with individual species whereas the control pots recorded the lowest content of NPK.

Similar to the NPK contents in the soil, the micro nutrients content also noticed higher values in the pots treated with the combination of *Nostoc sp.*, *Azolla sp.* and gypsum than the control treatment.

Further, there is an appreciable increase in the Organic Carbon in the soil samples from the treatments. The $Ca^{2+}$ and $Mg^{2+}$ ions have also gone up, which enhances the absorption of the essential nutrient ions from the soil.

## V. CONCLUSION

*Nostoc muscorum* and *Azolla microphylla* were cultured in controlled environment to show their various abilities as individual specimen and as a consortium too. It was done to explore the carbon sequestration potential of the specimens. The soil analysis was carried out to check the micro and macro nutrients in the treatments.

With reference to all the experimental results obtained, it is noted that *Nostoc sp.*, *Azolla sp.* sequester greater amount of carbon as calcite crystals. The enrichment of nutrients both micro and macro are found too. It is also noted in the individual specimens but not as much as in the mixture.

This is being proposed for its application in the "Green Belt" as it enhances the soil fertility and the biomass obtained can be further used to produce bio fuels. It has no ill effects on the soil and the various other organisms inhabiting it and ultimately the environment. Further the calcite precipitation goes into the fixed pool, which becomes biologically inactive and does not interfere in the metabolism of other microbes and other natural cycles.

The present investigation on *Nostoc sp.* and *Azolla sp.* prove them to be potential candidates for sequestering the atmospheric carbon, besides enriching the soil and improving the fertility. These organisms show notable diazotrophic characteristic by fixing nitrogen and enhancing the other soil nutrients.



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