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Original Article

ISOLATION OF HALOPHILIC ANAEROBIC BACTERIA FROM THE CORE DELTA AREA OF WEST BENGAL: ITS POSSIBLE IMPACT ON HUMAN HEALTH AND ENVIRONMENT

PATHIKRIT BANDOPADHYAY[1*]

[1]Department of Physical Education, Kalyani University, Kalyani 741235, Nadia, India. Email: pbandopadhyayku@gmail.com

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ABSTRACT

Objective: To identify bacteria which can be an impetus to solve the environmental problem in sunderban delta by protecting the mangrove as well as soil carbon recharging from the Sunderban Delta. To show its possible therapeutic impact on human health.

Methods: Total 5 different area marked as control and another 5 as experimental. Bacteria were isolated from soil sample and were allowed to grown and then increased the number in broth culture. Soil porosity, pH of soil, clay content, silt of soil, organic matter content, organic carbon content, phosphorous content of soil were measured prior and after application of bacterial broth culture in both the areas.

Results: Soil sample was colleceted at about 15cms root depth of plant.Isolation of Halophilic and anaerobic bacteria by progressive increment of saline percentage and CO_2 concentration.An orange colour bacterial strain that was grown on nutrient agar on normal incubation after about 72hrs,however the same was grown at 5gm% NaCl and 5.8% CO_2 in 24 hrs suggesting its anaerobic nature.It also inhibits the growth of *Salmonella sp* isolated from the control and experimental area when plated together.Halophilic nature was confered by the appearance of Poly Hydroxy Butyrate after Sudan Black B staining.

Conclusion: Isolated bacteria not only inhibit pathogenic bacterial growth but also have potential enviornmental impacts like stimulate mangrove growth, prevention of soil errosion as well as improve soil dynamics.Thereby it benefits human development in two ways:first by preventing the growth of pathogenic bacteria and second by potential positive impact on enviornment indirectly benefits human welfare.

Keywords: Halophilic anaerobic bacteria, Environment, Human helth.

INTRODUCTION

The Sundarban, covering about one million ha in the delta of the rivers Ganga, Brahmaputra and Meghna is shared between Bangladesh (~60%) and India (~40%), and is the world's largest coastal wetland harbouring varities of species starting from unicellular bacteria to multicellular mammals like Tiger and famous for Mangrove plants. Mangrove provides a unique ecological enviornments for diverse bacterial communities which largely control carbon,nitrogen,phosphorous and sulphur dynamics and contribute to soil and vegetation pattern [1] of which carbon retention in soil has several potential impacts.Salinity gradients change over a wide range of spatial and temporal scales causing several problems like land erosion mangrove plantation lost etc. Carbon sequestration is a geo-engineering technique for long-term storage of carbon dioxide or other forms of carbon to mitigate global warming. Carbon dioxide is usually captured from the atmosphere through biological, chemical or physical processes [2]. It has been proposed as a way to mitigate accumulation of greenhouse gases in the atmosphere, which are released by burning fossil fuels [3]. CO_2 may be captured as a pure by-product in processes related to petroleum refining or from flue gases from power generation [4].

The microbial contribution to soil Carbon storage is directly related to microbial community dynamics and the balance between formation and degradation of microbial byproducts. Soil microbes also indirectly influence Carbon cycling by improving soil aggregation, which physically protects soil organic matter (SOM). Consequently, the microbial contribution to Carbon sequestration is governed by the interactions between the amount of microbial biomass, microbial community structure, microbial byproducts, and soil properties such as texture, clay mineralogy, pore-size distribution, and aggregate dynamics. The capacity of a soil to protect microbial biomass and microbially derived organic matter (MOM) is directly and/or indirectly (i.e., through physical protection by aggregates) related to the reactive properties of clays. However, the stabilization of MOM in the soil is also related to the efficiency with which microorganisms utilize substrate Carbon and the chemical nature of the byproducts they produce. Several mechanisms of protection of microbial biomass in soils have been proposed: (i) clays promote microbial growth by maintaining the pH in the optimal range [5] (ii) clays adsorb metabolites inhibitory to microbial growth [6], (iii) clay-microbe interactions protect the organisms against desiccation [7], and (iv) microbes reside in small pores in which they are protected against predation by higher trophic groups, notably protozoa [8]. Since all of these mechanisms are directly or indirectly related to the reactive surface properties of clays, the protective capacity of soils for microbial biomass has often been related to clay content [9]; however, clay mineralogy may be as important as clay content for microbial biomass protection [10].

The influence of clay type has received much less attention than clay content, and the few studies that have been done have most often been conducted under in vitro conditions with single bacterial species [7]. Bentonite clay, which includes montmorillonite and other smectite group minerals, protected *Rhizobium* from predation by protozoa in a liquid culture and in soil amended with the clay [10]. Bacterial growth and activity were higher in the presence of montmorillonite clays compared with kaolinite clays and oxides [5, 7]. This differential effect is related to the greater cation exchange capacity and surface area of 2:1 clay particles [5], which presumably confer greater protection against desiccation [7] than 1:1 clays and oxides. Van Gestel *et al.* (1996) [11], however, suggested that clays do not provide protection against severe drying because water associated with clays is evaporated during drying events. Nevertheless, bacteria were protected in soil aggregates subjected to dry-wet cycles; whereas, fungi were not, presumably because they reside in larger pores and on the surfaces of the aggregates where they are more exposed to drying conditions [12].

The location of bacteria and fungi within the pore network is a key factor in their survival and activity, and the larger size of fungi may make them more vulnerable to predation. Bacterial populations are consistently high in small pores, but highly variable in large pores where they are vulnerable to being consumed [12]. Vargas and Hattori (1986) [13] suggested three mechanisms by which aggregates protect microbes against predation: (i) small pore neck sizes exclude protozoa and nematodes from entering, (ii) aggregates

are divided into "compartments" and "walls" which limit movement from one part of an aggregate to another and (iii) migration of protozoa and nematodes among aggregates is restricted by the extent that aggregates are connected by a continuous water film. In 1993 Nadelhoffer *et al.* [14] in a revere at nature showed that nitrogen deposition in the soil of the temperate forest has a minimal effect on carbon sequestration. In current science showed that plant roots are one of the greater contributors in the soil carbon recharging. They proposed that about 40% of the photosynthates synthesized in the plant parts are lost through the root system into the rhizosphere within an hour and the rate of loss is influenced by several factors, e.g. plant age, different biotic and abiotic stresses, etc. The rhizospheric environment of the plant is different compared to bulk soil with respect to physical, chemical and biological properties. Thus the aim of this study is to provide an insight on the contribution of plant roots for transfer of carbon from atmosphere to rhizosphere and further their finance in sustainable agriculture. In 2009 Taghavi *et al.* [15] had shown the beneficial role of endophytic bacteria in poplur tree growth. In 2006 Arora and Maheswari *et al*. [16] had shown the salinity induced PHB accumulation of PHB in the Rhizophora suggesting its role in protection in salinity stress. In this richest source of biodiversity there must be quite a large number of pathogenic as well as benifical bacterial occurrence is possible in this region. So, in the present study the attempt has been made to identify bacteria which can be an impetus to solve of the environmental problems in sunderban delta by protecting the mangrove as well as soil carbon recharging and to some extent protection against land erosion by carbon sequestration at the Sundarban area. On the other hand this study also makes an attempt to explore the possible therapeutic role of bacteria (if any).

MATERIALS AND METHODS

Materials

All the other chemicals used including the solvents, were of analytical grade obtained from Sisco Research Laboratories (SRL), Mumbai, India, Qualigens (India/Germany), SD fine chemicals (India), Merck Limited, Delhi, India.

Experimental Design

Samples were collected from the 10 different respective areas of Gosaba,Rangaberia of core area of Sunderban Delta.Each of the 10 different areas were again further divided into five small areas each named as control and experimental, and was marked.The basic objective was to separate those bacteria which mainly sequester carbon from the atmosphere and to observe there nature. Selected five smaller regions were identified in each of control and experimental area where the saplings of mangroves were predominant. The main reason behind the selection was to observe the productivity or the effects of the bacteria on the growing plants.In the next phase after isolation of the desired bateria,the plan was to increase that particular type/types of bacteria by 'Broth Culture' which was planned to be poured in the experimental area and not to be in the control area to compare the growth change in mangrove saplings and some soil parameters which favors the bacterial growth and improvement of soil quality making it less prone to erosion and also to isolate pathogenic strains from each of the area and to observe there growth pattern in presence of this isolated bacteria.

Collection of Sample

The samples were collected from the 10 different sites of Gosaba and Rangaberia of core Sunderban delta, sites were divided into 5 small area; namely control and experimental area as mentioned above. Both the sites are located proximal to the estuarine region but the sites are far above that contact with estuarine water.

Soil sampling can be done in two ways composite and representative sampling but as we were interested about the microbial isolation (those which utilizes CO_2 and salt) and their growth pattern and parameters that regulate their growth and occurrence, composite sampling was done. The soil samples were collected at a rooting depth of 10-15cm with the sterilized auger. The vials were then placed in the container containing ice pack after seal the mouth of the container. Leaf samples were collected for estimation of total chlorophyll content of that plant [16] and only the leaves of the saplings were collected and stored in a zip hold plastic bag and was placed in the container with ice packs.

Isolation of the bacteria from the soil sample

Preparation of Media: Initially Nutrient agar and was prepared of about 200ml each and were sterilized in autoclave at 15lb pressure for 15min.The sterilized media were then poured on the sterilized Petri plates and placed on the incubator at 37ºC for overnight incubation to observe whether any contaminations were being happened. Subsequent media preparation in the later stage the same procedure has been followed regarding sterilization.The same procedure was followed also for the selective media preparation.

Preparation of Soil suspension of the sample: In a sterilized test tube about 1gm of the soil(both form the control and experimental area) was taken and to it 10ml sterile water was added, the mixture was vortexed and was allowed to settle, after settling; about 0.1ml(for 2times) were taken from the liquid part above the settled material.

Culture of the bacteria: To each plates of Nutrient agar about 0.1ml of soil suspension from both the area was spreaded using a sterile glass spreader and was placed in the normal incubator at 37°C for overnight.However in the latter stage all the bacterial agar plates were placed in CO_2 incubator (ESCO) with different percentage of CO_2.

Growth of bacteria in selective medium: Nutrient agar was used for identification of all possible bacterial strains present in the soil sample.Additionally to isolate and identification of different pathogenic bacteria from this area different selective media was used,Bismuth sulphide for (Enterobactriacce family),Mackonkey Agar(for Gram positive),

Isolation of Desired Bacteria: Nutrient agar media containing 2.5, 5,10gm%Nacl were prepared and each of the strains was spreaded in these plates and incubates in 5.8%CO_2. Growth of bacteria of orange, color (from experimental sample) was observed in 24hrs incubation.

Estimation of Total Chlorophyll content

1gm of leaf extract was crushed in mortar pestle with 5ml of 80%acetone and kept at room temperature for 5-6hrs in a glass centrifuge tube. The clear extract was collected by low speed centrifuge tube (2000g for 5min) and the volume of the supernatant was made up to 5ml with 80% acetone.Optical density of the clear green extract was read at 650nm against a blank set containing 5ml of 80%acetone [17]. The quantity of total chlorophyll was calculated in milligrams/5ml (= 1gm fresh weight of leaf) through Arnon expression as under:

Total Chlorophyll content= OD at 650nm x 5 (in mg) per 5ml extract or 1gm of fresh weight of leaf by 34.5

Determination of Soil parameters

pH of the soil sample: To determine pH, at moisture saturation percentage of soil 50gm of 2-4mm soil (passed through 2-4 pore sieves) was taken in a beaker. Now small quantity of distilled water was added without stirring the soil, till a glistering layer appears on the surface of the soil. The soil sample was stirred with a glass rod to make uniform paste. pH of the solution was determined electrochemically using glass electrode pH meter. The results were directly expressed in pH units at 1:5 soil suspensions [18].

Total phosphorous content of soil sample: 1.5gm powdered soil was taken in 100ml Kjeldahl Flask and moisture the sample with little water to the consistency of a thin paste. To it 2ml ConcHNO_3 and 2ml of Conc.$HClO_4$ was added and was heated slowly on a hot plate until nearly dry. The flask was cooled and to it 1ml of $HClO_4$ and the solution was heated again until dry, rotate the flask several times during heating. The flask was cooled and to it 21ml of Conc.H_2SO_4 was added. The sample was boiled slowly for 10minutes and was cooled. The solution was filtered through Whatman No. 42 filter paper and make up the final volume to 250ml with distilled water in volumetric flask. Aliquot was taken and was diluted to 50ml

and the phosphorous was estimated.2ml of ammonium molybdate followed by 5drops of $SnCl_2$ solution. Blue coloration was developed reading was taken at 690nm on a spectrophotometer using a distilled water blank with same amount of chemicals. The reading was taken after 5minutes but before 12minutes of addition of the last reagent. The concentration was obtained from the standard curve [19].

Organic carbon and Organic matter content in the soil: Organic matter present in the sample was digested with excess K2Cr2O7 and H2SO4 and residual unutilized dichromate was then treated with ferrous ammonium sulphate. Oven dried soil sample was passed through 0.5mm non ferrous screen. Weighing of suitable quality of soil (roughly about 10gm) and was transferred to 500mL conical flask.10ml of 1N K2Cr2O7 was added along with addition of 2mL of Conc. H2SO4 and was mixed by gentle swing. The flask was kept for 30min for reaction. After the completion of the reaction the content was diluted with 200mL distilled water and to it 10mL of standard phosphate solution was added followed by addition of 1mL of diphenylamine as an indicator. The sample was titrated with 0.4N ferrous ammonium sulphate and the end point was determined when the color changes to brilliant green. A blank was run without soil but with the same chemicals [20].

Sand, silt and clay content in the soil: In the POM by loss on ignition (LOI) procedure of Kettler *et al.* [21], soil-particle dispersion is accomplished by adding HMP, at an aqueous concentration of 0.5% by weight, and shaking the soil sample (<2 mm) for 16 h (overnight) on a reciprocating shaker at 120 reciprocations per minute in a container with a 3:1 HMP (90 mL) to soil (30 g) ratio. After dispersion, the soil slurry is sieved through nested standard 0.5-mm mesh (no. 35) and 0.053-mm mesh (no. 270) sieves to separate sand particles and POM. The collected sand particles (>0.053 mm) are dried at 55°C to constant weight, then subjected to 450°C for 4 h to measure POM by LOI. The sand percentage is based on its fraction of the original sample mass and can be calculated using the mass of sand after either 55°C or 450°C. The mass after heating to 450°C will have greater accuracy, since any organic matter will have been oxidized at the higher temperature.

During sand-particle and POM separation, the solution and particles (silt + clay) passing the sieve are collected in a bucket and then transferred to a 1-L beaker. This solution is stirred thoroughly to achieve suspension of all soil particles. While stirring, a 45-mL sub sample is collected from the suspension using a 60-mL syringe and transferred to a 50-mL centrifuge tube. The sub sample is shaken vigorously (capped tightly) and then left undisturbed at room temperature (18–24°C) with a vertical orientation for at least 90 min but <6 h to allow silt particles to settle.

After the sedimentation period, the solution containing the suspended clay is decanted into a pre-weighed drying pan. The settled silt particles are then rinsed into another pre-weighed drying pan, and both are dried at 105°C to constant weight.

Calculation

The relative proportion of silt in the dried sub sample is calculated as: This is then used to calculate the percentages of silt and clay in the original sample:

Total Sub sample mass= (oven dry silt mass+oven dry clay mass)

Sub sample silt= oven dry silt mass

Total sub sample

Sand %=(Oven dry sand mass) X 100%

(Original sample mass)

(Silt+Clay)%=100- Sand %

Silt%=Sub sample silt X (Silt+Clay) %

Clay%=100-(Sand%+Silt %)

Determination of Water Holding Capacity of the soil

The clamp stands were set up to support a rail between them. It was ensured that the base of the clamp stand was facing forwards so that they support the weight of the funnels and do not topple forwards. Sufficient clamps were attached, suitable for supporting funnels, along the rail. Analysis was done with 3 replicates of each soil and at least 2 blanks.100 ml glass funnels were taken for 3 replicates per soil sample and at least 2 blanks and numbered sequentially with a marker pen. A short length of rubber tubing (~ 8 cm) was attached to the mouth of each funnel stem. A clip was placed on each piece of tubing and closes it completely. The funnels were placed along the stand. The glass wool was weighed out in lots of 0.25-0.30 g, but of all the same weight, for the number of funnels. The glass wool was rolled into a compact ball or cylinder and was placed it in the funnel at the top of the stem. The glass wool was tamped down firmly with a narrow spatula or other suitable implement. Weighing out of 3 moist portions of 50 g soil per replicate, and was placed in the funnels. The blanks should contain the glass wool only. 50 ml measuring cylinder was placed beneath each funnel ensuring that liquid from the tube will drip directly into the measuring cylinder (cylinders can be raised if necessary).50 ml of water was poured into each funnel via a 50 ml measuring cylinder and was leave for 30 minutes to saturate the soil. It was watched carefully to see if any liquid passes past the glass wool. After 30 minutes the clips were opened on the tubing, and the water was collected that drained out from each funnel for 30 minutes in a measuring cylinder held beneath the funnel stem. The final volume of water was collected in each cylinder after this time.

Calculation

The volume of water retained by the soil has been calculated by:

50 - (Volume water retained by glass wool + volume water collected) ml = A

Note: The volume of water retained by the glass wool = 50 ml - the volume of water collected from the blanks.

2.8 To calculate soil WHC (ml water held at 100% WHC per 100g oven dried soil)

2A + MC% = WHC (ml $100g^{-1}$ fresh soil) =B ml

Then: (Bml / soil DM) x 100 = mL of water held by 100g oven dried soil at 100% WHC)

Determination of Soil Porosity

Soil porosity is defined as the total amount of pore spaces or that portion of the soil volume not occupied by air and water. The term void is used to denote the pore space. Using the 100 ml of soil in the 250 ml beaker the following was completed. A 100ml. graduated cylinder was filled up to the mark with tap water.

The water was slowly poured onto the soil in the beaker with a glass rod.Addition of water was stopped when it was stand at the same level as the soil surface. Neither the beaker was not shaken nor was the soil stirred. The amount of water used to exactly fill the pore space in the 100cc volume of the soil was determined by subtracting the reading on the side of the graduated cylinder from the original volume.

Polyhydroxy butyrate production observation

PHB production was observed after preparation of PHB medium and containing high amount of saline and carbohydrate as soul energy source. After the growth of bacteria in the PHB medium the PHB inclusions are observed as blue black droplets after staining the sample with Sudan Black B.

Gram Staining of Isolated Bacteria

Isolated bacteria were stained by Gram stain to confer there gram positive or gram negative nature, the staining procedure followed by gram staining methods.

Statistical evaluation

Each experiment was repeated at least three times. Data are presented as mean ± S.E. Significance of mean values of different parameters between the treatments groups were analyzed using Student's t test. Pairwise comparisons were done by calculating the least significance. Statistical tests were performed using Microcal Origin version 7.0 for Windows.

RESULTS AND DISCUSSION

Mangrove forest is a woody community that can be periodically submerged in seawater of the inter-tidal zone of tropical and subtropical regions [22] belonging to the family**:** *Rhizophoraceae* genus: *Rhizophora*. They are the second highest source of primary production next to rainforests. They produce high detritus and release nutrients which are a food source for variety of organism. Mangroves provide homes to variety of marine and terrestrial organisms. They act as nurseries and feeding grounds for many fish, shrimp and crustaceans and non-resident fish enter the mangroves to feed at high tide. Mangroves are also prime nesting and migratory sites for hundreds of bird species. They not only provide a wealth of biodiversity, but are an essential part of the world's food web.

Isolation of Halophilic Anaerobic Bacteria

Initially 0.2ml of the soil sample was spread in each plates but too much growth of bacteria in a single plate was observed.The experiment was again repeated with 1:10 dilution of the sample, from it about 100µL of the samples were taken and spread on each of the plates ; the plates were incubated at 37ºC for overnight incubation. Almost after 48hrs the colonies were developed in the nutrient agar plates having characteristically of yellow and blue colour in the samples of control area but after 72hrs of incubation the only bacterial colony of orange colour were developed in the soil suspension of experimental area but no growth of orange bacterial strains were observed in control area.

The Orange colour bacterial colony was isolated from nutrient agar plates of experimental area and were grown on nutrient agar plates with gradually increasing concentration of saline starting from 2.5gm%Nacl and also of CO_2 i.e.starting from 2gm%.It was observed that at 5.8%CO_2 and 5gm% Nacl the appearance of orange color bacteria had occurred within 24hrs of incubation suggesting their anaerobic nature which is been correlated with the soil profile analysis as discussed later.

Fig.1 (a): Isolation of different bacteria from the soil samples

Fig.1 (b): Bacterial colony that appears after incubation for 72hrs in normal incubator at 37°C in control area

Fig.1 (c): Bacterial colony that appears from the control site were isolated.

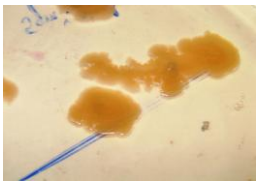
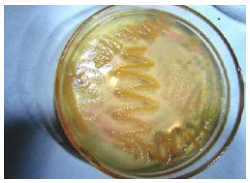


Fig.1 (d): Orange color strains identified from the experimental sites indicate the growth in normal incubation(after 72hrs) (marked by orange coloured circles).



The bacteria that grow at 5.8%CO_2 and 5gm%Nacl in 24hrs, (E) isolation of single colony from the bacterial colony mentioned in (D).

Moreover it has been proved that these bacteria are carbon sequestering bacteria as they not only grown in 5.8% CO_2 (0.03% CO_2 in atmosphere) but also they grow faster in this condition as the bacterial growth were observed after 72hrs in normal incubation where as only 24hrs in this condition truly signifies that CO_2 is beneficial and an essential component of their growth (Fig. 1A-E)

Measurement of Plant Chlorophyll content as an indicator of plant growth

Chlorophyll content in the leaf is an indicator of the plant growth, it has been observed that after the application of the bacterial broth the chlorophyll content of the saplings has been increased in the experimental area suggesting the possible role of this carbon sequestering microorganism to increment of the carbon content in the soil of the experimental area and stimulator of plant gorowth (Fig. 2A and 2B).

Assesment of Soil profile content to observe possible enviornmental impact

Hydrogen ion concentration in the soil is measured in terms of the pH scale. Soil pH ranges from 3 to 10. Pure water has a pH of 7 which is considered neutral, pH values greater than seven are considered basic or alkaline, below seven acidic. Most good agricultural soils have a pH between 5 and 7. Though acidic soils pose a problem due to their lack of nutrients, alkaline soils can pose a problem as well. Alkaline soils may contain appreciable amounts of sodium that exceed the tolerances of plants, contribute to high bulk density and poor soil structure. In this study it has been observed that both in the control and in the experimental area the soil pH does not differ too much and they are alkaline in nature but a small change has been observed in the experimental area after the application of the bacterial broth culture a decrement of the pH value is been observed i.e acidic pH of the soil in this area suggests the occurrence of the carbon utilizing anaerobic bacteria (Fig. 3A and 3B).

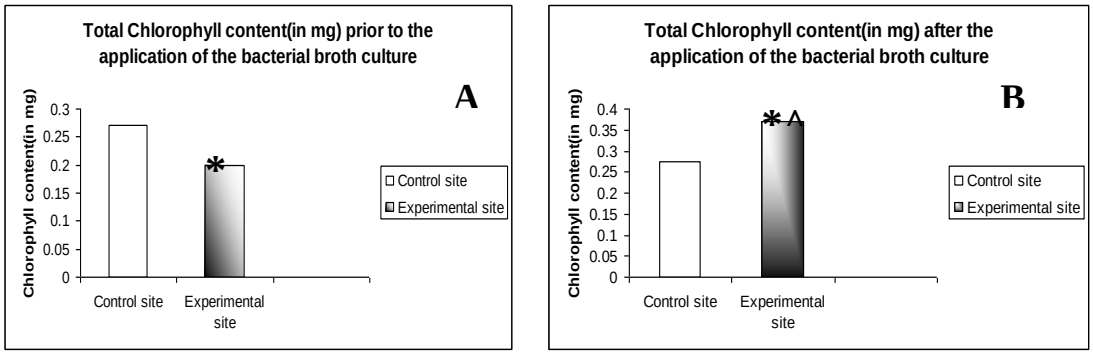


Fig. 2: Comparative Study of total chlorophyll content in the control and the experimental area prior (A) and after (B) the application of the bacterial broth culture. The values are expressed as Mean ± S.E.; * P ≤ 0.001; ^As compared to control site values using Student's t test.

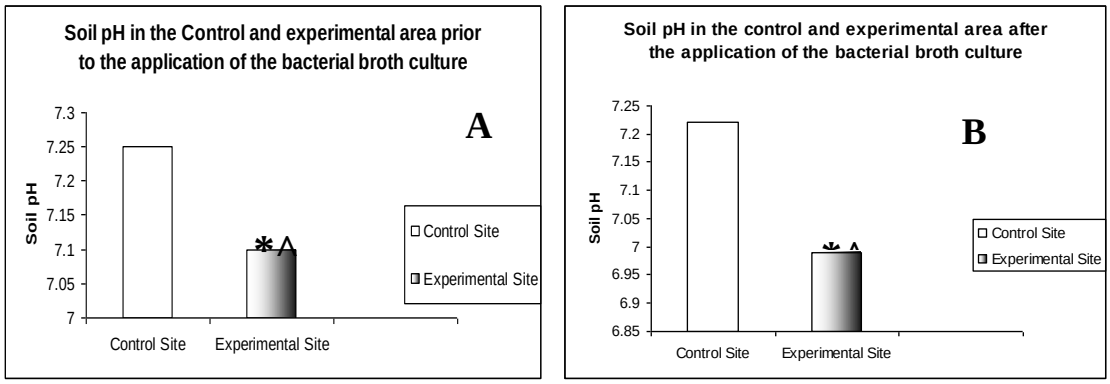


Fig. 3: Comparative study of soil pH in the control and experimental area prior (A) and after (B) the application of the bacterial broth culture. The values are expressed as Mean ± S.E.; * P ≤ 0.001; ^As compared to control site values using Student's t test.

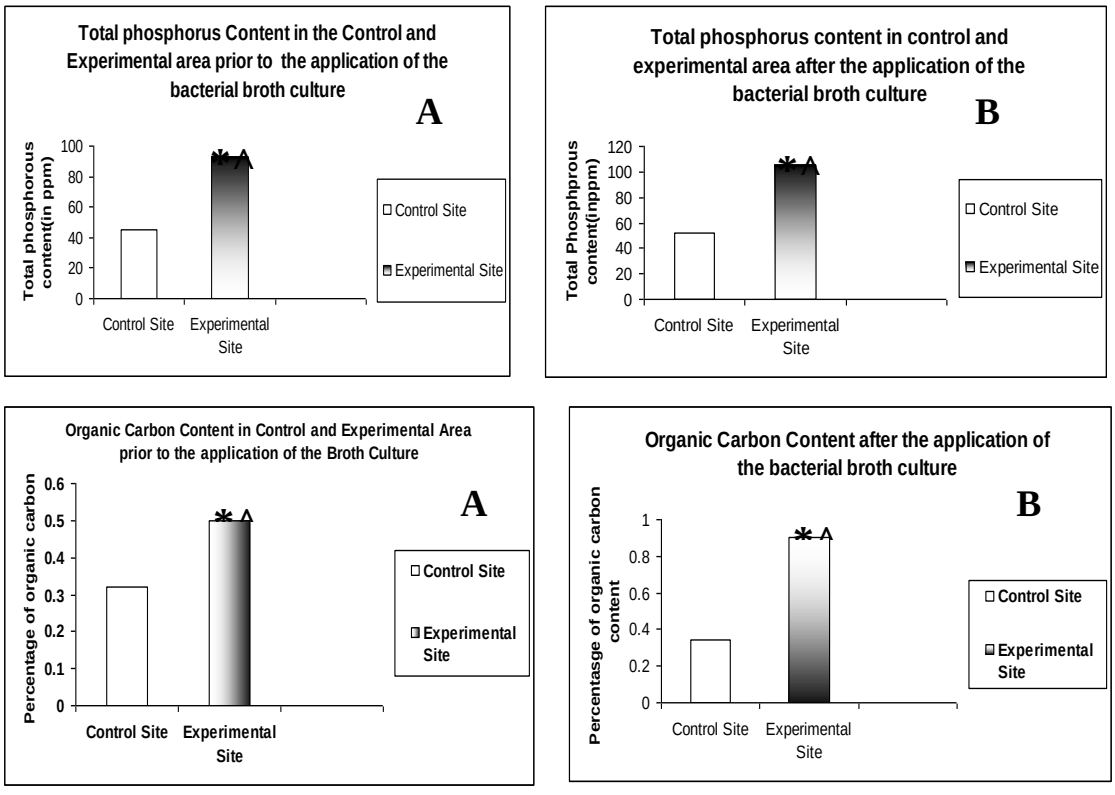


Fig. 4: Comparative study of Total phosphorous content in the control and experimental area prior (A) and after (B) the application of the bacterial broth culture. The values are expressed as Mean ± S.E.; * P ≤ 0.001; ^As compared to control site values using Student's t test.

The observations has showed that both the total phosphorous and total organic carbon content in the experimental area are greater than that of the control area, thus the experimental site is much more favorable for the growth of microorganism than that of the control area. Moreover increment of the value of the parameters in the experimental area after application of the bacterial broth gives an indication of the increment of the bacterial growth (Fig. 4A and 4B).These bacteria were grown in 5gm%Nacl suggesting their role in saline reduction of the soil which is beneficial for the growth of Mangrove vegetation. It was observed that when bacterial broth culture was applied to the experimental area, growth of mangrove saplings were stimulated (increment of chlorophyll content) suggesting their role in Mangrove plantation protection.

Moreover increment of soil carbon causing less prone to erosion, these bacteria when applied as large in number in broth culture the organic carbon has been increased suggesting that they might have an indirect role in prevention of soil erosion (Fig. 5A and 5B).

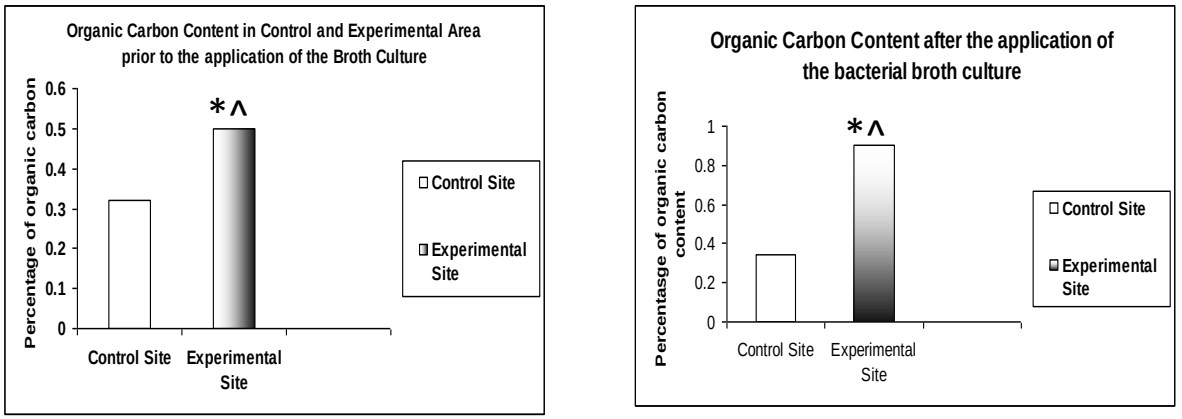


Fig. 5: Comparative study of organic carbon content in the control and experimental area prior (A) and after (B) the application of the bacterial broth culture. The values are expressed as Mean ± S.E.; * P ≤ 0.001; ^As compared to control site values using Student's t test.

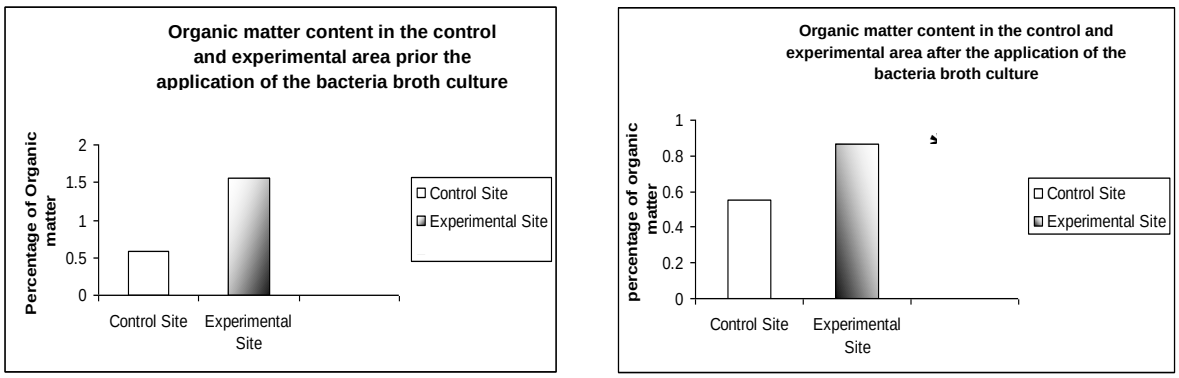


Fig. 6: Comparative study of organic matter in the control and experimental area prior (A) and after (B) the application of the bacterial broth culture. The values are expressed as Mean ± S.E.; * P ≤ 0.001; ^As compared to control site values using Student's t test.

As plant material dies and decays it adds organic matter in the form of humus to the soil. Humus improves soil moisture retention while affecting soil chemistry. Cations such as calcium, magnesium, sodium, and potassium are attracted and held to humus. These cations are rather weakly held to the humus and can be replaced by metallic ions like iron and aluminum, releasing them into the soil for plants to use. Soils with the ability to absorb and retain exchangeable cations have a high cations-exchange capacity. Soils with a high cation-exchange capacity are more fertile than those with a low exchange capacity. Soil organic matter represents a key indicator for soil quality, both for agricultural functions (i.e. production and economy) and for environmental functions (e.g., C sequestration and air quality). Soil organic matter is the main determinant of biological activity. The amount, diversity and activity of soil fauna and microorganisms are directly related to the organic matter. Organic matter, and the biological activity that it generates, have a major influence on the physical and chemical properties of soils. Aggregation and stability of soil structure increase with organic matter content. These in turn increase infiltration rate and available water capacity of the soil, as well as resistance against erosion by water and wind. Soil organic matter also improves the dynamics and bioavailability of main plant nutrient elements as after bacterial broth application increment of soil organic matter proves it beneficial role to improve soil quality (Fig. 6A and 6B).It has been observed that both in the control and the experimental area the percentage of clay, silt and sand content minute change has been occured after the application of the bacterial broth culture but in the control area percentage of sand is greater than that of the experimental area. Recalling the property of the sand it does not hold the water too much due to its lesser pore size and increment of sand percentage in the soil also makes it more porous; Regarding the growth of anaerobic bacteria in the soil more porous lesser will be favorable for the growth of them as we know percolation of water means more easier availability of dissolved oxygen with it and thus unfavours the growth of aerobic bacteria. Analysis of silt and clay content of the soil from the control and the experimental area it has been observed that both the clay and silt content in the soil are greater than that of the control site, as we

know silt is the granular material of a grain size between sand and clay derived from soil it has an adsorbing property to adhere, thus increment of clay and silt in the soil means the stickiness and hardness of the soil is increased and thus makes it less prone to errrosion. The diameter of clay is less than that of the sand and due to smaller size and large surface area it can absorb other materials and nutrients thus favors the growth of bacteria (Fig. 7A and 7B, Fig. 8A and 8B, Fig. 9A and 9B).

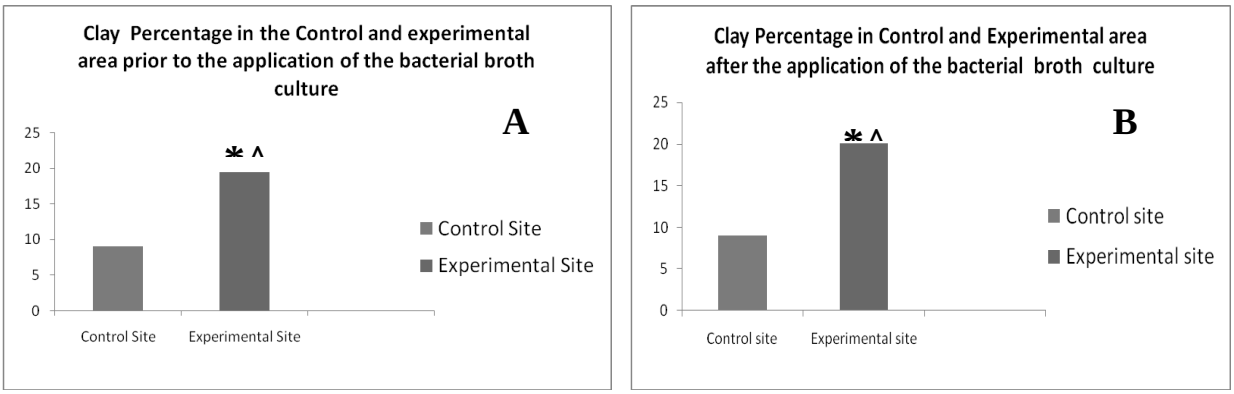


Fig. 7: Comparative study of clay percentage in the control and experimental area prior (A) and after (B) the application of the bacterial broth culture. The values are expressed as Mean ± S.E.; * P ≤ 0.001; ^As compared to control site values using Student's t test.

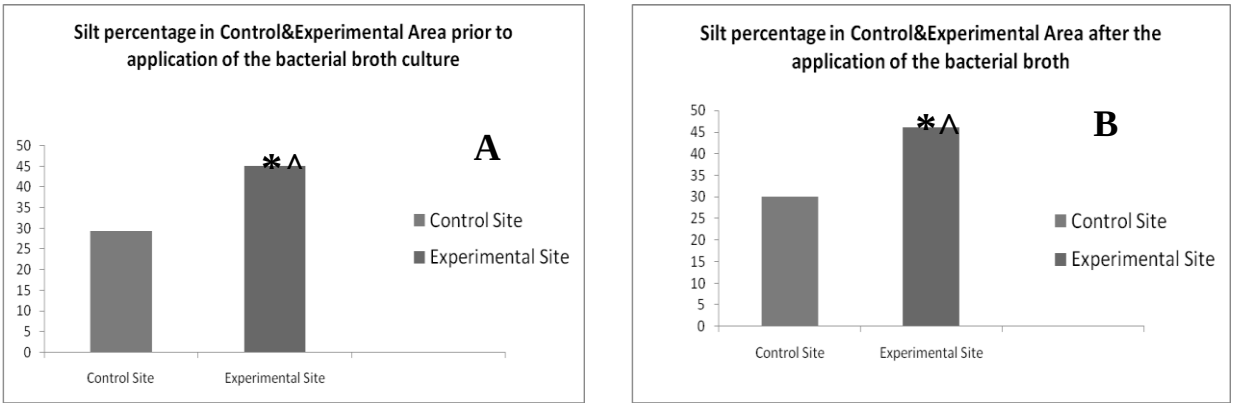


Fig. 8: Comparative study of silt percentage in the control and experimental area prior (A) and after (B) the application of the bacterial broth culture. The values are expressed as Mean ± S.E.; * P ≤ 0.001; ^As compared to control site values using Student's t test.

Regarding the water holding capacity it is been known to us that 30%Water holding capacity is essential for the growth of microbes, in the control and in the experimental area it has been observed that water holding capacity has been increased after the application of the bacterial broth culture; Moreover the water holding capacity in the control area is quite less than that of the experimental area so more the water holding capacity more the times soil holds the water and thus more decay of dissolved oxygen in water and thus favorable the growth of anaerobic bacteria (Fig. 10A and 10B) which is corelated with the growth of bacteria at 5.8%CO_2 within 24hrs as comapred to growth in normal incubation at 72hrs.

Soil texture effects many other properties like structure, chemistry, and most notably, soil porosity, and permeability. Soil porosity refers to the amount of pore, or open space between soil particles. Pores are created by the contacts made between irregular shaped soil particles. Fine textured soil has more pore space than coarse textured because one can pack more small particles into a unit volume than larger ones. More particles in a unit volume create more contacts between the irregular shaped surfaces and hence more pore space. As a result, fine textured clay soils hold more water than coarse textured sandy soils, it has been observed that soil porosity has been increased in the control area where as it has been decreased in the experimental area which might be due to growth of anaerobic bacteria and which deposits carbon and other organic material and have reduced the porous space in the experimental area (Fig. 11A and 11B).PHB staining with Sudan Black B has shown that the quite fair accumulation of PHB granules in the orange strains from experimental area indicating the role of PHB as an energy and carbon storage material accumulated in response to the limitation of an essential nutrient in high salinity [16] (Fig. 12).

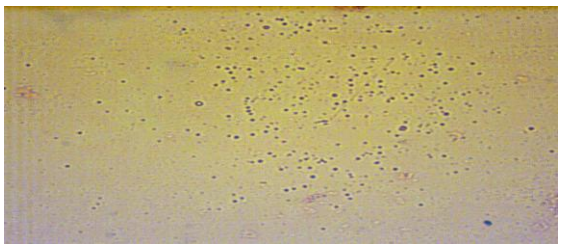


Fig. 12: Poly Hydroxy Butyrate accumulation observed in orange strains after Sudan Black B staining.

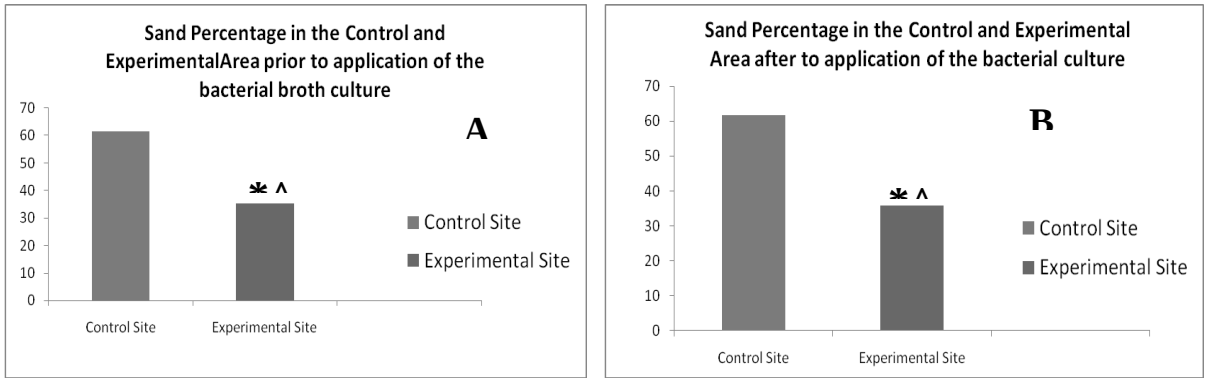


Fig. 9: Comparative study of sand percentage in the control and experimental area prior (A) and after (B) the application of the bacterial broth culture. The values are expressed as Mean ± S.E.; * P ≤ 0.001; ^As compared to control site values using Student's t test.

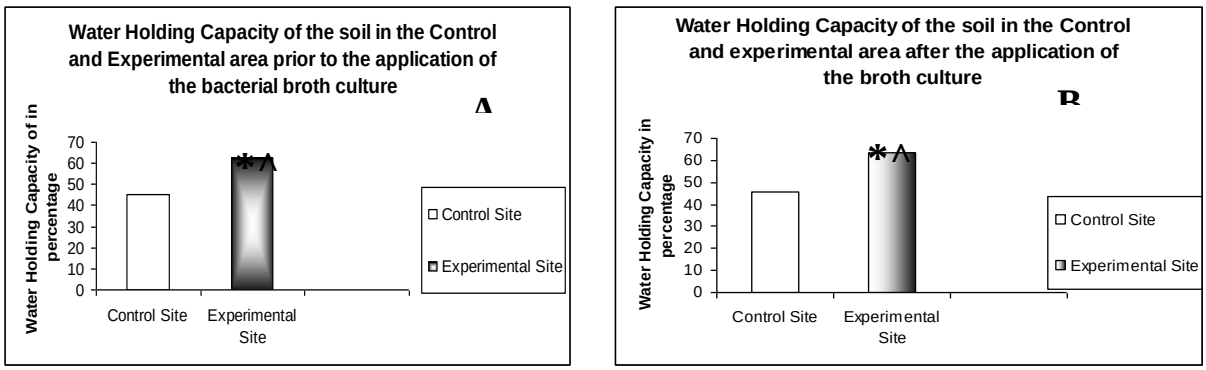


Fig. 10: Comparative study of Water holding capacity in the control and experimental area prior (A) and after (B) the application of the bacterial broth culture. The values are expressed as Mean ± S.E.; * P ≤ 0.001; ^As compared to control site values using Student's t test.

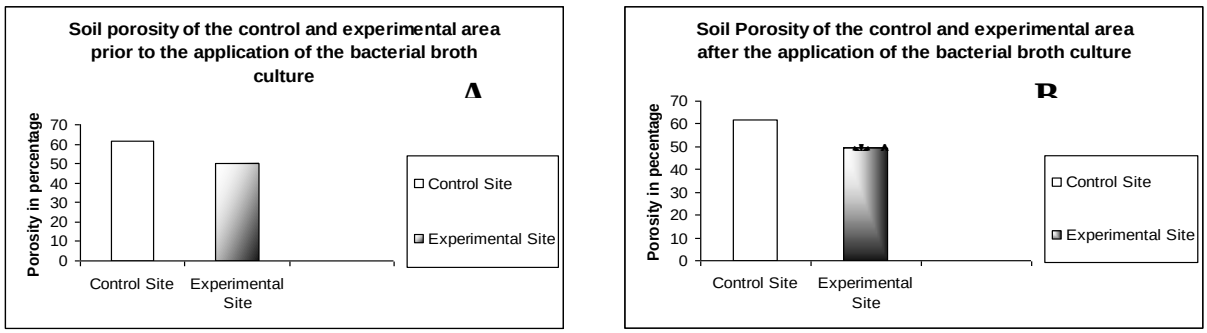


Fig. 11: Comparative study of soil Porosity in the control and experimental area prior (A) and after (B) the application of the bacterial broth culture. The values are expressed as Mean ± S.E.; * P ≤ 0.001; ^As compared to control site values using Student's t test.

Identification of Halophilic Nature of Bacteria

Possible Therapeutic Impact on Human Health

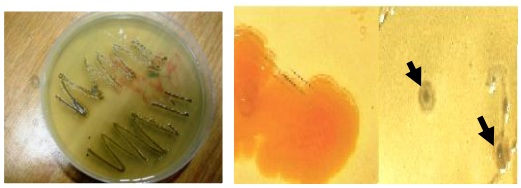


Fig.13: Inhibition of growth of *Salmonella sp*.in presence orange colour bacteria,(A)Growth of *Salomnella sp* on Bismuth Sulphite Agar and (B) inhibition of *Salmonella sp* (taken from the culture grown on Bismuth Sulphite agar fig A),arrow shows the inhibition of growth by orange bacteria when they plated together on same plate in Nutrient Agar medium.

The soil sample from both the control and experimental area was prepared by the procedure as mentioned earlier and was spreaded on different selective media like EMB, bismuth sulphide agar, appearance of bacteria in the bismuth sulphide agar proves the presence of *Salmonella sp* (Enterobacteriaceae family) in these area.It has been an interesting observation that when orange colour bacteria and *Salmonella sp* (from bismuth sulphide agar) were plated together in nutrient agar, distinct inhibition of growth was oberved in case of *Salmonella sp* species suggesting its possible beneficial role towards prevention of diseases, for eg.typhoid, salmonellosis (Fig. 13A and 13B). However no bacterial growth was observed in Mackonkey Agar maedium from the samples of both the area suggesting they are not of gram positive in nature. Mangrove forests continue to disappear all over the world. They were estimated to cover 18.1 million km^2 worldwide [23] but a more recent estimate indicates that the figure may now below 15 million km^2. The world mangrove experts are of the

opinion that the long term survival of mangroves is at great risk due to fragmentation of the habitats and that the services offered by the mangroves may likely be totally lost within 100 years [24].

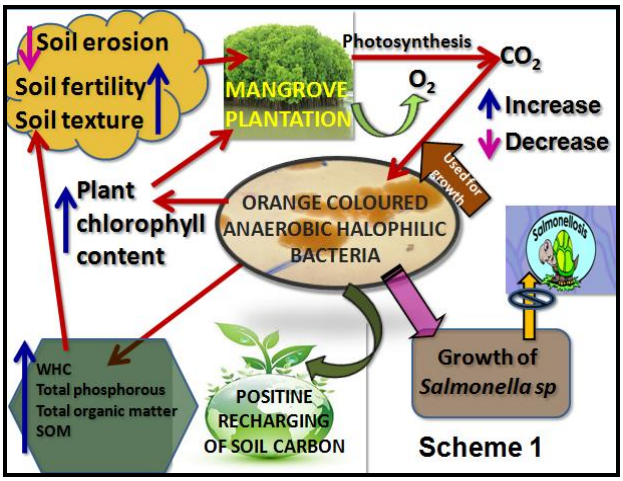
In the framework of the Kyoto mechanism, mangrove plantation is expected to be one of the options of the forestation CDM project. For the verification of a CDM sink project, it is necessary to present the land use history of project area at the end of 1989 and predict the CO_2 stock amount by the project activity. Compared to other land forest, mangrove ecosystem accumulates sequestered carbon in the sediment, thus the development of the determination and estimation methodology of CO_2 sequestration rate in mangrove sediment layer is expected.

Layers of soil and peat which make up the mangrove substrate have a high carbon content of 10% or more. Each hectare of mangrove sediment might contain nearly 700 metric tons of carbon per meter depth. When disturbed, carbon is released back into the atmosphere, further contributing to increased carbon emissions. In building large numbers of shrimp farms ranging in area from 1/2 hectare to several hectares in size, approximately of 2 meters of sediments are dug out.

The rate of loss in the recent past has reached alarmingly high rates [25]. To cite two examples, the Philippines lost 3155 km^2 of mangroves from 1968 to 1990 that was 70.4% of the initial stand, at a rate of 143 km^2 a year or 39 ha per day. The average annual destruction rate was calculated at 5.4% per year for the whole period. In Thailand the mangrove areas were lost by 1934 km^2 from 1961to 1991 and the reduction was 53% within 30 years. The average annual reduction was 65 km^2 or 18 ha per day and the average loss rate were 2.5% per year. These rates of mangroves loss are much higher than other tropical forests and coral reefs.

CONCLUSION

In conclusion it can be said carbon sequestering microorganism at the Sundarban can protect the land erosion as well as it can solve the environmental problem by protecting the mangrove and also favours plant growth (as observed from increase in chlorophyll content) and these bacteria can inhibit the growth of one of bacteria of Enterobacteriaceae family (Ex.*Salmonella sp*). Therefore, these bacteria might have the potential role in prevention and spreading of the disease caused by Enterobacteriaceae family bacteria like typhoid, shigelosis, and salmonellosis etc in these area which sometimes turns endemic.Therefore isolated bacteria not only benefits human health directly by prevention of certain diseases but also its impact on postive soil carbon recharge stimulates mangrove plantation,soil erosion prevention,soil fertility and by that way indirectly helps human habbitat by emmitting carbon di oxide,potentiate agricultural benefits.Further detailed genetic and biochemical investigation is necessary to open a new avenues.The schematic representation below will theoritically concludes the discussion.



CONFLICT OF INTEREST

There is no conflict of interest.

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