Impacts of Climate Change Induced Salinity Intrusion on Nitrogen Fixing Microbial Community of *Sesbania bispinosa* and Histo-Architecture Changes of *Eichhornia crassipes* and *Enydra fluctuans* tissues

Senior Thesis submitted as a Course Requirement for Bachelor of Science in Environmental Sciences at Asian University for Women, Chittagong, Bangladesh.

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Senior Thesis

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1. Abstract

The salinity intrusion due to the effect of global climate change is a major concern in the coastal regions of the world. It has severe effect of making surface-water and ground-water saline which hampers the ecosystems and extinctions of different species. The salinity intrusion effect on the two species of freshwater plants *Eichhornia crassipes* and *Enydra fluctuans* and a terrestrial plant species *Sesbania bispinosa* was examined in this study by introducing them in salt stress. For the terrestrial legume plant *Sesbania bispinosa,* the effect of the salinity in the survival and growth of Nitrogen fixing *Rhizobium* in the nodules of the plants was examined. Salt concentrations which were introduced in the YEMA medium were 10, 20, 30 and 40ppt and the *Rhizobium* bacteria showed effect for it. Four rhizobial strains showed growth in all the concentrations (Ses 17, Ses 18, Ses 19 and Ses 27) which define that these are high salt tolerant *Rhizobium* species and can be used to make better and sustainable manure from them. Other strains showed either growth or incomplete growth in 10 ppt of salt (Ses 20, Ses 21, Ses 22, Ses 23, Ses 24, Ses 26 and Ses 28 to Ses 30) but couldn't survive in the higher concentration of salt than 10ppt. One species (Ses 25) couldn't survive in any of the salt concentration. Beside the *Rhizobium* survivability study, the histological study in the freshwater plants showed deformity in the root and tuber tissue structure of those plants. The epidermis of the both plant's tissue was thickened at 30ppt of salt due to the osmotic pressure created by salt stress. There was expansion in the vascular bundle of those tissues which was identified at 30ppt salt which was caused due to the inhibition of the process of water up-take of those plants. These findings indicate that salinity intrusion constrains *Rhizobium* growth and causes histo-architecture changes in the root tissues of plants which can help to combat against the salinity intrusion in the coastal region countries like Bangladesh..

2. Introduction

With the rapid increase of several anthropogenic activities releasing the higher rate of green-house gases is making the world warmer day by day. This process of changing the climate system with the increase of temperature than the average one is creating global warming, thus, global climate change. Global climate change has severe effect in our environment by changing the various physical factors of the environment. The current situation of global warming has increased the surface temperature 2° Fahrenheit which is 1.1° Celsius from the late 19th century and it has greatly been greatly impacted by the increase in all the green-house gas. It is found that the main factor lying behind the huge rise in green-house gas emission is anthropogenic activities (95% of the factors). This percentage has rapidly increased in last few decades since the rise in industrialization, urbanisation, deforestation and etc. (Santer, 1996). It creates the glaciers and permafrost to melt down and rise in the sea level causing flood. It creates effect in increasing the salinity of the sea water and also puts an ample effect on freshwater aquifers. The change in the natural hydrological system and hydrodynamic because of sea level rise due to climate change can do alteration of making fresh water saline (Xiao, 2017). The salinity intrusion happens in a process where the freshwater aquifers which either don't contain any salt concentration or contain in a very little amount become rich in salinity due to the intrusion of seawater towards fresh water. In the coastal regions, the fresh-water and sea-water interference is very close to each other for which any imbalance in the fresh-water aquifer leads the seawater to intrude in freshwater. The groundwater pumping close to the coastal regions for the municipal, agricultural and industrial use cause the reduction in the freshwater level and leads the seawater intrude in the groundwater (Werner, 2013). Thus the salinity concentration increases in ground water and also in surface water level. In Bangladesh, the coastal region consists of 32% of the whole country and 35 million of people live there. The salinity intrusion has become a serious problem in the coastal areas as the salinity affected area has severely increased in the last few decades (In 1973, it was 83.3 million hectares and in 2009, it was recorded 105.6 million hectares of salinity affected area in Bangladesh). Due to the salinity intrusion in the freshwater aquifers of the coastal areas, salinity concentration has also increased in

soil which has resulted in the reduction of crop production and availability of freshwater plants (Mahmuduzzaman, 2014).

The salinity concentration increase in the freshwater bodies and ground water bodies can hamper the survival and the growth of the plants which are related to those water-bodies. It limits the plant growth through disrupting the usual osmosis function of up-taking water and minerals. It also hinders the use of the freshwater bodies and groundwater bodies as a source of irrigation. The excessive amount of salt in the water not only impacts the plant by disrupting the osmotic function, but also makes the soil-water toxic for the plants. The salt is dissolved in the water as cation Na^+ and anion Cl^- which makes the water toxic for the plants and damages the metabolic system of the plants. The metabolic disorder in the plants can cause failure to the photosynthesis system which ultimately can turn into the death of those plants. The studies in the salt stressed plants are very necessary, because the development of the germplasms of different species which are salt tolerant can help the survival of different fresh-water and terrestrial plants. It will provide the food security of a climate change affected country (Bernstein, 2017).

To understand the salt water intrusion effect on the plants two different experiments were done on different fresh-water and terrestrial plants. In those experiments, the connection between salinity intrusion and effect on aquatic and terrestrial plants were investigated. The two studies gave a better understanding of the relation between the saline water increase and histological and microbial community changes in the aquatic plants.

2.1 Objectives

I. Examining the Nitrogen fixing *Rhizobium* colonies in the roots of the plant's samples and contrasting the result after treating the plants with salt water
II. Investigating the relation between the salinity increase in water and the microbe community of the terrestrial plants
III. Investigating the histological changes in the tissues of freshwater plants due to increase amount of salinity

3. Literature Review

The direct effect of salt water intrusion due to climate change can cause severe changes in the population of different plants including both aquatic and terrestrial. In the article regarding the exposure of *Eichhornia crassipes* (Mart.) Solms to the saline water and its implications, the author investigated high salinity in *E. crassipes* plants. The author found significant physiological changes in that plant where E. crassipes population decays with the increase of salinity in the water. The decomposition process of dead plants was also investigated in this research. The author exposed the plants to saline water with tidal action to do this research (Imchen, 2017). In another article, the author describes about the salt effect on the physiological changes in plants and the ways behind affecting the plant growth. The excessive amount of salt concentration was seen in that experiment leading to toxicity of the plant for the excessive amount of Cl^- there. The toxicity and metablic disturbance in functioning inhibited the growth of some terrestrial plants (Sheldon, A. R., 2017). In the article, about the growth-promoting bacteria which confer resistance in tomato seedlings to salt stress, the author describes about the experiment which was done on the tomato plants to examine whether salt stress impacts the rhizosphere bacteria, *Achromobacter piechaudii*. The studies showed the result that the concentration of the salt concentration upto a certain level, 172 mM helped the growth of the bacterium which promoted the growth of tomato seedlings. But the reverse of this happened after the increase of more salt in that experiment causing the water efficiency increase and changes in seedlings (Mayak, 2004).

For this experiment, three different kinds of plants are used in which two plants are fresh water plants and one is terrestrial plant. The fresh water plants are used to examine the histological changes in the plant tissues due to salinity intrusion in the fresh –water body. Besides this, the terrestrial plant is used to examine the microbial changes due to salinity intrusion in ground water and also fresh-water.

The fresh water plants that are chosen in this experiment are *Eichhornia crassipes* and *Enydra fluctuans.* These plants are widely found in the stagnant water bodies in Bangladesh. As these plants are usually abundant in number, they were chosen for this experiment to see the changes.

3.1 *Enydra fluctuans* (Helencha):

Enydra fluctuans is a floating species on water which is also indentified as marshy herb. This plant can grow 2.5 to 7.5 cm long with the white flower. This plant has several uses, such as ascites, dropsy and anasarca are some field in which it is used. It is also eaten as a vegetable or food source which can help to develop a better immune system and prevent diseases. The leaves have several medicinal values and can help in curing several health problems like inflammation, bronchitis, bronchitis, and many more. It also helps in maintaining the skin care and nervous affections and besides this it is very useful in maintaining the torpidity of the human liver. As this plant's leaves are very useful, people also make some medicines from its leaf paste to reduce inflammation (Yusuf, 2007).

3.2 *Eichhornia crassipes* (Kachuripana):

Eichhornia crassipes is one of the widespread species in all over the bangalades and is identified as a weedy species. It is a floating plant which has a whitish-purple color flower and the leaves are very distinct in structure. The floating part of the plant comes from the petioles which are spongy and filled with mesophyll spongy tissues which help it to float in the water. This plant is so much abundance that it covers the water bodies and don't let other native plant to survive there. The huge abundance of it creates Eutrophication and destroy aquatic ecosystem. Thus small amount of it can be beneficial for the herbivorus fishes but the abundance of it is harmful for the water bodies (Basak et al, 2015).

The only plant which was chosen to check the salt water intrusion in the terrestrial plant in their nitrogen fixing microbial community is the *Sesbania bispinosa* which is locally called as dhaincha plant. The description of this plant is written below.

3.3 *Sesbania bispinosa* (Dhaincha):

It is a shrub type plant with dinctinct think kind stems. The stem of this plant is very useful and is used in the paper industries. It has high potentiality to serve as green manure for the crops and other kinds of plants. This is a widely cultivated and beneficial plant as it is a legume plant which has nodule to fix Nitrogen in the

atmosphere into ammonia. It also has the capacity to suppress the weeds. Besides these the plant also has medical benefits (Pugalenthi, 2004).

Sesbania bispinosa has great role in fixing the atmospheric Nitrogen which can be disturbed due to the salinity intrusion in the freshwater and also ground water level. The Nitrogen fixation process and why it is so much beneficial for the plants are described below.

3.4 The Nitrogen Fixation Process and the Significance of It:

Nitrogen is one of the most essential elements of which plants need to uptake for the photosynthesis process and also for the protein formation process. It is required in different factors and elements for the growth and survival of the plants. Also Nitrogen is needed to keep the diversity, productivity of different ecosystem. Though there is a huge amount of nitrogen present in the atmosphere but plant can't uptake the nitrogen directly from the atmosphere. The Nitrogen atoms require triple bond to form a nitrogen molecule. This bond is highly stable and hard to break it for providing nitrogen as nutrition to the plants. The nitrogen molecule is up-taken by the plants in cation forms through the process in which nitrogenase catalyze helps to break the nitrogen bond and forms hydrogen bonds with three hydrogen atoms. Thus this process actually converts Nitrogen (N_2) to Ammonia (NH_3). The conversion of ammonia helps plants to uptake the reduced form of nitrogen from the roots (Wagner, 2011). The reduction process happens as the Nitrogen molecule (N_2) breaks into two nitrogen atoms by nitrogenase and each nitrogen atom transfers six electrons. For this reason, 12 ATP is needed in this process of fixing nitrogen molecule. Then hydrogen molecule H_2 also breaks down into two atoms of hydrogen and transfers two electrons which adds up with 6 electrons of nitrogen. Thus the reduction process needs 8 electrons to get transferred and 16 ATP in total requires to be hydrolysed. So the reaction of the total process is written below:

$$N_2 + 8H^+ + 8e^- + 16ATP \leftrightarrow 2NH_3 + H_2 + 16ADP$$

(Biswas, 2014).

This reduction process can taken place in different ways i,e, applying nitrate or ammonia fertilizer in the soil, the natural lightening process and most importantly

through nitrogen fixation by microbes. The microbes i,e, *Rhizobium*, *BradyRhizobium, Azotobacter*, and some cyanobacteria helps to convert the nitrogen into ammonia without the addition of extra manure which can further pollute the environment (Wagner, 2011).

This process can be destroyed due to salinity intrusion the ground water level. Thus the effects of the salt water intrusion in these freshwater and terrestrial plants are described below:

3.5 The Effect of Salt water Intrusion on the Fresh water Terrestrial Plants:

The salinity increase in the water can cause different changes in the plants as the higher amount of salt doesn't help plant survive naturally. The effect of salinity increase can show physiological changes in plants throughout their lifetimes. It severely impacts the growth of the plants through osmotic pressure affecting the water uptake of plants and creating toxicity for specific ions. The osmotic pressure which is created in the plants due to the high salt concentration in the soil water prevents the plants from up taking the water. It further decreases the osmotic pressure in the soil which leads to more decrease in the water uptake amount. After the natural process of soil drying, the soil concentration increases in a high rate which makes it totally resistant for the plants to uptake water. Thus it inhibits plants growth, leading to the death of that plant. Some of the plants tolerate high concentration of salts and they are called halophytes, such as mangrove and other coastal plants. But some plants are very much sensitive to the salt concentration and only needs organic solutes for the growth. These plants are called glycophytes which die even in a very low salt concentration. Other than these two extreme types, most plants are in between glycophyte and halophyte which can tolerate limited level of salt concentration. Also up-taking a high level of Na^+ and Cl^- can cause toxicity for the plants by leading them the death of the leaves through chlorosis and metabolic disturbances. The high level of the salinity can damage the leaf tissue which can disrupt the photosynthesis system trough inhibiting nitrate reductase activity. It can lead to specific changes due to the dehydration and cells can die because of theses (Sheldon, 2017).

4. Materials and Methods

4.1 Isolation, Purification and the Evaluation for the Survival of *Rhizobium* Bacteria Species in Saline Medium

The *Sesbania bispinosa* plant in which the yellow flowers were bloomed those plants was taken for the experiment. An individual of *Sesbania bispinosa* was uprooted and the nodules were separated from the root carefully.

Figure 1: The photo of a legume plant, Dhaincha in BINA (Bangladesh Institute of Nuclear Agriculture) campus,Mymensingh

Among the nodules, only the nodules which were reddish in color were taken for the experiment. The picture of the root with nodules, collected from BINA experimental firm are shown below:

a

b

Figure 2(a, b): The nodules of the Dhaincha plant

The nodules from *Sesbania bispinosa* were collected from three different places which are: Bangladesh Agricultural University (BAU) campus, Bangladesh Institute of Nuclear Agriculture (BINA) experimental field, and farmers field from Khagrachari. The nodules were preserved in the scientific way of using Silica gel. The preservation technique of nodules which was used in the experiment is described below:

In this process, a glass jar was taken in which the first layer of the silica gel was placed. After that a layer of cotton was placed above the silica gel. The nodules were sandwiched between the cottons. And then the cap was tightly closed. In this way the nodules were preserved until bacterial isolation.

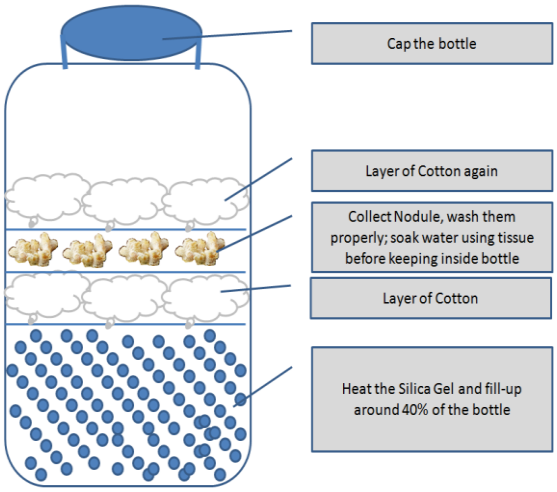


Figure 3: The preservation Process of nodules

4.1.1 Surface Sterilization and Streaking:

The nodules were preserved in a glass bottle with Silica gel. After that they were washed with water for several times in a conical flask. Then the nodules were kept in sterile water for 2 to 3 hours.

Then the nodules were washed three times with a 1.5% of NaOCl solution for 3 minutes, ethanol for 1 minute and at last they were rinsed with dH2O (distil water) for 6 times. These steps were done for sterilizing the nodules before taking them for the isolation of *Rhizobium* bacteria and-salinity tolerance analysis.

A porcelain plate was taken where there were 12 wells. In each well, a single nodule was placed and then 50µl of dH2O was placed there. After that the nodules were crushed and a suspension was created to streak in a plate containing YEMA agar medium. The plates were incubated for 24hr in 30 Celsius.

The medium preparation for the plates in which the nodule suspension was streaked is written below:

Medium Preparation:

The medium in which the suspension was streaked was “Yeast Extract Mannitol Agar”. For 1 litre of the solution, 10 grams of the Mannitol [$C_6H_8(OH)_6$] was mixed . After that 1 gram of yeast extract, 0.5 gram of K2HPO4, 0.2 gram of MgSO4.7H2O, 0.2 gram of NaCl, 18 grams of agar mixed in the solution mixed accordingly. The pH of the solution was adjusted with NaOH and HCl to make it neutral (pH = 7.00) before adding agar.

The medium was prepared again where the salt was not mixed. After that, the medium was separated in 6 conical flasks where each flask contains 15mL of the medium. To create the salinity concentration of 10ppt, 20ppt, 30ppt, 40ppt, 50ppt (ppt = parts per thousand) in a 150 ml of the medium solution, 1.5 g, 3 g, 4.5 g, 6 g, 7.5 g of salt (NaCl) were mixed.

4.1.2 Inoculum Preparation:

From the crushed nodule solution, a loop was dipped and streaked in the YEMA plates (these YEMA plates were not mixed with salt). The plates were incubated after that at 30 °C.
After the incubation of 24-48 hours, the plates were taken and the bacterial growth was observed. This gave the result of the presence of bacteria in 0ppt salt. On the basis of colony morphology, single colony was selected and preserved as a strain.

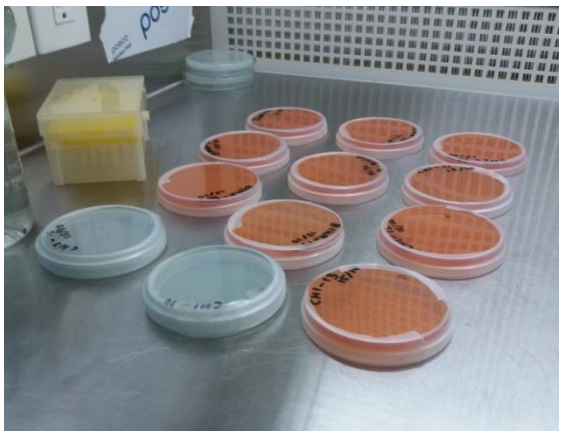


Figure 4: Streaking of bacteria in plates

Some conical flasks were taken where 25 ml of medium (YEMA medium without agar) was poured. The flasks were sealed with cotton and Al foil paper and were autoclaved. After that they were taken in LAF (Laminar Air Flow) for rhizobial inoculation.

Figure 5: Preparation of medium for broth culture

The plates from which the pure bacterial growth was identified were taken and a loopful colony or creamy portion of bacteria was taken from there. It was then mixed in the conical flask's suspension one by one. For proper optimal growth, the flasks were kept in incubator shaker and then incubated at 30 °C.

The suspensions were again brought under the LAF and the YEMA plates containing 10, 20, 30, 40ppt NaCl were also taken. The YEMA plates containing different concentration of NaCl were labelled according to the strain numbers and then those plates were inoculated where 5µl of suspension was placed in each spot. The plates were then incubated for 48 hours at 30 °C. After that the bacterial growth was examined at the plates.

Figure 6: Incubating the inoculated plates

4.2 Examination of the Structural Changes of the Root Tissues of *Enydra fluctuans* and Tuber Tissues of *Eichhornia crassipes*

The freshwater plants were collected from the place Agrabad Dhebarpara Dighi. The set-up of the experiment was done in 20J building, M.M Ali Road, Chittagong.

The plants were set-up in the bowls following aquaponic systematic way by using cork sheet and plastic cups. Other than the control one which was without any salt (0ppt), the saline concentration in which the plants were exposed was 30ppt. Replications were done of those plants to avoid the experimental error.

Figure 7: The set up of the experiment

A small portion approximately 5 cm of the tuber of water hyacinth and the root of the Helencha plant was harvested and preserved in FAA solution. The FAA solution was made according to the different proportion of ethanol, glacial acetic acid, formalin and water. The proportion is written below:

For a 20 ml of FAA solution,

Ethanol (50%)_____ 10 ml

Glacial Acetic Acid (5%)_____ 1ml

Formalin (10%) _____ 2ml

Distil Water_____ 7ml

For preserving the plant tissues of roots and tubers, some 25 ml of small bottles were taken and each bottle was filled up with 20ml of FAA solution. The plant samples were dipped into the solution and preserved for 4 days in 26° Celsius.

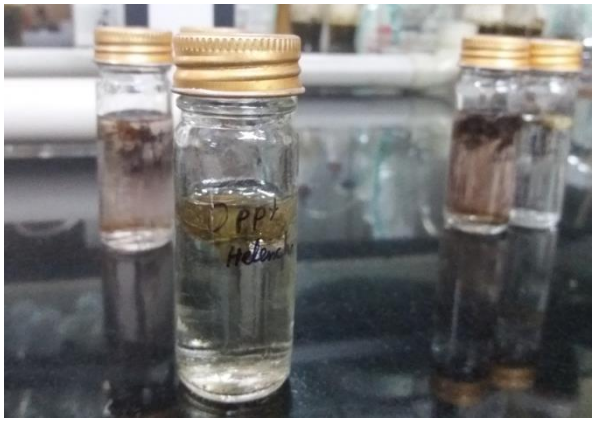


Figure 8: Preservation of Plant Tissues

After preserving the samples with FAA preservative, the manual sectioning was done. The sectioning was done using very sharp blade and was stained with Safranine and placed on a glass slide which was mounted with a drop of glycerine. After placing the sectioning in glass slides, cover slips were placed over them and were taken under microscope for observation. The tissues were observed in 10X ocular and 10X objective lens and the pictures were taken for further analysis.

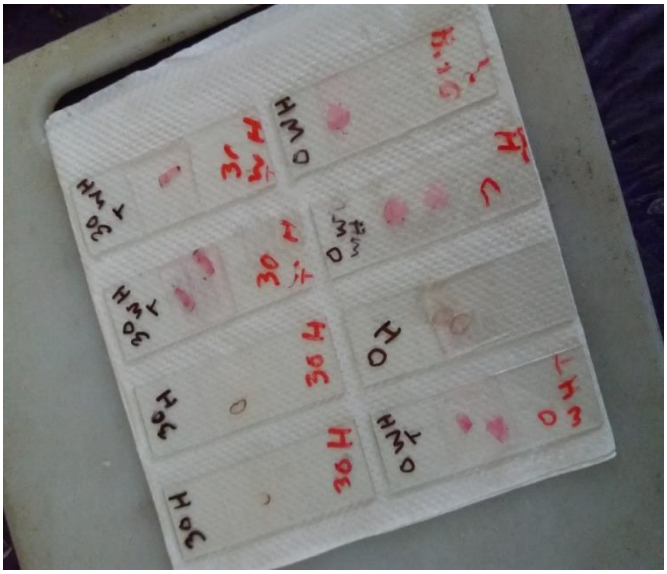


Figure 9: Tissue Slides

5. Result

5.1 Examination of the Survival of *Rhizobium* Bacteria Species in the Saline Medium

The plates which were streaked after crushing the nodules were identified with the presence of bacterial growth. Besides this, the salt exposed plates showed bacteria survival and death for different strains of *Sesbania bispinosa* after 48 hour of incubation. The result of the tolerance of salinity for the strains of *Rhizobium* of those *Sesbania bispinosa* from different locations is enlisted below:

Table1: Growth/ Death of Salinity Exposed *Rhizobium* Bacteria Collected from Different Places of Bangladesh

Stain no.	Place	0ppt	10ppt	20ppt	30ppt	40ppt
Ses 17	BINA	+	+	+	+	+
Ses 18	BINA	+	+	+	+	+
Ses 19	BINA	+	+	+	+	+
Ses 20	BINA	+	+	-	-	-
Ses 21	BINA	+	+	-	-	-
Ses 22	BINA	+	±	±	-	-
Ses 23	BINA	+	±	-	-	-
Ses 24	BINA	+	±	-	-	-
Ses 25	BAU	+	-	-	-	-
Ses 26	BAU	+	±	-	-	-
Ses 27	Khagrachari	+	+	+	+	+
Ses 28	Khagrachari	+	+	-	-	-
Ses 29	Khagrachari	+	+	-	-	-
Ses 30	Khagrachari	+	+	-	-	-

Abbreviations: BINA: Bangladesh Institute of Nuclear Agriculture, BAU; Bangladesh Agricultural University.

Here, the symbol "+" signifies the full growth of the nitrogen fixing bacteria, "-"signifies that the growth is absent in the given concentration and "±" signifies that there is doubt of the growth or survival of the bacteria. The rows which are colored (Ses 17, Ses 18, Ses 19 and Ses27) showed high tolerance to saline water by showing bacterial growth up-to 40ppt salinity.

5.1.1 Rhizobium Bacteria Tolerance at 0ppt:

The *Rhizobium* bacteria were growth in the Petri-dishes containing YEMA agar in 0 ppt of salt concentration. Some of those bacteria showed single colonies even after streaking from the nodule suspension. The photos of the *Rhizobium* growth in 0ppt are shown below:

Figure 10: Here, all the *Rhizobium* bacteria in 0 ppt showed positive result in the bacterial growth. The creamy portions are the clustered bacterial colonies and the single colonies prove the *Rhizobium* bacteria growth in 0ppt. The red color plates containing YEMA media with congo red while white plates contains YEMA without congo red.

5.1.2 *Rhizobium* Bacterial strains Tolerance at 10 ppt:

a b

Figure 11 (a,b): The strains of *Rhizobium* bacteria Ses17, Ses 18, Ses 19, Ses20, Ses21 from BINA and Ses 27, Ses 28, Ses29, Ses 30 from Khagrachari showed positive result or bacterial growth in 10 ppt of the medium. Some of the strains Ses 22 to Ses 26 which were from both BINA and BAU are showed no bacterial growth/ very poor doubtful growth of *Rhizobium* bacteria.

5.1.3 *Rhizobium* Bacteria Tolerance at 20 ppt:

a b

Figure 12(a, b): The strains of *Rhizobium* bacteria Ses17, Ses 18, Ses 19, from BINA and Ses 27, from Khagrachari showed positive result or bacterial growth in 20 ppt of the medium. Some of the strains such as, Ses 20, Ses 21, Ses 22, Ses 23, Ses 24, Ses 25 Ses

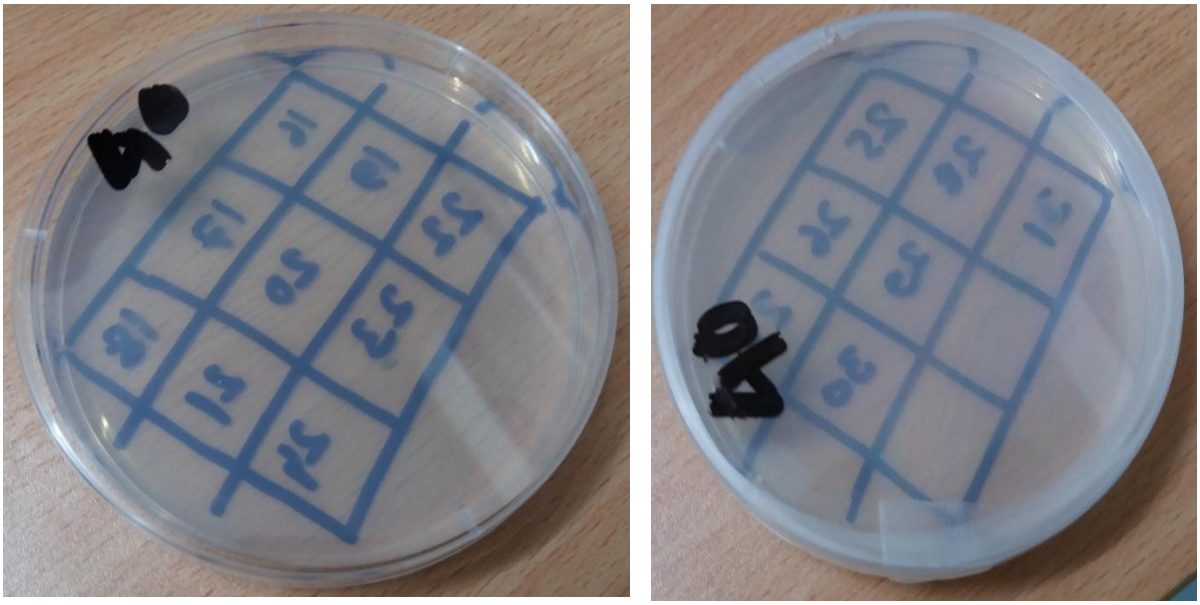
26 which were from both BINA and BAU and Ses 28, Ses 29, Ses 30 which were from Khagrachari showed no bacterial growth/ very poor doubtful growth of *Rhizobium* bacteria.

5.1.4 *Rhizobium* Bacteria Tolerance at 30 ppt:

a b

Figure 13(a, b): The strains of *Rhizobium* bacteria Ses17, Ses 18, Ses 19, from BINA and Ses 27, from Khagrachari showed positive result or bacterial growth in 30 ppt of the medium. Some of the strains such as, Ses 20 to Ses 26 which were from both BINA and BAU and Ses 28 to Ses 30 which were from Khagrachari showed no bacterial growth/ very poor doubtful growth of *Rhizobium* bacteria.

5.1.5 Rhizobium Bacteria Tolerance at 40 ppt:



a b

Figure 14(a,b): The strains of *Rhizobium* bacteria Ses17, Ses 18, Ses 19, from BINA and Ses 27, from Khagrachari showed positive result or bacterial growth in 40 ppt of the medium. Some of the strains such as, Ses 20 to Ses 26 which were from both BINA and BAU and Ses 28 to Ses 30 which were from Khagrachari showed no bacterial growth/ very poor doubtful growth of *Rhizobium* bacteria.

5.2 Examination of the Structural Changes of the Root Tissues of *Enydra fluctuans* and Tuber Tissues of *Eichhornia crassipes*

5.2.5 Root Tissues of Enydra fluctuans in 0ppt:

The microscopic photo of the tissue showed a clear view of outer layer of epidermis, cortex containing parenchyma cells, then sclerenchyma cells or blast fibre and the darker part in middle clearly showed vascular tissues xylem and phloem. The clear view of the cross section of *Enydra fluctuans* in 0ppt showed below:

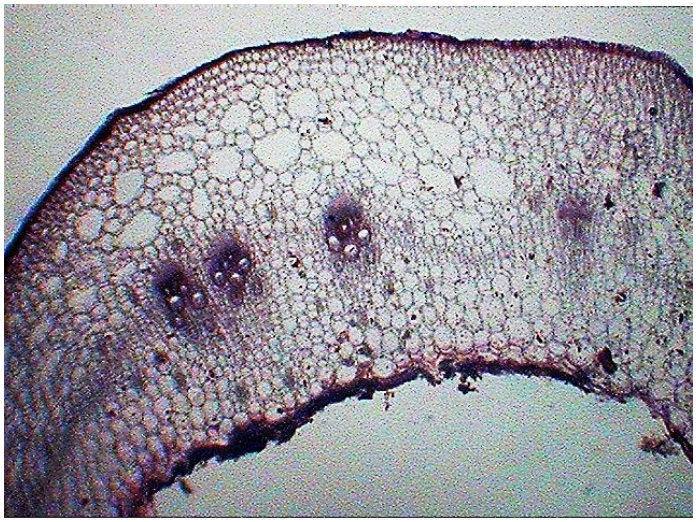
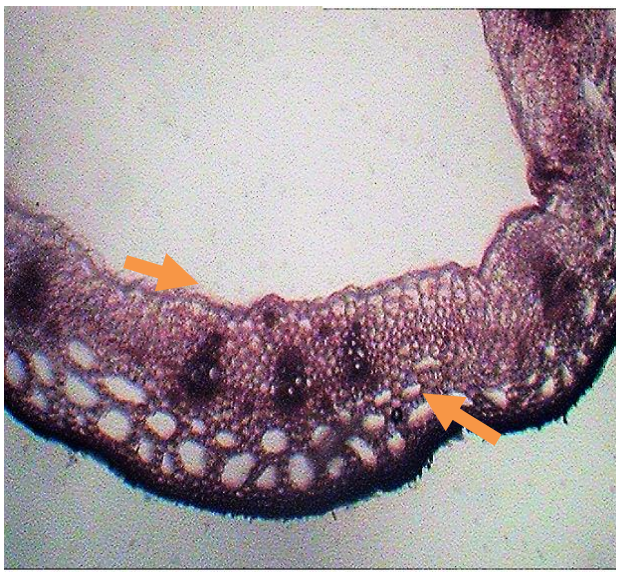


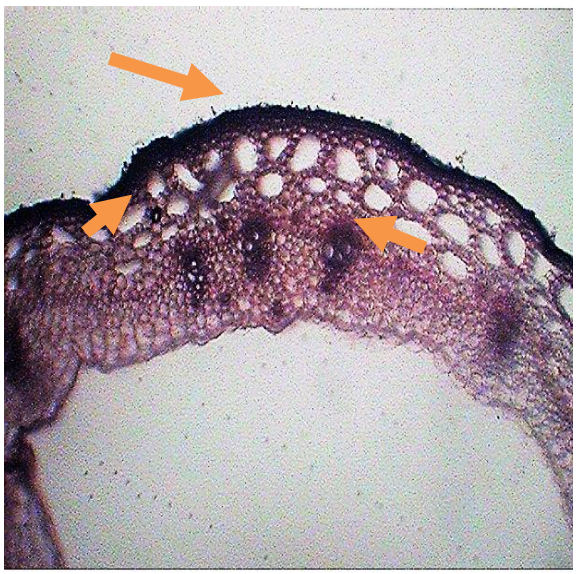
Figure 15: *Enydra fluctuans* tissue in 0ppt

5.2.6 Root Tissues of *Enydra fluctuans* in 30ppt:

There is a great comparison can be seen in the root tissue of *Enydra fluctuans* which is exposed in the 30ppt of saline water. The deformity in the cell structure is seen in the thick layer of epidermis and dense structure of the cortex. Though there is not any specific changes is detected in the xylems cells besides the diameter of xylem cells are reduced, the phloem cells are increased in number. The collenchyma are seen to be thickened in wall and densely surrounding the parenchyma cells. The microscopic view clearly shows the differences in the tissue of 30ppt from the control one. The differences are marked in the pics.



a

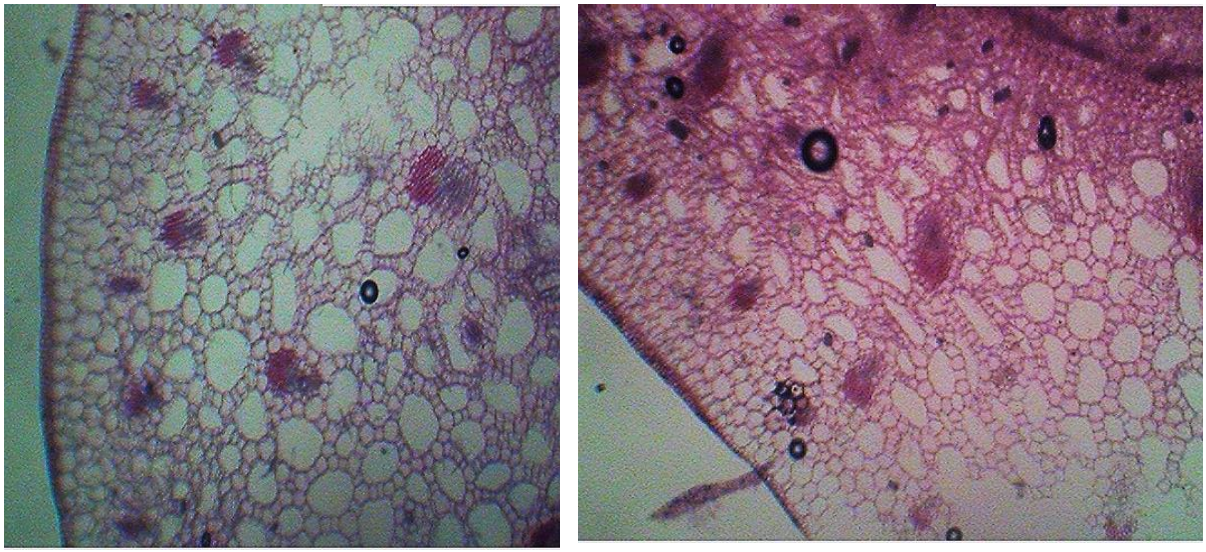


b

Figure 16 (a,b): *Enydra fluctuans* Root Tissue in 30 ppt, showing the differences in the thick epidermis, cell density and vascular bundle expansion

5.2.7 Tuber Tissues of *Eichhornia crassipes* in 0 ppt:

Under the microscope, the tissue showed a clear view of outer layer of epidermis, cortex containing arenchyma and parenchyma cells. The aerenchyma is one of the hydromorphic trait which was found in the tissue of *Eichhornia crassipes* in 0ppt. This aerenchyma is also called air spaces which were clearly indentified under the microscope. The darker part in middle showed vascular bundle including xylem and phloem which were hard to be identified due to not good sectioning. The clear view of the cross section of *Eichhornia crassipes* in 0ppt showed below:



a b

Figure 17 (a,b): Water Hyacinth Tuber Tissue 0ppt

5.2.8 Tuber Tissues of *Eichhornia crassipes* in 30 ppt:

The distinctive differences can be seen in the tuber tissue of *Eichhornia crassipes* which was exposed in the 30ppt of saline water. The changes in the cell structure showed the disruption in the epidermis cells. The thick layer of epidermis and dense structure of the cortex were identified in the tissues. The aerenchyma cells were found squeezed and not in the specific structures. But there was not any distinct changes

detected in the vascular bundle, besides the diameter of the vascular bundle was increased in number. The outer cortex are seen to be thickened in the cell wall and densely surrounding the parenchyma cells.

The microscopic view clearly shows the differences in the tissue of 30ppt from the control one. The differences are marked in the photos.

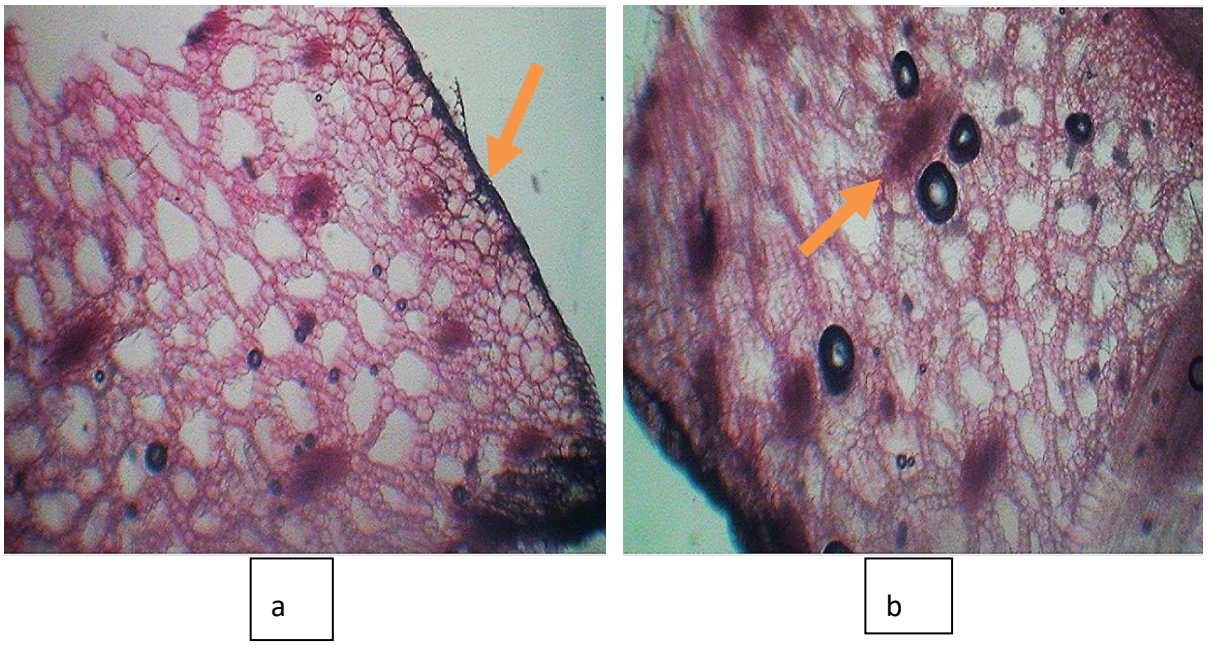


Figure 18 (a,b): Water Hyacinth Tuber Tissue 30 ppt, differences are showing in the epidermis and the vascular bundle of the tissue

6. Discussion

The salinity stress effect was clearly shown the survival of the *Rhizobium* bacteria and also the histological changes in the plant tissues. The data of the *Rhizobium* survival and the histological photos have shown the differences in the saline water from the control one. The discussions for the two different experiments are written below:

6.1 Examination of the Survival of *Rhizobium* Bacteria Species in the Saline Medium:

From the experiment, it is clearly seen that most of the strains of the *Rhizobium* bacteria died with the increase of salt concentration. Though there were poor growth of the bacteria found in Ses 22, Ses 23, Ses24, Ses 25 (bacteria showed dead in Ses 25), most of the bacteria survived in this concentration. The reason for the survival of this is the minerals found from the mixture of NaCl in a limited amount which helps the bacteria to grow. Another experiment was done by Mayak on 2004 which showed that a limited amount of salt helped the bacterial growth and further enhanced the tomato seedlings growth. The limited amount which was identified in that experiment was 172mM, after that the experiment showed that the increase of the salt inhibited bacterial growth (Mayak, 2004).

If the concentration of the salt is converted from mM to ppt, 172mM NaCl means 10ppt of NaCl concentration. Thus, the experiment with *Rhizobium* bacteria in the 10ppt salinity also showed mostly growth in the bacteria strains, and the growth of the bacteria which were unsure might be very salt sensitive bacteria or there could be some handling faults. Thus, the growth of the bacteria in 10ppt was same like the experiment which was done by Mayank on 2004 with bacteria in tomato seedlings. Also another experiment which was done by Bernstein on 2017 with the pathogenic bacteria Salmonella couldn't identify the growth changes in 30mM NaCl (Bernstein, 2017).

Besides these experiments with tomato seedlings and pathogenic bacteria, other studies which were done specifically to examine the survival of the *Rhizobium* in salinity also showed similar experimental result. The experiment was conducted by Singleton, et al. on the salinity effect on *Rhizobium* growth and in survival. In that research work, only three strains among eleven strains of *Rhizobium* species died in

different concentration of sea-water and other strains survived in the saline water. The research led to the conclusion that most of the *Rhizobium* species have potentiality of high tolerance to salinity unlikely other terrestrial legume plants (Singleton, P. W., 1982). In another research which was done by Cardoso on salinity intrusion effect on rhizobia and its impact on the nitrogen symbiosis in 2015. The research showed that the plasmids of the some strains of *Rhizobium* survived in salinity (2.1-3.6 %) which gives the connotation that they are salt tolerant, and some of the species of *Rhizobium* were extremely salt tolerant (≥5.4 %). Other than these, only few species were sensitive and some were extremely sensitive (≤0.15 %) which demonstrates that these few species of *Rhizobium* died due to high salinity (Cardoso, P, 2015). Similarly, the research which was done by Radha Krishnan on 2017 showed that some bacteria (glycophytic plants) can adapt the mechanism of fighting against salt stress and survive in the saline soil.

Therefore, the research results which were done by Cardoso and Singleton matched with the result that I have conducted. The *Rhizobium* bacteria which died in the 20, 30 and 40ppt of the salt concentration (Ses 20, Ses 21, Ses 22, Ses 23, Ses 24, Ses 25, Ses 26 and Ses 28 to Ses 30) were inhibited the cell's growth by the high salt concentration introduced there. These bacteria were not high salt tolerant for which the osmotic pressure and toxicity due to high salinity cause death in those cells of *Rhizobium* bacteria. But the *Rhizobium* bacteria which survived in the 20, 30 and 40ppt of the salt concentration (Ses 17, Ses 18, Ses 19 and Ses 27) can be marked as high salt tolerant or adapted the mechanisms to tackle against salt stress.

Besides this, a number of rhizobial strains were isolated from Dhaincha and evaluated fourteen strains for their salt tolerance in the thesis study that I have performed with my co-supervisor. We have found four rhizobial strains those are very high salt (40ppt) tolerant. Thus, these strains could be used for the preparation of bio-fertilizers for salt stress areas since they can survive at high salt concentration. These bio-fertilizers could increase agricultural productivity by enhancing biological nitrogen fixation of Dhaincha at saline conditions because the legumes as green manure can potentially increase crop productivity in saline environments by up-taking nearly 100 % of total plant N from symbiotic nitrogen fixation at salinity level 20 dS (Bruning 2015).

6.2 Examination of the Structural Changes of the Root Tissues of *Enydra fluctuans* and Tuber Tissues of *Eichhornia crassipes:*

The differences were found in the fresh water plant tissue due to salinity increase in those plants. The common histological change which was found in both *Eichhornia crassipes* and *Enydra fluctuans* was the thickening of the epidermis cells. The introduction of the salinity caused the reduction of certain necessary minerals of Ca^{+} and K^{+} which led the root cells limited from the deficit minerals. Also osmotic pressure increased in the cells of the roots of the plants which prohibited the water uptake from the soil. Thus the unfavourable condition which was created due to salinity intrusion in plants, the cell wanted to prevent it through defence mechanism of building a thicker epidermis layer and not letting salt to enter in the tissue. Similarly, an experiment was done by Shabala also describes the extension of the epidermis for the salinity effect (Shabala, 2006).

Besides the epidermis extension, the diameter of the vascular bundle also increased due to salt stress. The both plants *Eichhornia crassipes* and *Enydra fluctuans* showed the expansion of the vascular bundle in the salt stress. The reason can be described according to the experiment done by Sánchez. The xylem expansion was seen in his experiment which can happen because of lignifications and scarcity of water to uptake (Sánchez, et al, 2014). Moreover another experiment which was done by Kiegle, on the response of cells to salt in the *Arabidopsis* root showed the result that salt stress created huge differences in the cell structure. The salt concentration which was provided to the plants was 220mM NaCl. The cells of the root of the plants showed prolonged endodermis and pericycle. This prolonged structure was distinct from the other control plant's cells.

The same reason can be given behind the expansion of the xylem of vascular bundle in 30 ppt of *Eichhornia crassipes* and *Enydra fluctuans* . The water shortage due to the osmotic pressure created because salt water increase, has led the changes in the structure of the vascular bundle tissues. Thus there were distinct changes in the epidermis and vascular bundle of the freshwater was detected in this study due to high salinity. The effect of osmotic pressure and defence mechanism in the cell of those plants resulted in their histo-architecture changes. There were some resource and time limitation factors present in this study for which the histology technique with

dehydrating, embedding, sectioning with microtome and standard staining processes couldn't be followed. The limitation in this study was one of the factor which was the constraint in identifying the changes in a very cellular basis. Thus this study needs to be continued further by using new and recent techniques of histology for identifying the precise changes in the salinity affected plant tissues.

7. Conclusion

Global climate change has become one of the major problems of this century which has compelled various problems in the environment i,e, global temperature rise, ocean acidification, sea level rise, shrinking ice-sheets, salinity intrusion etc. Among these serious issues which are introduced to the environment, salinity intrusion is one of them which have affected severely the coastal regions of the world. Salinity intrusion is the process which makes the freshwater contaminated with salt load. It hampers the living beings and even can cause extinction of different species. The plants which are the producer of food in the ecosystem can be severely hampered with the increase of saline concentration in freshwater and groundwater aquifers. It will create the whole ecosystem of a place dysfunction leading to the extinction of different species. Thus the impact of the salinity intrusion was examined in this study from the microbiological and histological ways.

For the microbiological studies, the survival of the nitrogen fixing bacteria *Rhizobium* in the salinity was examined using the nodules of the legume plant *Sesbania bispinosa* (Dhaincha). The study was effective to identify the different concentration of salt stress in the *Rhizobium* survival. There were four rhizobial strains which were detected with high salt tolerant, some were low salt tolerant (survived in 10ppt) and others not salt tolerant which led to the death of those *Rhizobium* strains. Thus the result which was found in this study can help in future to choose the nodules from the legume plants which are salt tolerant. It will help to use them as a bio-fertilizer which can prove to be effective to combat with climate change and provide growth of the other plants.

Besides this, the another study which was conducted to examine the histological changes in the tissue structure of two freshwater plants *Eichhornia crassipes* and *Enydra fluctuans* showed changes in the root and tuber tissues of these plants. The

epidermis of those tissues was thickened and vascular bundle was expanded for the introduction of the saline water in the plants. A distinct type of defence mechanism was developed in the plant tissue which was acted against the salt intrusion in the tissues. Thus this study should have been done by using the standard technique of histology. It could have helped to identify more changes in the tissues and precisely in the cells of those tissues. For this reason, further research is recommended with the new techniques of plant histological examination. It will help to draw an effective conclusion of the climate change induced salinity intrusion in both freshwater and terrestrial plants.

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