JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(1), 234–247
https://doi.org/10.12911/22998993/195575
ISSN 2299–8993, License CC-BY 4.0

Received: 2024.10.12
Accepted: 2024.11.15
Published: 2024.12.01

Responses of aquatic macrophyte *Egeria densa* to saline waters and biochar amended substrate

S. M. Mahmudur Rahman[1,2*], Mahfuza Parveen[1], Mashura Shammi[2]

[1] Department of Environmental Science and Disaster Management, Daffodil International University, Daffodil Smart City, Birulia, Savar, Dhaka – 1216, Bangladesh
[2] Department of Environmental Sciences, Jahangirnagar University, Savar, Dhaka – 1342, Bangladesh
* Corresponding author's e-mail: mahmudur.esdm@diu.edu.bd

ABSTRACT

In freshwater environments, salinity is a significant environmental stressor that has an impact on aquatic plant development and survival. Elevated salinity levels, frequently originating from natural processes or human actions, can lead to diminished plant growth, poor photosynthesis, and even plant mortality. Hence, the present study aimed to analyze the salinity tolerance level of the macrophyte *Egeria densa*, assess the biochar induced growth of *E. densa* in different saline condition (5 and 10 ppt) in addition of biochar (15 and 30 gm). The increased interest in biochar as an environmentally friendly and sustainable amendment to improve plant resilience in harsh environmental circumstances is the driving force for this work. The study analyzed different water quality parameters, shoot weight gain rate (SWR), shoot growth rate (SGR), root growth rate (RGR), total chlorophyll, carotenoids, and availability of florescence dissolved organic matter (fDOM). Experimental analysis was conducted in a setup at a laboratory scale for 10 days. Slight difference was observed in water quality parameters (pH, electrical conductivity, total dissolved solids and dissolved oxygen) and the identified salinity threshold level of *E. densa* were 5 ppt. SWR (0.014 gm/day), SGR (0.14 cm/day), RGR (0.015 cm/day), total chlorophyll (0.34 mg/g fresh weight) and carotenoids (0.16 mg/g fresh weight) concentration substantially increased with the application of 5% biochar in the 5 ppt saline water. Fulvic acid, p-Cresol like substance, and Protein-like extracellular polymeric substance (EPS), and anthracene-like peaks were found in the fDOM. According to the study, biochar increased plant growth at 5 ppt salinity, however neither *Egeria densa* nor biochar reduced the salinity of the water. Biochar aided *E. densa* in adapting to salt stress by increasing shoot length, root development, and chlorophyll levels.

Keywords: salinity, biochar, *E. densa*, water quality, chlorophyll.

INTRODUCTION

Among the most abiotic stresses that affecting the plant's survival throughout the world now a days is salinity (Ali et al., 2017a; Parihar et al., 2014; Parkash and Singh, 2020; Rizwan et al., 2016; Saifullah et al., 2018). Because of the global climate change, the ocean surface is expanding, icecaps are melting, and the ocean water intruding towards the land surfaces, contaminating the sources of groundwater and soil. Halophytic plant species grow in saline water by using several adaptation methods such as resistance and tolerance (Hasanuzzaman et al., 2014; Sundararaju, 2019). A few studies conducted intensively on the distribution, exploitation, and physiology of macrophytic plant species for their salt tolerant capacity (da Silva et al., 2008; Kefu et al., 2002; Koca et al., 2007; Yensen, 2008). In recent years, attention has also turned toward biochar as a soil amendment, given its high salt adsorption capacity, which could complement the natural salt tolerance mechanisms observed in these plants (Thomas et al., 2013). Different studies have evaluated the application of biochar as a soil amendment (Agbna et al., 2017; Ali et al., 2017b; Anwari et al., 2019; Hunt et al., 2010; Nehela et al., 2021; Sun et al., 2020). A very limited number of studies have evaluated the application of biochar as a water amendment. While some studies

have explored the potential of biochar as a water amendment, research specifically addressing its impact on the saline tolerance of aquatic macrophytes, such as *Egeria densa*, remains scarce. Furthermore, the salinity threshold levels for halophytic and macrophytic species have yet to be systematically analyzed, leaving a critical gap in understanding how biochar might influence these plants' responses to saline environments. That's why, we want to examine the threshold limit of the saline water tolerance by the macrophytic plant (*Egeria densa*), and want to see the impact of biochar implication to observe the growth pattern of the macrophyte.

According to the study of (Parkash and Singh, 2020), biochar (softwood and hardwood) might be utilized to reduce the negative effects of salt stress on eggplant. This is because treatments with biochar produced greater root development, shoot growth, and fruit production than non-biochar (control) treatments. While releasing mineral elements including potassium, calcium, and magnesium into the soil solution, biochar addition decreased transient sodium ions through adsorption. Because biochar additions boosted vegetative growth, yield, and quality metrics under saline irrigation water regimes and reduced the effects of salt stressors on crop growth, they have the potential to reduce salt stress and improve tomato output. Application of biochar increased the effectiveness of irrigation water consumption. Therefore, adding biochar to soil might be a good way to ameliorate the salty soil that affects croplands since biochar application increased tomato plant height, stem diameter, fresh and dry weights, and yield components (Agbna et al., 2017). That means, quite a few works have been done on the potential of aquatic plants and macrophytes for the treatment of water, especially saline water. But, a study on the implication of biochar for the treatment of saline water is a raising study area. That means, the idea is quite new in terms of the saline water treatment. This study aimed to analyze the salinity tolerance level of the macrophyte *Egeria densa*, and to assess the biochar-induced growth of *E. densa* exposing to different saline conditions.

MATERIALS AND METHODS

A framework was followed, a constructive framework that helped the study to carry out with a methodological and systematic approach (Fig. 1).

Preparation of sand

Soil samples were collected from a construction site on the DIU_Smart City Campus (23° 52' 36.3" N, 90° 19' 20.9" E). In that dry state, the sandy soil was sieved through a No. 200 (0.075 mm) sieve to separate the fine sand. Later, the sand was washed with fresh filtered water two times and distilled water two times to remove nutrients and organisms (Imakumbili et al., 2020). Finally, the cleansed soil was dried and used in the subsequent experiments.

Preparation of saline solution

Distilled water has been used as a solvent to prepare the saline water solution for different concentrations (5, 10 and 15 ppt). 1000 ml of distilled water was heated through a magnetic hotplate stirrer at 100 ℃ for 20 minutes. When the water became light warm, 6.5, 13 and 19.5 gm NaCl (laboratory grade) were added as solutes with the warm water to prepare 5, 10 and 15 ppt saline solution, respectively (Erin, 2013). The solute dissolved using a magnetic hotplate stirrer at 500 RPM for 10 minutes. The same procedure has repeated for each saline solution preparation.

Plant cultivation and preparation

Egeria densa plant samples were collected from an aquarium of the Environmental Science and Disaster Management Laboratory, Daffodil International University. In a tank (60 × 45 × 45 cm^3), plants were cultured at room temperature (23 ± 3 ℃) with 12: 12-hour light: dark condition using fluorescent tube light (Parveen et al., 2017a). The light concentration was upheld at about 2600 flux during the plant culture and in the final experimental chamber (Parveen et al., 2022b). Plant samples for the experimental setup were attained from the tank and clipped with the apical tips (≈ 12.7 cm), as 1000 ml beaker were used for the experimental setup. The plants were rinsed with distilled water thoroughly before their plantation at the experimental beakers.

Biochar collection and preparation

Biochar was collected from the Bangladesh Biochar Initiative (BBI), made from wood chips and bamboo using Top-lit Updraft Gasifier Cookstove (TULD). The Biochar has been prepared through the cooking stove at a temperature of

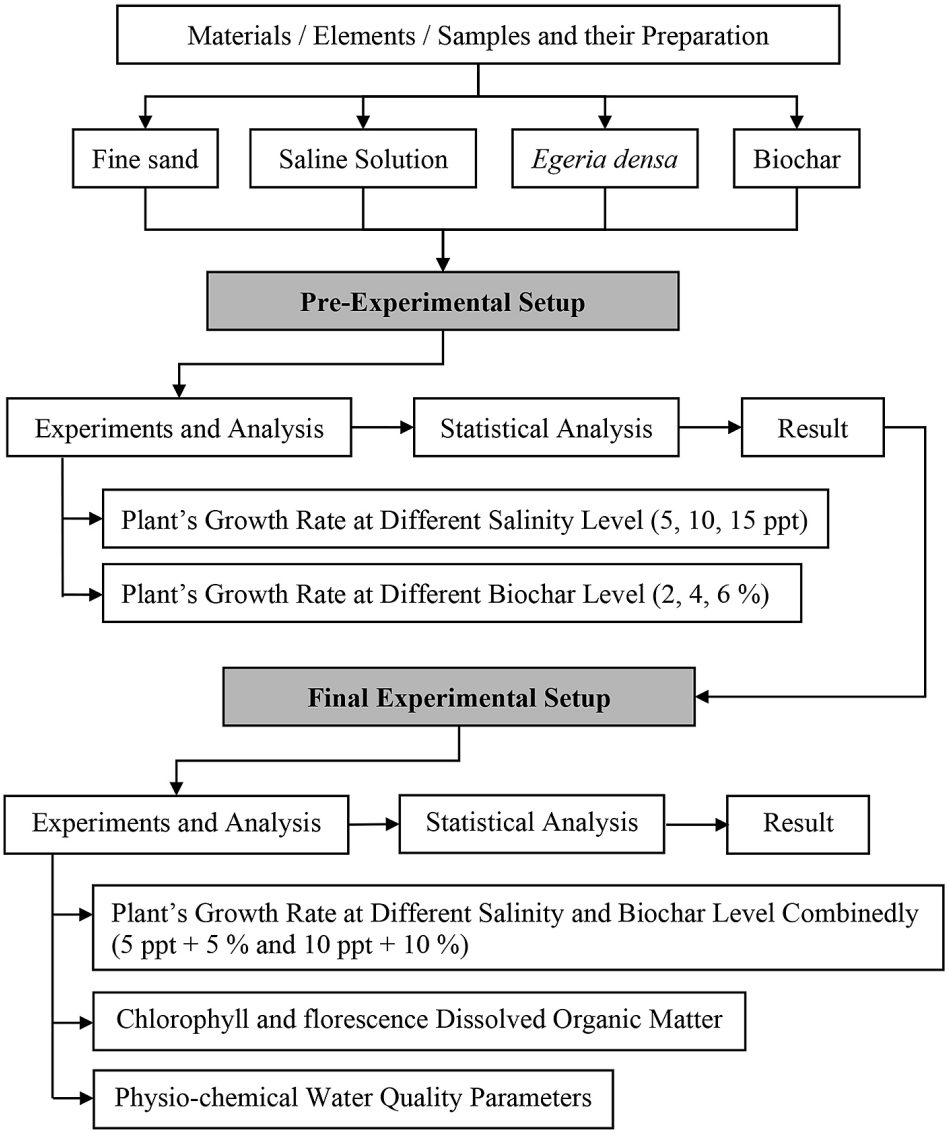

Figure 1. Conceptual framework of the study

450–750 °C with an anaerobic condition (pyrolysis) or, in restricted oxygen state (gasification) (BBI, 2022; Tasnim et al., 2021).

Plant's morphological parameter analysis

The final shoot length (stem length) and root length of the plant samples was determined using a ruler for the estimation of the plant's growth rate. The shoot growth, and root growth rate of the plants were estimated using the following equation.

$$Shoot\ or, Root\ Growth\ Rate(SGR\ or, RGR) = = \frac{Final\ Length(cm) - Initial\ length(cm)}{Time\ (Days)} \quad (1)$$

Both the shoot and root growth rates were attained from the variance between the final and initial length divided by the number of days and it was calculated in cm/day (Atapaththu et al., 2018; Parveen et al., 2019).

The final weight of the plant (stem and root) was determined using a Digital Analytical Balance (Model: FA 2004; Range: 0-200 gm; Readability: 0.1 mg) for the estimation of the plant's growth by means of biomass production. The weight gain of the plants was estimated through the following equation.

$$Shoot\ Weight\ Rate\ (SWR) of\ Plants = = \frac{Final\ weight\ (gm) - Initial\ weight\ (gm)}{Time\ (Days)} \quad (2)$$

Plant's weight gain was attained from the variance between the final and initial weight divided by the number of days and was calculated in gm/day (Parveen et al., 2022b).

Analysis of chlorophyll contents

The chlorophyll content was determined spectrophotometrically using three different wavelengths (470, 646, 663 nm) through a UV-VIS Laboratory Spectrophotometer (HACH – DR 6000). Chlorophyll was extracted into 10 ml of acetone (80%) using fresh leaves in a dark at room for 24 hours at temperature 23 ± 3 °C (Hu et al., 2013; Mur et al., 2010; Pechar, 1987; Rashid et al., 2010). Chlorophyll a, b, carotenoids, and total chlorophyll (a+b) were estimated through the following equations by (Porra et al., 1989).

$$Chlorophyll - a = 12\ (Abs_{663}) - 3.11\ (Abs_{646}) \quad (3)$$

$$Chlorophyll - b = 20.78\ (Abs_{646}) - 4.88\ (Abs_{663}) \quad (4)$$

$$Carotenoid = \frac{1000\ (Abs_{470}) - 3.27\ (Chl\ a) - 104\ (Chl\ b)}{229} \quad (5)$$

$$Chlorophyll\ (a+b) = 17.67\ (Abs_{646}) + 7.12\ (Abs_{663}) \quad (6)$$

The chlorophyll content was measured in mg/g FW (fresh weight) unit (Parveen et al., 2017b). Where Abs represents the absorbance of the wavelengths in the equation. OPTIKA Microscope – Italy (4083.B1) were used for the observation of the leaf status and for the imaging as well. The scale was 2000 µm at 10× and 500 µm at 40× magnification.

EEM spectra analysis

A fluorescence spectrophotometer (F – 4600, HITACHI, Japan) in scan mode with a 700-voltage xenon lamp at room temperature was used to capture the water sample's three-dimensional fluorescence (excitation-emission matrix, 3D-EEM) spectra. Using an excitation range of 200–400 nm and an emission range of 250–500 nm by every 1 nm, the EEM spectra were obtained in steps of 5 nm. Bandpass values of 5 nm were chosen for the excitation and emission slits, respectively. 1200 nm min^{-1} were scanned every second. Prior to analysis, Milli-Q water was used to rinse each cuvette. The EEM spectrum of the sample was subtracted from the Milli-Q water blank spectrum. Using FL solution software and 3D-EEM spectra created by Sigmaplot 10.0, fluorescence peak data were acquired, and the peaks were then identified (Guo et al., 2016; Niloy et al., 2022, 2021; Yu et al., 2020; Zhu et al., 2022).

Determination of the physio-chemical properties of water

The water qulaity parameters were determined in electrometric method. pHep tester – pocket pH tester (HI 98107) was used for the determination of pH. The water salinity was determined through the portable refractometer (REF 211). *HANNA* GroLine pH/EC/TDS/temperature multipara meter probe (HI9814) was used to measure the TDS value of the water sample separately. *HANNA* EC tester (HI98304) was used to measure the EC value of the water sample separately. Lutron DO meter (D0 – 5509) was used to determine the dissolved oxygen content of the water samples (Mili et al., 2024; Parveen et al., 2022a; Pasha et al., 2023, 2022; Rezwan et al., 2022).

Statistical analysis

The experimental data were presented as the mean ± SD (n = 3). Before doing the statistical analysis, the data were examined for normality. The data were analyzed using one-way ANOVA to determine mean differences at the 0.05 significance level. Pearson's correlation coefficients were calculated between total chlorophyll content and SGR. The data were analyzed using Excel 2016 for Windows.

Pre-experimental setup and analysis with result

That phase was conducted to understand the saline water and biochar effect on the *E. densa*, separately. That pre-experimental phase has been conducted for seven (07) days and monitored every day. After 7 days, plant samples were collected from each beaker for further analysis.

Each beaker was filled with 300 gm of prepared sand and applicable biochar concentration mentioned in Table 1. Three plants were clipped (≈ 12.7 cm) for each beaker with their apical tips and planted in a 1000 ml glass beaker (≈ 18.5 cm). Initial morphological parameters (plant length, weight, root number and length, branch number and length) of the plants were determined and recorded. Beakers were filled with 1000 ml applicable solutions mentioned in Table 1, and distilled water were used to fill the evaporation gap of water in the beakers (Alam and Hoque, 2017; Parveen et al., 2022a).

In the SP1 beakers, the plant was survived, and the plants length was increased as well (Fig. 2b). But the plants weight has decreased in all the beakers (Fig. 2a). On the other hand, in the SP2 and SP3 beakers, the plants length was stunned. Means, salinity causes the decrease of the plants

Table 1. Setup for the pre-experimental phase (n = 3)

Control (C)	Saline solution with slants (SP)			Biochar with slants (BP)		
	5 ppt	10 ppt	15 ppt	2%	4%	6%
C1	SP1a	SP2a	SP3a	BP1a	BP2a	BP3a
C2	SP1b	SP2b	SP3b	BP1b	BP2b	BP3b
C3	SP1c	SP2c	SP3c	BP1c	BP2c	BP3c

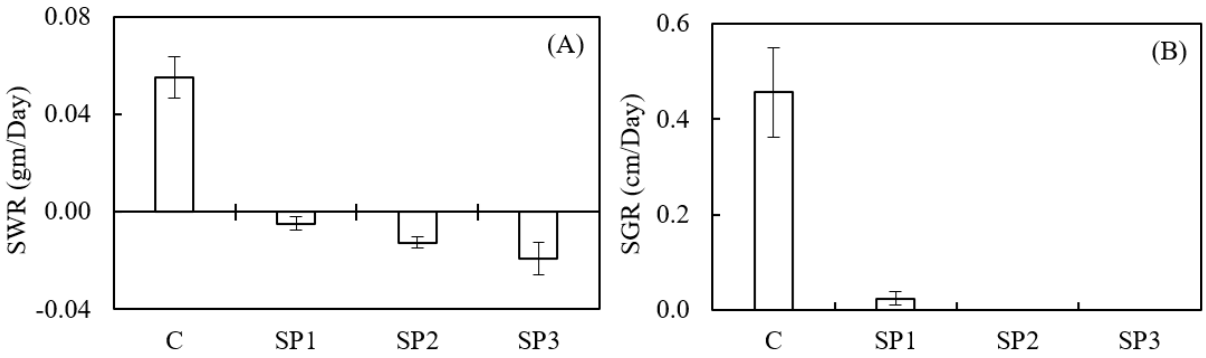

Figure 2. Plant's growth rate at different salinity level: (a) SWR, (b) SGR

weight and as salinity level increased the weight of the plants was decreased gradually. Also, except SP1, in all other beakers, the plants died (Fig. 2).

In BP1, the plants weight and length has increased significantly (Fig. 3a, 3b), and root was formed as well (Fig. 3c). In BP2 and BP3, the plants weight and length increased as well but not as BP1, and root also formed in BP2 beakers but not as significant as BP1. No root was formed in BP3. Means, biochar helped the plants to increase their weight and length, and root formation as well, but plants weight and length was decreased as biochar concentration level increased (Fig. 3). So, the fact is biochar had a significant impact on the plant's growth.

Final experimental setup and analysis

The final experimental setup was designed based on the outcome from the analysis of the pre-experimental phase of the study. That final experimental phase was conducted for ten (10) days and monitored every day. After 10 days, plant and water samples were collected from each beaker for further analysis. Each beaker was filled with 300 gm of prepared sand with applicable biochar concentration and filled with 1000 ml applicable solutions mentioned in Table 2. All other preparation were done following the procedure described before. The parameters and properties that has been analyzed are mentioned in the Table 3.

RESULTS AND DISCUSSION

Result for final experiments and analysis

The status of pH, salinity, EC, TDS and DO in the beakers were determined each day of the experimental phase (10 days) to observe any

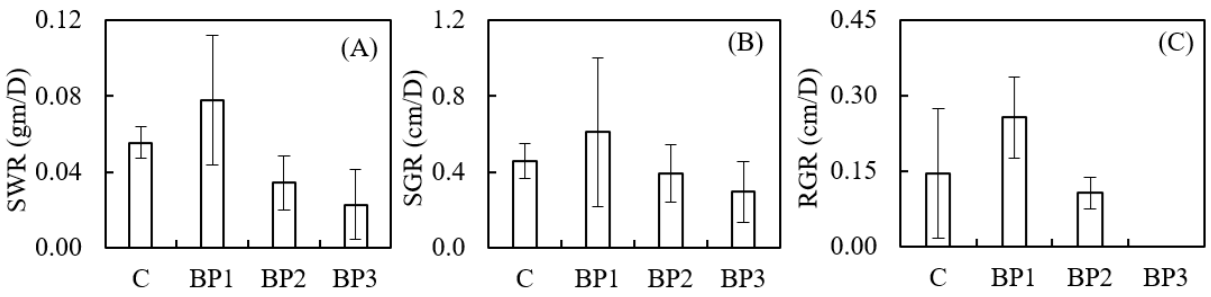

Figure 3. Plant's growth rate at different biochar level: (a) SWR, (b) SGR, (c) RGR

Table 2. Setup for the final experimental phase (n = 3)

Sample beakers	300 gm sand in a beaker with
C1	Distilled water (0 ppt)
P1	Distilled water (0 ppt) + plants
B1	Distilled water (0 ppt) + 5% biochar (15 gm)
BP1	Distilled water (0 ppt) + 5% biochar (15 gm) + plants
C2	Saline water solution (5 ppt)
P2	Saline water solution (5 ppt) + plants
B2	Saline water solution (5 ppt) + 5% biochar (15 gm)
BP2	Saline water solution (5 ppt) + 5% biochar (15 gm) + plants
C3	Saline water solution (10 ppt)
P3	Saline water solution (10 ppt) + plants
B3	Saline water solution (10 ppt) + 10% biochar (30 gm)
BP3	Saline water solution (10 ppt) + 10% biochar (30 gm) + plants

Table 3. Final experimental analysis for the final experiment phase

Analysis	Parameters/Properties
Physicochemical water quality	pH, salinity, EC, TDS and DO
Plants morphological parameters	Height, weight, and root length
Chlorophyll content	Chlorophyll a, b, (a+b) and carotenoids
Fluorescent dissolved organic matter (fDOM)	EEM spectra analysis

significant changes in the samples. The measured water quality parameters data for the initial (before starting of the experiment) and final (after 10 days of the experiment) stage are given in the Table 4. There were no substantial changes detected among all the samples in the experimental setup.

Egeria densa can be able to survive and grow well in the treatment of 5 ppt saline water (P2, BP2) and eventually die in the treatment of 10 ppt saline water (P3, BP3) (Fig. 4). A substantial increase in shoot weight gain rate (SWR, calculated by gm/day) at BP2 was identified when the macrophytes exposed to 5 ppt saline water with 5% of biochar. A substantial decrease (SWR and SGR) was observed when the macrophytes exposed to 10 ppt saline water with 10% of biochar (P3, BP3)

Table 4. Analysed water quality parameters before starting and after finishing of the experiment (10 days). The results expressed as Mean ± SD, where n = 3, I = initial, F = final

Sample	pH (Mean ± SD)		Salinity (ppt) (Mean ± SD)		EC (mS/cm) (Mean ± SD)		TDS (ppm) (Mean ± SD)		DO (mg/L) (Mean ± SD)	
	I	F	I	F	I	F	I	F	I	F
C1	7.00	7.30 ± 0.17	0	0	0.02	0.11 ± 0.02	10	103 ± 6	7.80	7.90 ± 0.36
P1	7.00	8.73 ± 0.31	0	0	0.02	0.13 ± 0.02	10	110 ± 10	7.80	6.80 ± 0.10
B1	7.00	8.53 ± 0.25	0	0	0.02	0.27 ± 0.05	10	200 ± 26	7.80	5.70 ± 0.17
BP1	7.00	8.80 ± 0.20	0	0	0.02	0.32 ± 0.05	10	240 ± 20	7.80	7.70 ± 0.30
C2	6.60	7.73 ± 0.50	5	5 ± 0	12.74	13.10 ± 0.26	6160	6720 ± 17	7.00	7.60 ± 0.20
P2	6.60	8.10 ± 0.36	5	5 ± 0	12.74	13.30 ± 0.17	6160	6753 ± 23	7.00	6.70 ± 0.10
B2	6.60	8.30 ± 0.26	5	5 ± 0	12.74	13.20 ± 0.20	6160	7080 ± 20	7.00	5.63 ± 0.06
BP2	6.60	8.30 ± 0.17	5	5 ± 0	12.74	13.45 ± 0.33	6160	6993 ± 12	7.00	7.60 ± 0.26
C3	5.60	7.90 ± 0.26	10	10 ± 0	24.5	25.90 ± 0.20	17010	17997 ± 38	5.00	7.40 ± 0.26
P3	5.60	7.90 ± 0.20	10	10 ± 0	24.5	24.32 ± 0.19	17010	17520 ± 20	5.00	6.40 ± 0.10
B3	5.60	8.00 ± 0.30	10	10 ± 0	24.5	24.82 ± 0.19	17010	17280 ± 26	5.00	5.60 ± 0.26
BP3	5.60	8.10 ± 0.26	10	10 ± 0	24.5	27.02 ± 0.28	17010	18717 ± 31	5.00	7.40 ± 0.36

(Fig. 4A, 4B). No root formation was observed in the beaker with only the 5 ppt saline water (P2). But, at BP2, where 5% biochar were applied with 5 ppt saline water, two (02) roots were observed to form that grow 0.45 cm in combine in 10 days of the experiment (Fig. 4C). Means, the macrophytes were adopting with the salinity stress and the biochar were supporting the plants to grow their root in salinity concentration of 5 ppt. Besides, the SGR and RGR ratio also backing-up the outcome in the experiment (Fig. 4D).

Higher salinity increases the stress on *Arbidopsis thaliana* plant. Lower salinity stimulates the growth of the plants through improving the assimilation process of Carbon (C) and Sulfur (S), and influences the plant's metabolism in a decent manner (Hongqiao et al., 2021). Plants receiving higher concentration of salinity resulted into the reduction in shoot weight and shoot growth compared to the plants which were exposed at lower concentration of salinity (Lee and Van Iersel, 2008).

The concentration of total chlorophyll was almost similar in P1 (0.15 mg/g FW), P2 (0.16 mg/g FW) and BP1 (0.35 mg/g FW), BP2 (0.34 mg/g FW), separately (Fig. 5A). Also,

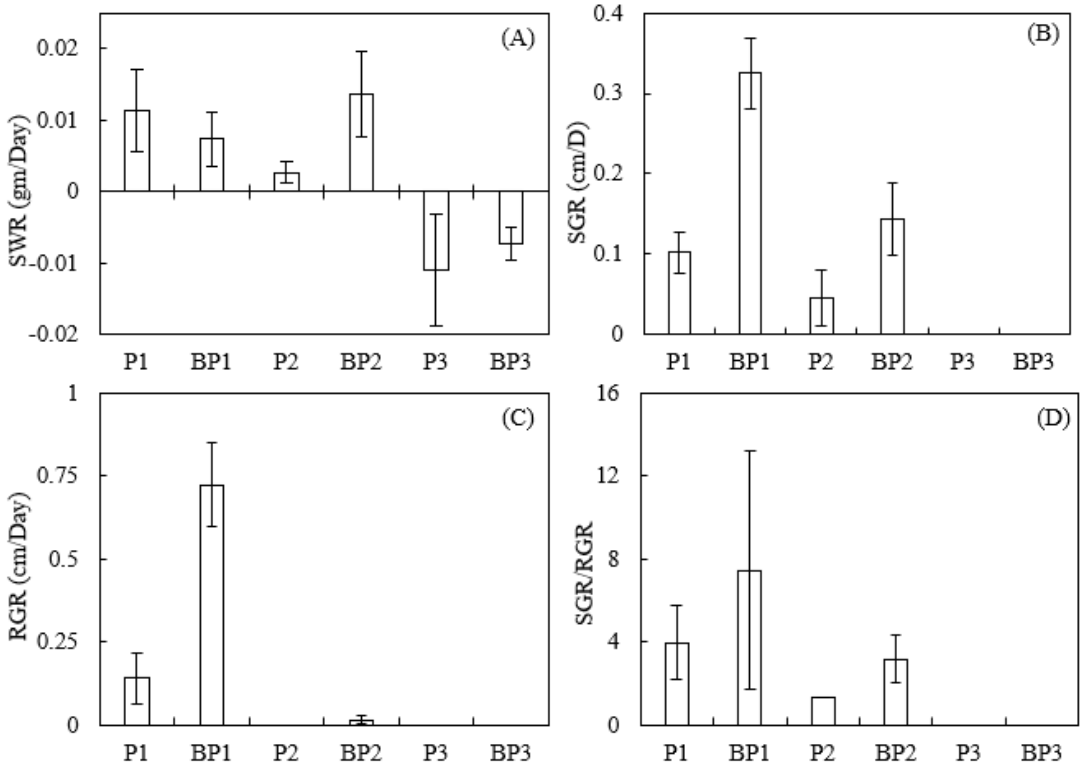

Figure 4. Morphological parameters of *E. densa* after 10 days of the experiment (n = 3): (a) SWR, (b) SGR, (c) RGR, (d) SGR/RGR

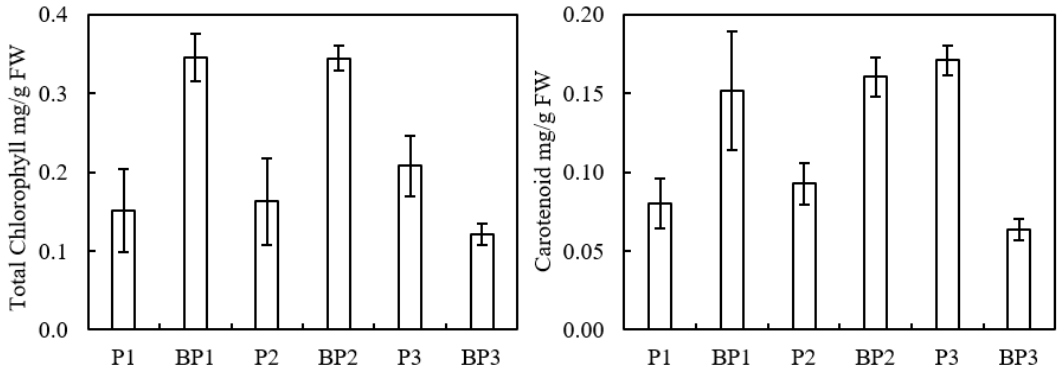

Figure 5. Status of the chlorophyll contents on the plants of the sample: (a) total Chlorophyll, (b) carotenoid

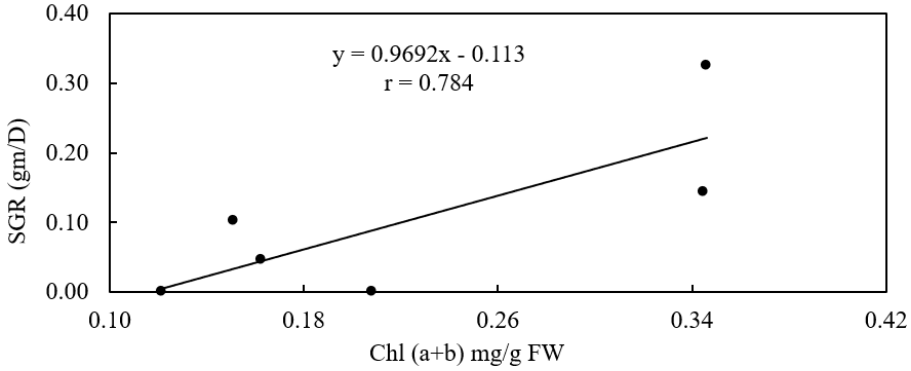

Figure 6. Correlation between the shoot growth rate and chlorophyll (a + b)

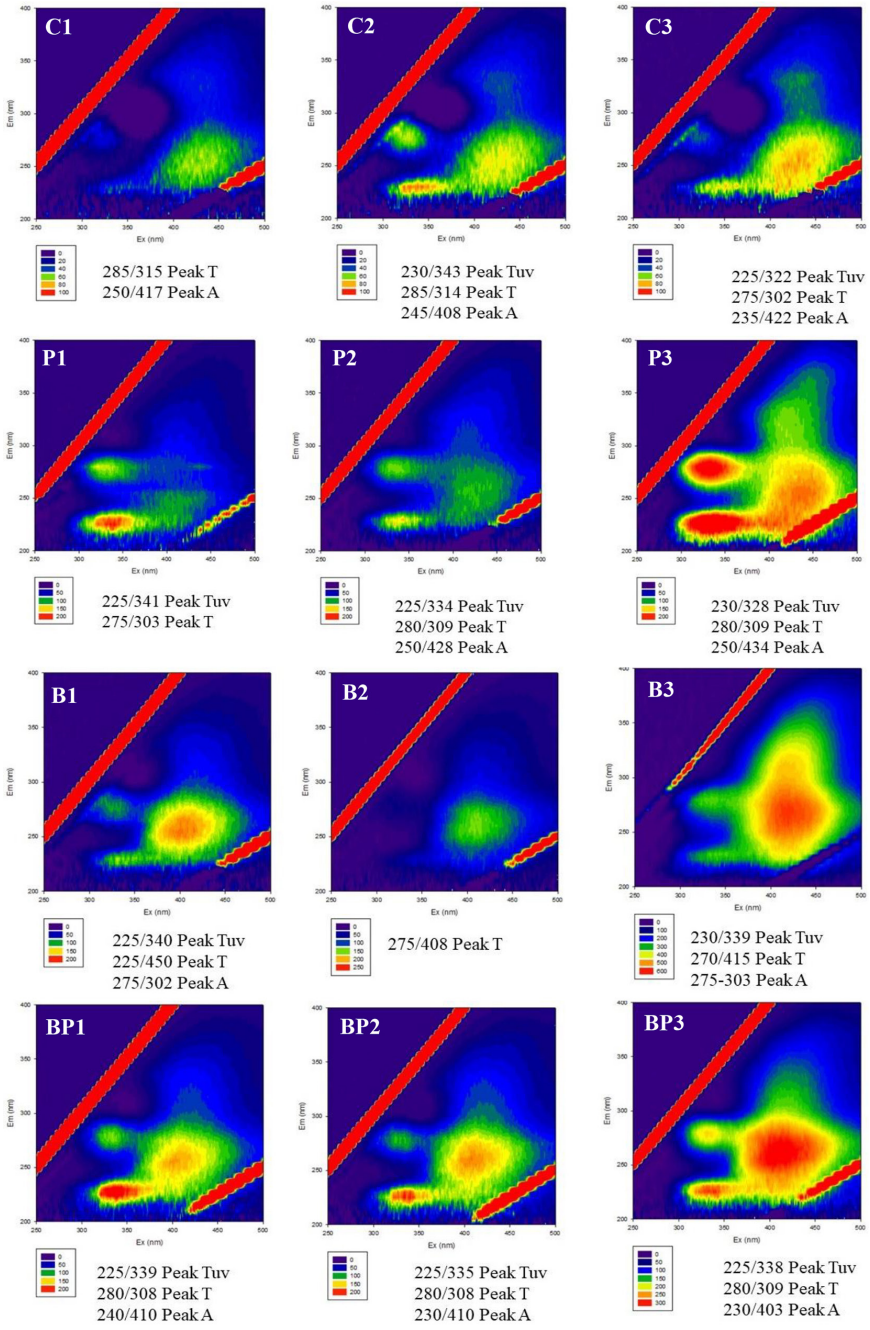

Figure 7. Analysis of the fluorescent spectra for C, P, B and BP samples

the concentration of carotenoids was almost similar in P1 (0.08 mg/g FW), P2 (0.09 mg/g FW) and BP1 (0.15 mg/g FW), BP2 (0.16 mg/g FW), separately (Fig. 5B). These observed results indicates that the macrophytes in 5 ppt saline water solution act well in terms of keeping balance of the total chlorophyll and carotenoids content in the macrophytes of P2 and BP2, where 5% of biochar were applied. Means, biochar support macrophytes to adopt with the salinity stress and balance the total chlorophyll and carotenoid contents (Fig. 5).

As salinity increases gradually the chlorophyll content decreased in equal manner. It happens when salinity influences plants metabolism and stunted the growth (Heidari, 2012; Taïbi et al., 2016). According to (Oo et al., 2017; Parry et al., 2014), there was no influence of salinity in the increase or decrease of chlorophyll content in the plant's leaf until there is no influence on

Table 5. Fluorescence excitation/emission (Ex/Em) wavelengths interpretation of different peaks with reference substances

Sample	Possible substances	Peak position Em/Ex (nm)	Peak region	Reference
C1	p-Cresol like	285/315	Peak T	(Mostofa et al., 2013)
	Fulvic acid type	250/417	Peak A	(Schwede-Thomas et al., 2005)
C2	Protein-like	230/343	Peak Tuv	(Liu and Fang, 2002)
	p-Cresol like	285/314	Peak T	(Mostofa et al., 2013)
	Fulvic acid type	245/408	Peak A	(Schwede-Thomas et al., 2005)
C3	Tryptophan like	225/322	Peak Tuv	(Mostofa et al., 2013)
	o-Cresol like	275/302	Peak T	(Mostofa et al., 2013)
	Fulvic acid type	235/422	Peak A	(Schwede-Thomas et al., 2005)
P1	Similar to algae and bacteria	225/341	Peak Tuv	(Determann et al., 1998)
	Tyrosine-like	275/303-4	Peak T	Nakajima, 2006
P2	Similar to algae and bacteria	225/334	Peak Tuv	(Determann et al., 1998)
	Tyrosine-like	280/309	Peak T	Nakajima, 2006
	Fulvic acid like	250/428	Peak A	(Mostofa et al., 2013)
P3	Similar to algae and bacteria	230/328	Peak Tuv	(Determann et al., 1998)
	Tyrosine-like	280/309	Peak T	Nakajima, 2006
	Fulvic acid like	250/434	Peak A	(Mostofa et al., 2013)
B1	Aromatic protein or soluble microbial by-products like	225/340	Peak Tuv	(Mayer, 1999)
	Fulvic acid type	225/450	Peak A	(Sugiyama et al., 2005)
	p-Cresol like	275/302	Peak A	(Mostofa et al., 2013)
B2	Fulvic acid type	275/408	Peak A	(Sugiyama et al., 2005)
B3	Aromatic protein or soluble microbial by-products like	230/339	Peak Tuv	(Mayer, 1999)
	Fulvic acid type	270/415	Peak A	(Sugiyama et al., 2005)
	p-Cresol like	275-303	Peak A	(Mostofa et al., 2013)
BP1	Protein like EPS	225/339	Peak Tuv	(Mostofa et al., 2013); (Shammi et al., 2017a, 2017b)
	Protein like EPS	280/308	Peak T	(Mostofa et al., 2013); (Shammi et al., 2017a, 2017b)
	Anthracene-like	240/410	Peak A	(Mostofa et al., 2013)
BP2	Protein like EPS	225/335	Peak Tuv	(Shammi et al., 2017a, 2017b)
	Protein like EPS	280/308	Peak T	(Shammi et al., 2017a, 2017b)
	Anthracene-like	230/410	Peak A	(Mostofa et al., 2013)
BP3	Protein like EPS	225/338	Peak Tuv	(Mostofa et al., 2013); (Shammi et al., 2017a, 2017b)
	Protein like EPS	280/309	Peak T	(Mostofa et al., 2013); (Shammi et al., 2017a, 2017b)
	Anthracene-like	230/403	Peak A	(Mostofa et al., 2013)

the plant's growth. Shoot growth rate (SGR) and total chlorophyll has a positive correlation between them. As the shoot growth rate increased, the total chlorophyll concentration was increased too, with the comparison of shoot growth rate. So, both of the parameter consists of a strong (positive) correlation, where the correlation coefficient is r = 0.784, and the regression equation is $y = 0.9692x - 0.113$ (Fig. 6). In EEM-spectra analysis, sample C1, C2 and C3, p-Cresol like or o-Cresol like substances were found at the Peak T region. All three samples had a Fulvic acid type substance at the Peak A region. C2 and C3 had peaks at the Peak Tuv region probably belonging to the group protein-like EPS from plant or tryptophan-like substances (Fig. 7C1, C2, and C3). In sample P1, P2 and P3, algae and bacteria were found at Peak Tuv region, Tyrosine-like substances at Peak T region and Fulvic acid like substances at Peak A region. It is obvious that salinity had the role to create such peaks associated with plants (Fig. 7P1, P2, and P3). A full list of substances identified with the excitation/emission (Ex/Em) wavelengths interpretation of different peaks is shown in the Table 5 with the reference substances.

In B1, B2 and B3, one common unidentified peak at the Peak A region probably belong to the Fulvic acid type substances (Schwede-Thomas et al., 2005; Sugiyama et al., 2005). This peak might have originated from the biochar. B1 and B3 had a common peak at the Peak A region probably belong to the p-Cresol like substance originated from microbial origin of the biochar. In addition, both B1 and B3 had aromatic protein or soluble microbial by-products like substance at the Peak Tuv region (Fig. 7B1, B2, and B3). In BP1, BP2 and BP3, with biochar and macrophyte, all the common peaks belong to Protein like EPS at Peak Tuv region, Protein like EPS at Peak T region and anthracene-like substance at Peak A region (Fig. 7 BP1, BP2, BP3).

Excitation-emission (Em/Ex) patterns in biochar-derived dissolved organic matter (BDOM) frequently show distinct fluorescence peaks based on the organic chemicals present. Humic-like molecules in BDOM typically glow at excitation/emission wavelengths of 240–260 nm/400–500 nm, whereas protein-like compounds (such as tryptophan) emit at 280 nm/330–350 nm. Variations in these Em/Ex patterns can be influenced by the biochar's source material and manufacturing conditions, such as pyrolysis temperature, which influences aromatic content and hence shifts or amplifies certain fluorescence peaks in EEM analysis (Peng et al., 2020).

In a previous study, different biochar samples' EEMs displayed a range of fluorescence traits and revealed compositions linked to humic-, fulvic-, and protein-like compounds (Rajapaksha et al., 2019). The biochar's were originated from soybean stover, garlic stem, rice husk, tea waste, perilla, wood pine chip, etc. and oak wood.

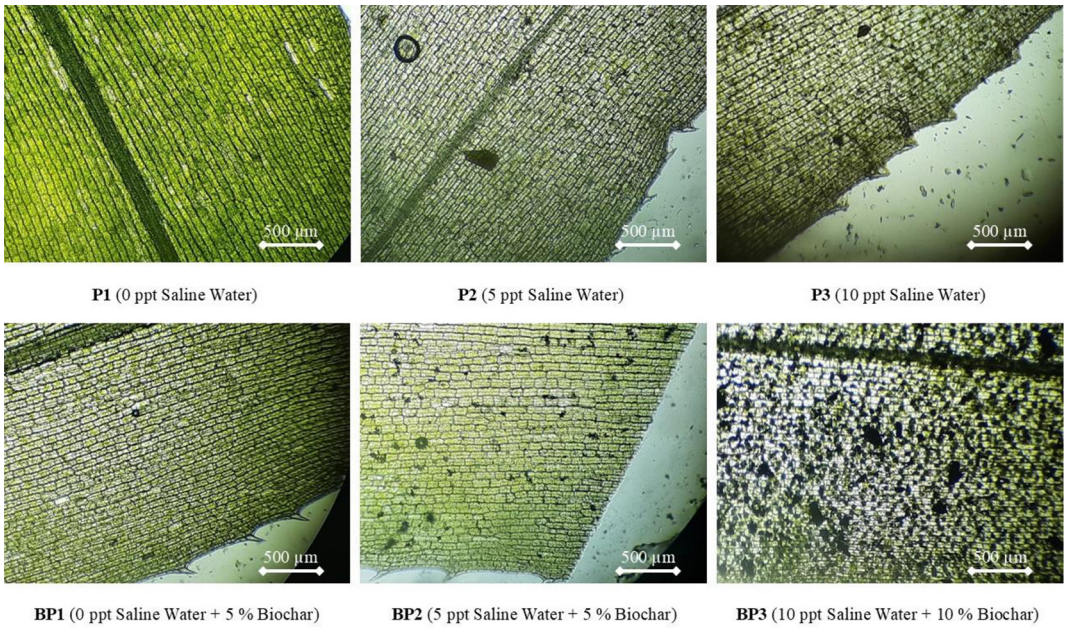

Figure 8. Plant's leaf status after 10 days of the experiment at 40x magnification

After 10 days of the experiment, microscope imaging shown the status of the macrophytes leaf. As the concentration of the salinity increased, the green leaf color has been faded more (P1, P2, and P3). In the macrophytes, which were exposed to saline water (0 ppt, 5 ppt and 10 ppt) with the application of biochar (5% and 10%), the plants green leaf color not has been faded as observed in P1, P2 and P3. But plants leaf adsorbed the biochar particles, and adsorption increased with the increase of biochar application (5% to 10%) (Fig. 8). As (Heidari, 2012; Taïbi et al., 2016) mentioned the stunted of the plant's growth due to the increasing of the salinity, the imaging in this study backing up the result as the plant's leaf were faded with the gradual increase of the salinity level.

CONCLUSIONS

In this research work, the salinity threshold level of the *E. densa* macrophyte were analyzed. *E. densa* survived in 5 ppt and eventually die at 10 ppt saline water concentration. *E. densa* and biochar in combine weren't able to reduce the water salinity from the sample beakers. Means, in this study, the study finds that the plants and biochar didn't reduce the salinity from the water. Plant's weight, shoot length were increased in biochar application with the saline water. Means, plants were not only survived in 5 ppt saline water but also plants growth rate has been increased sufficiently by the application of biochar. Root was started to form in the application of biochar at 5 ppt saline water. Means, biochar supports macrophytes to adopt with the salinity stress and after adapting with the condition macrophytes started to grow root. Total chlorophyll and carotenoids concentration also increased in the biochar application with 5 ppt saline water solution and as the shoot growth rate increased, the concentration of total chlorophyll and carotenoids increased in the samples.

Acknowledgements

All of the experiments and experimental setup has been done at the Environmental Science and Disaster Management Laboratory of Daffodil International University. Besides, EEM spectra analysis has been done at the Hydrobiogeochemistry and Pollution Control Laboratory of Jahangirnagar University.

REFERENCES

1. Agbna, G., Ali, A., Bashir, A., Eltoum, F., Hassan, M., Agbna, G.H., Ali, A.B., Bashir, A.K., Hassan, M.M., (2017). Influence of biochar amendment on soil water characteristics and crop growth enhancement under salinity stress, *International Journal of Engineering Works Kambohwell Publishers Enterprise.*
2. Alam, A.K.M.R., Hoque, S., (2017). Phytoremediation of industrial wastewater by culturing aquatic macrophytes, Trapa natans L. and Salvinia cucullata Roxb. *Jahangirnagar University J. Biol. Sci 6*, 19–27.
3. Ali, S., Riwan, M., Qayyum, M., Ok, Y.S., Ibrahim, M., Riaz, M., Arif, M., Hafeez, F., Al-Wabel, M., Shahzad, A., (2017a). Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review. *Environmental Science and Pollution Research 24*. https://doi.org/10.1007/s11356-017-8904-x
4. Ali, S., Rizwan, M., Qayyum, M.F., Ok, Y.S., Ibrahim, M., Riaz, M., Arif, M.S., Hafeez, F., Al-Wabel, M.I., Shahzad, A.N., (2017b). Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review. *Environ Sci Pollut Res Int 24*, 12700–12712. https://doi.org/10.1007/S11356-017-8904-X
5. Anwari, G., Mandozai, A., Feng, J., (2019). Effects of biochar amendment on soil problems and improving rice production under salinity conditions. *Advanced Journal of Graduate Research 7*, 45–63. https://doi.org/10.21467/ajgr.7.1.45-63
6. Atapaththu, K.S.S., Parveen, M., Asaeda, T., Rashid, M.H., (2018). Growth and oxidative stress response of aquatic macrophyte myriophyllum spicatum to sediment anoxia. *Fundamental and Applied Limnology 191*, 289–298. https://doi.org/10.1127/FAL/2018/1070
7. BBI, (2022). Bangladesh Biochar Initiative [WWW Document]. URL https://www.biochar-bangladesh.org/ (accessed 1.27.22).
8. da Silva, E.C., Nogueira, R.J.M.C., de Araújo, F.P., de Melo, N.F., de Azevedo Neto, A.D., (2008). Physiological responses to salt stress in young umbu plants. *Environ Exp Bot 63*, 147–157. https://doi.org/10.1016/J.ENVEXPBOT.2007.11.010
9. Determann, S., Lobbes, J. örg M., Reuter, R., Rullkötter, J. ürgen, (1998). Ultraviolet fluorescence excitation and emission spectroscopy of marine algae and bacteria. *Mar Chem 62*, 137–156. https://doi.org/10.1016/S0304-4203(98)00026-7
10. Erin, L., (2013). Preparing Saline Solution. University of Michigan Health System.
11. Guo, X., Wang, X., Liu, J., (2016). Composition analysis of fractions of extracellular polymeric substances

from an activated sludge culture and identification of dominant forces affecting microbial aggregation. *Sci Rep 6*. https://doi.org/10.1038/srep28391

12. Hasanuzzaman, M., Nahar, K., Alam, M.M., Bhowmik, P.C., Hossain, M.A., Rahman, M.M., Prasad, M.N.V., Ozturk, M., Fujita, M., (2014). Potential use of halophytes to remediate saline soils. *Biomed Res Int*. https://doi.org/10.1155/2014/589341
13. Heidari, M., (2012). Effects of salinity stress on growth, chlorophyll content and osmotic components of two basil (*Ocimum basilicum* L.) genotypes. *Afr J Biotechnol 11*, 379–384. https://doi.org/10.5897/AJB11.2572
14. Hongqiao, L., Suyama, A., Mitani-Ueno, N., Hell, R., Maruyama-Nakashita, A., (2021). A low level of NaCl stimulates plant growth by improving carbon and sulfur assimilation in arabidopsis thaliana. *Plants 10*. https://doi.org/10.3390/PLANTS10102138/S1
15. Hu, X., Tanaka, A., Tanaka, R., (2013). Simple extraction methods that prevent the artifactual conversion of chlorophyll to chlorophyllide during pigment isolation from leaf samples. *Plant Methods 9*, 1–13. https://doi.org/10.1186/1746-4811-9-19/FIGURES/8
16. Hunt, J., Duponte, M., Sato, D., Kawabata, A., 2010. Soil and Crop Management SCM-30 The Basics of Biochar : A Natural Soil Amendment.
17. Imakumbili, M.L.E., Semu, E., Semoka, J.M.R., Abass, A., Mkamilo, G., (2020). Plant tissue analysis as a tool for predicting fertiliser needs for low cyanogenic glucoside levels in cassava roots: An assessment of its possible use. *PLoS One 15*, e0228641. https://doi.org/10.1371/JOURNAL.PONE.0228641
18. Kefu, Z., Hai, F., Ungar, I.A., 2002. Survey of halophyte species in China. *Plant Science 163*, 491–498. https://doi.org/10.1016/S0168-9452(02)00160-7
19. Koca, H., Bor, M., Özdemir, F., Türkan, I., (2007). The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ Exp Bot 60*, 344–351. https://doi.org/10.1016/J.ENVEXPBOT.2006.12.005
20. Lee, M.K., Van Iersel, M.W., (2008). Sodium chloride effects on growth, morphology, and physiology of chrysanthemum (Chrysanthemum ×morifolium). *HortScience 43*, 1888–1891. https://doi.org/10.21273/HORTSCI.43.6.1888
21. Liu, H., Fang, H.H.P., 2002. Characterization of electrostatic binding sites of extracellular polymers by linear programming analysis of titration data. *Biotechnol Bioeng 80*, 806–811. https://doi.org/10.1002/BIT.10432
22. Mayer, L.M., (1999). Extent of coverage of mineral surfaces by organic matter in marine sediments. *Geochim Cosmochim Acta 63*, 207–215. https://doi.org/10.1016/S0016-7037(99)00028-9
23. Mili, S.A., Rahman, S.M.M., Pasha, A.B.M.K., Chowdhury, M.A.H., (2024). Assessment on the effect of rapid urbanization on groundwater quality of mirpur area, Dhaka City, Bangladesh. *INTI JOURNAL,* 1–9. https://doi.org/https://doi.org/10.61453/INTIj.202433
24. Mostofa, K.M.G., Liu, C. Qiang, Mottaleb, M.A., Wan, G., Ogawa, H., Vione, D., Yoshioka, T., Wu, F., (2013). Dissolved organic matter in natural waters. *Environmental Science and Engineering* 1–137. https://doi.org/10.1007/978-3-642-32223-5_1/COVER
25. Mur, L.A.J., Aubry, S., Mondhe, M., Kingston-Smith, A., Gallagher, J., Timms-Taravella, E., James, C., Papp, I., Hörtensteiner, S., Thomas, H., Ougham, H., (2010). Accumulation of chlorophyll catabolites photosensitizes the hypersensitive response elicited by Pseudomonas syringae in Arabidopsis. *New Phytologist 188*, 161–174. https://doi.org/10.1111/j.1469-8137.2010.03377.x
26. Nehela, Y., Mazrou, Y.S.A., Alshaal, T., Rady, A.M.S., El-Sherif, A.M.A., Omara, A.E.D., El-Monem, A.M.A., Hafez, E.M., (2021). The integrated amendment of sodic-saline soils using biochar and plant growth-promoting rhizobacteria enhances maize (*Zea mays* L.) resilience to water salinity. *Plants 10*. https://doi.org/10.3390/plants10091960
27. Niloy, N.M., Haque, M.M., Tareq, S.M., (2021). Characterization of dissolved organic matter at urban and industrial rainwater of Bangladesh by fluorescence spectroscopy and EEM-PARAFAC modeling. *Environmental Challenges 5*, 100250. https://doi.org/10.1016/J.ENVC.2021.100250
28. Niloy, N.M., Shammi, M., Haque, M.M., Tareq, S.M., 2022. Biogeochemistry of the dissolved organic matter (DOM) in the estuarine rivers of Bangladesh–Sundarbans under different anthropogenic influences. *Heliyon 8*, e10228. https://doi.org/10.1016/J.HELIYON.2022.E10228
29. Oo, Y.Y.N., Su, M.C., Kyaw, K.T., (2017). Extraction and determination of chlorophyll content from microalgae. *International Journal of Advanced Research and Publications 1*.
30. Parihar, P., Singh, S., Singh, R., Singh, V.P., Prasad, S.M., (2014). Effect of salinity stress on plants and its tolerance strategies: a review. *Environmental Science and Pollution Research 22*(6), 4056–4075. https://doi.org/10.1007/S11356-014-3739-1
31. Parkash, V., Singh, S., (2020). Potential of biochar application to mitigate salinity stress in eggplant. *HortScience 55*, 1946–1955. https://doi.org/10.21273/HORTSCI15398-20
32. Parry, C., Blonquist, J.M., Bugbee, B., (2014). In situ measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship. *Plant Cell Environ 37*, 2508–2520. https://doi.org/10.1111/PCE.12324

33. Parveen, M., Abdullah, M., Rahman, S.M.M., Chowdhury, M.A.H., Khan, M.S.I., Pasha, A.K., (2022a). Improvement of wastewater quality of Dhaleswari river, Bangladesh using submerged macrophyte Egeria densa. *Journal of Sustainability Perspectives 2*, 449–458. https://doi.org/10.14710/jsp.2022.15547
34. Parveen, M., Abdullah, M., Rahman, S.M.M., Haque, A., Khan, S.I., Kamal, P.A.B.M., (2022b). Improvement of wastewater quality of Dhaleswari river, Bangladesh using submerged macrophyte Egeria densa. *Journal of Sustainability Perspectives 2*, 449–458. https://doi.org/10.14710/jsp.2022.15547
35. Parveen, M., Asaeda, T., Rashid, M.H., (2017a). Effect of hydrogen sulfide exposure on the growth, oxidative stress and carbohydrate metabolism of Elodea nuttallii and Egeria densa. *Fundamental and Applied Limnology 191*, 53–62. https://doi.org/10.1127/FAL/2017/1046
36. Parveen, M., Asaeda, T., Rashid, M.H., 2017b. Hydrogen sulfide induced growth, photosynthesis and biochemical responses in three submerged macrophytes. *Flora 230*, 1–11. https://doi.org/10.1016/J.FLORA.2017.03.005
37. Parveen, M., Miyagi, A., Kawai-Yamada, M., Rashid, M.H., Asaeda, T., (2019). Metabolic and biochemical responses of Potamogeton anguillanus Koidz. (Potamogetonaceae) to low oxygen conditions. *J Plant Physiol 232*, 171–179. https://doi.org/10.1016/j.jplph.2018.11.023
38. Pasha, A.B.M.K., Abdillahi, M.M., Rahman, S.M.M., Mozumder, S., Chowdhury, A.H., Fuente, J.A. Dela, Parveen, M., 2022. Studies on physicochemical properties of Buriganga river water and the vegetation coverage of surrounding Area, Dhaka, Bangladesh. *Sci. Int.(Lahore) 34*, 73–78.
39. Pasha, A.B.M.K., Mustafa, S.O., Rahman, S.M.M., Abdullah, M., Chowdhury, Md.A.H., Parveen, M., 2023. Analysis of water quality of Hatirjheel Lake, Dhaka, Bangladesh. *Nature Environment and Pollution Technology 22*, 245–252. https://doi.org/10.46488/NEPT.2023.v22i01.000
40. Pechar, L., (1987). Use of an acetone: methanol mixture for the extraction and spectrophotometric determination of chlorophyll- a in phytoplankton. Algological Studies 46/Archiv für Hydrobiologiie Supplement 78, 99–117.
41. Peng, N., Wang, K., Tu, N., Liu, Y., Li, Z., (2020). Fluorescence regional integration combined with parallel factor analysis to quantify fluorescencent spectra for dissolved organic matter released from manure biochars. *RSC Adv 10*, 31502–31510. https://doi.org/10.1039/D0RA02706E
42. Porra, R.J., Thompson, W.A., Kriedemann, P.E., (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica et Biophysica Acta (BBA) - Bioenergetics 975, 384–394. https://doi.org/10.1016/S0005-2728(89)80347-0
43. Rajapaksha, A.U., Sik Ok, Y., El-Naggar, A., Kim, H., Song, F., Kang, S., Tsang, Y.F., (2019). Dissolved organic matter characterization of biochars produced from different feedstock materials. *J Environ Manage 233*, 393–399. https://doi.org/10.1016/j.jenvman.2018.12.069
44. Rashid, M.H., Asaeda, T., Uddin, M.N., (2010). Litter-mediated allelopathic effects of kudzu (Pueraria montana) on Bidens pilosa and Lolium perenne and its persistence in soil. *Weed Biol Manag 10*, 48–56. https://doi.org/10.1111/J.1445-6664.2010.00366.X
45. Rezwan, S.M., Chowdhury, M.A.H., Rahman, S.M.M., (2022). Assessment of ecosystem services, plant diversity pattern, and water quality of an urban water Body in Dhaka, Bangladesh. *Advances in Science, Technology and Innovation* 151–163. https://doi.org/10.1007/978-3-030-86499-6_14/COVER
46. Rizwan, M., Ali, S., Qayyum, M.F., Ibrahim, M., Zia-ur-Rehman, M., Abbas, T., Ok, Y.S., (2016). Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: a critical review. *Environ Sci Pollut Res Int 23*, 2230–2248. https://doi.org/10.1007/S11356-015-5697-7
47. Saifullah, Dahlawi, S., Naeem, A., Rengel, Z., Naidu, R., (2018). Biochar application for the remediation of salt-affected soils: Challenges and opportunities. *Science of The Total Environment 625*, 320–335. https://doi.org/10.1016/J.SCITOTENV.2017.12.257
48. Schwede-Thomas, S.B., Chin, Y.-P., Dria, K.J., Hatcher, P., Kaiser, E., Sulzberger, B., (2005). Characterizing the properties of dissolved organic matter isolated by XAD and C-18 solid phase extraction and ultrafiltration. *Aquat Sci 67*, 61–71. https://doi.org/10.1007/s00027-004-0735-4
49. Shammi, M., Pan, X., Mostofa, K.M.G., Zhang, D., Liu, C.Q., (2017a). Seasonal variations and characteristics differences in the fluorescent components of extracellular polymeric substances from mixed biofilms in saline lake. *Sci Bull (Beijing) 62*, 764–766. https://doi.org/10.1016/J.SCIB.2017.04.016
50. Shammi, M., Pan, X., Mostofa, K.M.G., Zhang, D., Liu, C.Q., (2017b). Photo-flocculation of microbial mat extracellular polymeric substances and their transformation into transparent exopolymer particles: Chemical and spectroscopic evidences. *Sci Rep 7*. https://doi.org/10.1038/S41598-017-09066-8
51. Sugiyama, Y., Anegawa, A., Inokuchi, H., Kumagai, T., (2005). Distribution of dissolved organic carbon and dissolved fulvic acid in mesotrophic Lake

Biwa, Japan. *Limnology 6*, 3(6), 161–168. https://doi.org/10.1007/S10201-005-0155-8

52. Sun, Y. peng, Yang, J. song, Yao, R. jiang, Chen, X. bing, Wang, X. Ping, (2020). Biochar and fulvic acid amendments mitigate negative effects of coastal saline soil and improve crop yields in a three year field trial. *Sci Rep 10*. https://doi.org/10.1038/s41598-020-65730-6
53. Taïbi, K., Taïbi, F., Ait Abderrahim, L., Ennajah, A., Belkhodja, M., Mulet, J.M., (2016). Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in Phaseolus vulgaris L. *South African Journal of Botany 105*, 306–312. https://doi.org/10.1016/J.SAJB.2016.03.011
54. Tasnim, U.F., Shammi, M., Uddin, M.K., Akbor, M.A., (2021). Effect of biochar amended vermicomposting of food and beverage industry sludge along with cow dung and seed germination bioassay. *Pollution 7*, 355–365. https://doi.org/10.22059/poll.2021.315530.961
55. Thomas, S.C., Frye, S., Gale, N., Garmon, M., Launchbury, R., Machado, N., Melamed, S., Murray, J., Petroff, A., Winsborough, C., (2013). Biochar mitigates negative effects of salt additions on two herbaceous plant species. *J Environ Manage 129*, 62–68. https://doi.org/10.1016/J.JENVMAN.2013.05.057
56. V Sundararaju, (2019). Propagate and protect halophytes [WWW Document]. URL https://www.downtoearth.org.in/blog/forests/propagate-and-protect-halophytes-63711 (accessed 1.14.22).
57. Yensen, N.P., (2008). Halophyte uses for the twenty-first century. *Ecophysiology of High Salinity Tolerant Plants* 367–396. https://doi.org/10.1007/1-4020-4018-0_23
58. Yu, J., Xiao, K., Xue, W., Shen, Y. xiao, Tan, J., Liang, S., Wang, Y., Huang, X., (2020). Excitation-emission matrix (EEM) fluorescence spectroscopy for characterization of organic matter in membrane bioreactors: Principles, methods and applications. *Front Environ Sci Eng 14*. https://doi.org/10.1007/s11783-019-1210-8
59. Zhu, Y.-Y., Liu, Y., Xu, J., Ni, B.-J., (2022). Three-dimensional excitation-emission matrix (EEM) fluorescence approach to probing the binding interactions of polystyrene microplastics to bisphenol A. *Journal of Hazardous Materials Advances 5*, 100046. https://doi.org/10.1016/J.HAZADV.2022.100046