Exposure of *Eichhornia crassipes* (Mart.) Solms to salt water and its implications

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In this article, we discuss the effect of salinity on the viability and decomposition of *Eichhornia crassipes* plant under normal photoperiod, dark condition and physiological response. Highest concentration of total organic carbon (27.43 mg C l^{-1}) was recorded in 15 psu salinity after 45 days. The TOC output was more in case of leaf (3.6 mg C l^{-1}) than petiole (2.39 mg C l^{-1}) under dark condition, after 21 days in freshwater. Salt stress was found to enhance the superoxide dismutase activity at 20 psu in both leaf and petiole. Enzyme activity declined when salt-stressed plants were transferred to nutrient enriched freshwater. This indicated that 20 psu could be a plant's salt tolerance limit. The potential transfer test conducted in this study showed that *Eichorrnia* introduction through shipping activities is less likely.

Keywords: *Eichhornia crassipes*, salinity stress, superoxide dismutase, total organic carbon.

EICHHORNIA CRASSIPES (Mart.) Solms (water hyacinth) is an invasive aquatic macrophyte. The ability of the plant to flourish in any condition has made it a noxious and invasive weed in tropical and subtropical regions[1,2]. The plant has a wide range of tolerance to temperature, lighting conditions, pH, drought resistance and salinity[1,3]. The backwaters in Cochin including Cochin Port area, Kerala-India, become infested by this plant during monsoon when the sluice gates of Thannermukkom salt-water barrier are opened. The sluice gates prevent the *E. crassipes* plants growing upstream to move downstream. As a consequence, the plant is subjected to a varying range of salinities both in the upstream and downstream areas. The high salinity condition affects the plant and it starts decaying. Further, these plants are also subjected to physical damage, breaking into pieces, due to movement of mechanized boats, trawlers and ships in the area. The dead plants generate detrital matter, which is a major organic source in an aquatic system. The extent of detritus decomposition depends on the type of metabolic activities taking place in the system[4]. During decomposition of the plants, three main types of microbial activity occur – aerobic, facultative anaerobic and anaerobic. Besides these, various factors both external and biochemical are also involved in the process of decomposition[4–6], biotic and abiotic[7,8]. However, some authors have indicated that tissue chemistry influences the decomposition process more than environmental conditions[3,9,10]. Earlier decomposition studies on *E. crassipes* showed that physical leaching and biological degradation are the two principal processes involved[11–13]. Litter decomposition is also influenced by nutrient concentration[14], water salinity[15] and seasonal periodicity[16]. The sudden appearance of *E. crassipes* during monsoon in the Port area and its subsequent disappearance, add to the complexity of the ecosystem. It therefore, becomes imperative to investigate the organic matter supply to ecosystem due to plant decomposition. In view of the perennial *E. crassipes* infestation in the Cochin backwaters, the present study was conducted to gain an insight into the problem. In this regard, the concentration of total organic carbon (TOC) due to *E. crassipes* decomposition was studied in the laboratory. Although the characteristics of the Cochin backwater system change after monsoon due to sea-water intrusion, the plant survives at the southern end which is fed by perennial freshwater. In this system, the plants are exposed to regular fluctuation in salinity due to tidal action. This phenomenon of alternate action, by sea water and freshwater, could have an effect on the plants. Therefore, in this study, the effect of salinity on plant decomposition, its tolerance and physiological response to varied salinity levels were assessed in the laboratory using TOC and superoxide dismutase (SOD) as proxies. In addition, an experiment was also performed to test the transfer potential of the plant through ballast water of ships.

Materials and methods

Effect of salinity on plant decomposition

The plants were subjected to seven different levels of salinity (5, 10, 15, 20, 25, 30 and 35 psu) using fresh sea water (Figure 1). Freshwater was used for equilibration and another set with a plant was maintained as control. Five litre plastic containers (v/v) were filled with equilibrated sea water. Plants of similar physiological age, size and an equal number of leaves (~10 g wet weight) were cleaned to remove debris and other detrital matter, and used for the study. One plant each with 4–5 leaves was maintained in each container containing 5 litres of water

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Figure 1. Experimental set-up. *Eichorrnia crassipes* plants under a series of salinity conditions. FW, Freshwater; 1, 5 psu; 2, 10 psu; 3, 15 psu; 4, 20 psu; 5, 25 psu; 6, 30 psu; 7, 35 psu.

to evaluate the salinity effect. This was assessed by estimating TOC in the experimental water as a measure of the extent of plant decomposition. The containers were kept in the open area under normal photoperiod (14/10, light and dark cycle). TOC was measured at 15 days interval using TOC analyzer (Shimadzu, Model TOC LCPH). This works on the principle of combustion catalytic oxidation of carbonaceous materials and the concentration of carbon was determined against potassium hydrogen phthalate as a standard using non-dispersive infrared detector (NDIR). Another set of the same range of salinity (0–35 psu) was prepared using sodium chloride in distilled water (referred as salt water or artificial sea water). Although sea water has a consortium of salts, the reason for using only sodium chloride was because salinity effect is mainly osmoregulatory in function. Thus the salt water enabled us to quantify the TOC concentration more precisely. The effect of salinity on the decomposition of *E. crassipes* was studied for a period of 60 days. In addition, the effect of dark condition on decomposition of the plant was also studied, so as to understand and equate the effect of dense mat of plant beds in natural environment. The duration of the study was 49 days. In this set-up, four salinity levels of salt water (0, 15, 25, 35 psu) were used. Leaf and petiole of ~2 g (wet weight) each were incubated separately in a 1 litre jar containing 400 ml (v/v) water to assess their individual contribution to environment degradation through TOC. The jars were covered with double-layered black plastic bag. A set of control (without plants) was also maintained. TOC was measured at seven days interval. Due care was taken to prevent exposure to light at the time of sampling. Dry weight of leaf and petiole was recorded at the end, after oven-drying at 60°C for 48 h.

Salt stress on E. crassipes

The physiological response of *E. crassipes* to salt stress was studied using four different salinity levels of salt water (0, 5, 10 and 20 psu). All four media were enriched by F/2 nutrient medium without silicate. Enriched freshwater was used as zero salinity. The stress due to salt water was determined by measuring the changes in the activity of SOD enzyme. SOD activity was analysed according to the protocol given in the assay kit (Sigma-Aldrich, USA) and expressed in terms of percentage. The absorbance was read at 450 nm. SOD activity was calculated using the equation

$$\text{SOD activity (inhibition rate \%)} = \{[(A_{\text{blank1}} - A_{\text{blank3}}) - A_{\text{sample}} - A_{\text{blank2}}]/(A_{\text{blank1}} - A_{\text{blank3}})\} \times 100/\text{wet weight}.$$

E. crassipes plants of similar physiological age with equal number of petioles per plant were selected for salt-stress test. The plants were manually cleaned to remove any attached detritus matter and washed with filtered water. Plants were acclimatized overnight prior to use. Keeping in mind the diurnal tidal cycle, the total duration of the study was 12 h. It was further sub-divided into 6 h each. Just before the start of the experiment, the samples were collected from the acclimatized plants. The plant samples were removed with a sharp blade from the base of the petiole; they were further sub-divided into petiole and leaf. These samples were designated as control. The samples were put into a zip-lock plastic bag and stored in deep-freeze until further use. Plants were then exposed to different salinity conditions. On completion of the first 6 h exposure to salt water, samples were collected from all the sets. After this, salt water was replaced with nutrient-enriched freshwater. The plants were maintained in this condition for another 6 h. At the end of 12 h, samples were collected from all the plants. Samples from zero salinity (freshwater) were collected only at the beginning and at the end of 12 h.

Transfer potential through ship ballast

A part of the Cochin backwaters serves as a commercial seaport. Some of the seaports in the region are freshwater

ports. Keeping this in mind, the transfer potential of *E. crassipes* through ship ballast or as stowaway was tested under laboratory conditions. Plants of similar size were incubated in two separate dark chambers containing sea water and freshwater respectively. Three plants each were removed at regular intervals and transferred to nutrient-enriched freshwater. Survival and growth of the plants were visually monitored.

To determine the combined effect of salinity and time on the decomposition of *E. crassipes* and the subsequent release of TOC, two-way analysis of variance (ANOVA) was used. The effect and response of the plants to salinity were analysed by one-way ANOVA. These analyses were performed using StatSoft software (v8). Normality of data was checked prior to statistical analysis.

Results

Measurement of extent of plant decomposition in terms of TOC

TOC concentration recorded as a result of plant decomposition did not show any definite trend temporally in all the salinity conditions tested (Figure 2 *a*). High TOC concentration of 27.43 mg C l^{-1} at 15 psu was recorded after 45 days, followed by 23 mg C l^{-1} at 35 psu. The effect of time and salinity was not statistically significant ($P > 0.05$). Similarly, in the second supplementary experiment, no significant interactive effect of time and salinity was recorded ($P > 0.05$). The highest TOC concentration (3.84 mg C l^{-1}) was recorded after 45 days at 25 psu (Figure 2 *b*). However, data analysis showed that the output pattern of TOC concentration due to decomposition in the both cases was similar (Figure 2 *a* and *b*).

Effect of dark condition on the decomposition process

Time and salinity had a significant effect on the decomposition of leaf ($P < 0.001$) and petiole ($P < 0.001$) in the TOC output under dark condition. However, salinity alone had no significant effect on the decomposition of leaf and petiole. Maximum TOC concentration was recorded after the 21st day under freshwater condition followed by 35 psu. TOC from leaf decomposition in freshwater was 3.6 mg C l^{-1} (Figure 3 *a*). Similarly, the highest TOC concentration from petiole decomposition was 2.39 mg C l^{-1} after the 21st day under similar conditions (Figure 3 *b*). However, assessment of loss in weight (dry weight) of leaf and petiole at the end of the experiment showed that the overall decomposition process occurred at a much faster rate in zero salinity, which decreased with increase in salinity (Figure 4). The results showed that the decomposition rate was higher in petiole than in leaf.

Effect of salt stress

Analysis of the effect of salt stress under changing salinity indicated elevated SOD activity. The salinity effect on SOD activity was highly significant in both leaf ($P < 0.01$) and petiole ($P < 0.01$). Similarly, there was a significant interactive effect of time and salinity on the response and activity of SOD enzyme in both leaf ($P < 0.01$) and petiole ($P < 0.01$). SOD activity was recorded more in leaf (33.5%) than in petiole (12%). Salinity of 20 psu elicited maximum activity in both leaf and petiole (Figure 5 *a* and *b*). SOD enzyme activity declined when the plants were transferred to freshwater conditions. Consequently, these plants produced new shoots when maintained in freshwater.

Test of transfer potential

E. crassipes plants in both freshwater and sea water conditions decomposed after incubation in the dark. There was high chlorophyll degradation (visual observation) and the plants turned yellow before decomposition. The degradation was more in freshwater conditions. No growth and recovery was recorded after its transfer to nutrient-enriched freshwater even after several days of transfer. From the fifth day, chlorophyll was completely degraded (turned yellow). The observations of subsequent days indicated that degradation of plant material accelerated further, emanating foul odour from the incubation tank. As a result, the experiment was discontinued after 11 days.

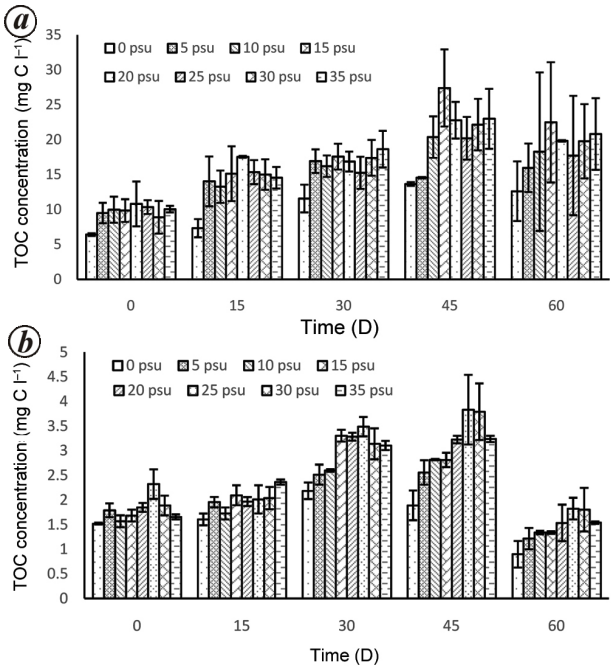

Figure 2. Temporal variation in the concentrations of total organic carbon (TOC), representing the extent of decomposition in *E. crassipes* plants exposed to different salinities of (***a***) natural sea water and (***b***) salt water (artificial sea water).

Discussion

TOC is the amount of carbon present in a suite of organic compounds. In the present case, organic carbon enters the medium through plant decomposition, and the release of dissolved compounds is an essential process in the ecosystem[17,18]. TOC is the main source of organic matter for the organisms present in the aquatic ecosystem. The decomposition rate of *E. crassipes* varies with tissue N and fibre content[5]. The loss in dry weight was attributed to the decomposition of organic compounds[4]. The decomposition rate of aquatic plants is strongly dependent on the content of highly resistant materials such as fibre, hemicellulose, cellulose and lignin[19]. In concurrence with the stated literature, even though tissue chemistry was not studied in the present investigation, it showed that the decomposition process differed in leaf and petiole. It was high in petiole and low in leaf. This difference could be due to high content of fibre, lignin and cellulosic material in leaf, which might have contributed to low decomposition. After 21 days, TOC concentration declined, whereas in the first experiment (where whole plant was used) TOC concentration was recorded till the 60th day. This was possibly due to difference in biomass used. Studies have shown that salinity negatively affects decomposition[15,20]. Consistent with earlier reports, it was found that TOC contribution was not only from the decomposition of plant matter, but also from leaching. Plants exposed to high salinity decomposed less, though TOC levels were high. The effect of salinity on decomposition may be attributed to low microbial activity[8].

High salinity severely affects plant growth[21] and productivity[22], though some plants have physiological mechanisms to tolerate and recover from high salt stress[23]. On the other hand, the ameliorative role of SOD against oxidative stress is well documented[24,25]. The visible effect of salt stress in the present study was wilting of the leaf margin, a result of osmoregulatory injury at high salinity. The salt injury effect was visible in the first 3 h after expose to salt water. The affected margin turned necrotic in severe cases. This indicated that stress was more on the leaf. Maximum SOD activity at 20 psu in both leaf and petiole indicated that this could be the threshold limit of salt tolerance of the plant. The change in SOD activity under salt water and freshwater conditions indicated that SOD could play a pivotal role in reversing the salt injury on return to an ambient condition. This also showed that the length of exposure time is important in addition to high salinity. Subsequently, the plants transferred to

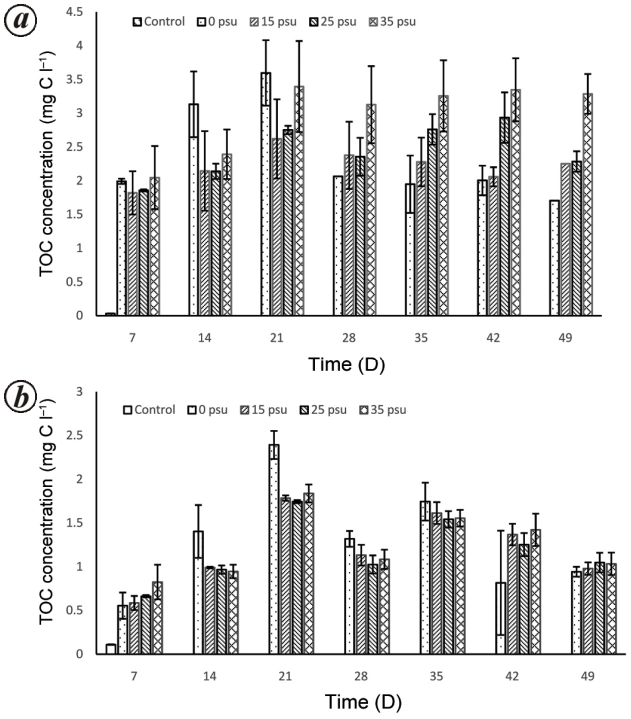

Figure 3. Temporal variation in the concentrations of TOC, representing the extent of decomposition of *E. crassipes* leaf (***a***) and petiole (***b***), exposed to different salinity levels under dark condition.

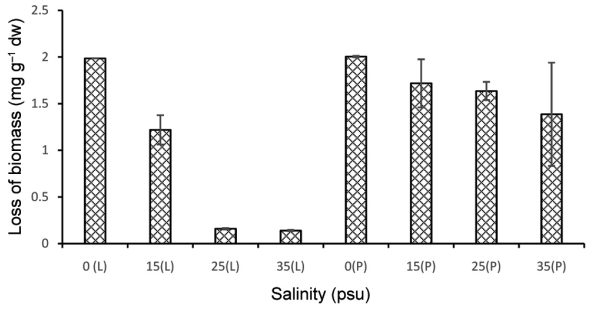

Figure 4. Extent of loss in weight (dry weight, dw) of *E. crassipes* leaf and petiole subjected to different salinities of salt water (artificial sea water) under dark condition. Bar indicates standard deviation. L, Leaf; P, Petiole.

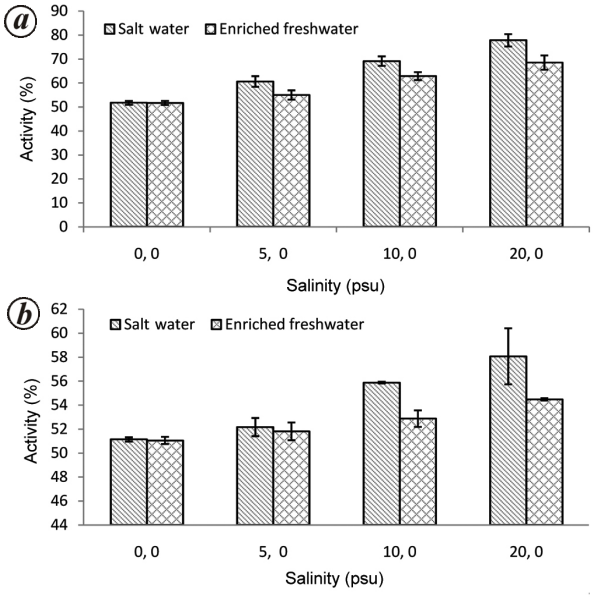

Figure 5. Variations in superoxide dismutase activity in *E. crassipes* leaf (***a***) and petiole (***b***) on exposure to salt water (artificial sea water) of different salinities and F/2 nutrient enriched freshwater. Bars indicate standard deviation.

freshwater conditions regained normal growth with new shoots. An earlier study[26] showed that *E. crassipes* can tolerate salinities less than 10 psu, but the present study showed that regular flushes of freshwater will increase the probability of survival.

Invasive species are the primary drivers of ecological change[27]. Although *E. crassipes* is a highly invasive plant, the present study showed that it is less likely to be transported as a stowaway or through ship ballast water. Therefore, translocation and introduction of the plant require direct human-mediated intervention.

The consequent implication of TOC following the collapse and decomposition of *E. crassipes* biomass is expected to be detrimental on the ecosystem. This could be true in the case of the Cochin backwaters where *E. crassipes* infestation is perennial, and the flushing is reportedly weak[28]. Under such conditions, the organic material is expected to remain for longer periods in the system. Considerable increase in biological oxygen demand has been recorded post-monsoon in the Cochin backwaters[29,30], which coincided with the collapse of *E. crassipes*. Such conditions can be detrimental to the overall productivity of the ecosystem.

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