



REGULAR ARTICLE

# EFFECT OF SALINITY ON MINERAL NUTRIENT DISTRIBUTION ALONG GROWING SEEDLINGS OF *BRUGUIERA CONJUGATA* LAMK.

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## SUMMARY

*Bruguiera conjugata* (Rhizophoraceae) is a salt tolerant plant species which is widely distributed in Inland and coastal salt marshes area. This study reports the effect of salinity (0, 200, 400, 600 and 800 mM) on the growth and ionic content. Fresh and dry weight of plants increased with an increase in salinity. The optimal growth of *B. conjugata* plants were recorded at 400 mM NaCl and the growth declined with further increase in salinity. The leaf, stem and root had Ca<sup>2+</sup>, P, Mg<sup>2+</sup>, Nitrogen and micronutrients such as Fe<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup> and Mn<sup>2+</sup> content increased upto the optimum level and thereafter decreased gradually. In Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> content increased upto optimal level of 800 mM.

**Keywords:** *Bruguiera conjugata*, halophyte, growth, mineral nutrients and salt stress

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## 1. Introduction

Mangroves are special intertidal woody communities, common in tropical and subtropical coastal environments. They are important not only in protecting coasts from erosion by fierce tides but also in promoting the diversity of marine organisms and fishery by contributing a quantity of food and providing favourable habitats for animals [1]. A variety of mechanisms contributes to the salt tolerance of halophytes. Adaptation of halophytes to the saline environment includes high tolerance for the negative effect of salinity as well as positive reaction towards it. It is

suggested that compartmentation of ions in vacuoles and accumulation of compatible solute in the cytoplasm, as well as presence of genes for salt tolerance, confer salt resistance to halophytes [2 and 3].

[4] found that fresh and dry weight of root and shoot of *Halopyrum mucronatum* were the greatest for plants grown at 90 mM NaCl, but growth was inhibited at higher salinities and all plants died at 360 mM NaCl. Osmotic adjustment occurred under increased salinity and was with an increase in the sodium and chloride concentrations of shoots, decreased shoot potassium concentration, and decreased shoot succulence [5]. The accumulation of high salt concentrations in the organ of grasses

usually serves to achieve osmotic balance under saline conditions by adjusting the plant's water potential to more negative levels than that of the growth medium [4].

Increasing NaCl salinities have increased at optimal level of the potassium content of several halophyte such as *Atriplex griffithii* [6]; *Salicornia rubra* [7]; *Haloxylon recurvum* [8]. On the other hand, increasing salinities have decreased the potassium content of some halophytes such as *Spergularia marina* [9] and *Rhizophora mangle* and *Laguncularia recimosa* [10]. In general,  $K^+$  is accumulated in response to low soil moisture, whereas  $Na^+$  is accumulated under saline conditions [11].

Calcium plays an important role in the response of plants to salinity which is well documented. The reduction of growth when  $Ca^{2+}$  of the cell membrane is replaced by  $Na^{2+}$  is attributed by  $K^+$  leakage from the cell. The imbalance of ions may cause a loss in cell turgor pressure and leads to growth inhabitation [12]. Very little is known about calcium being a toxic constituent of salinity. The plants affected with chloride salinity show increased content of  $Ca^{2+}$ . Increasing external salinity has decreased the  $Ca^{2+}$  content of *Allenrolefea occidentalis* [13] and *Atriplex griffithii* [6].

Chloride is essentially required for photosynthetic  $O_2$  evolution [14], chloride content is also increased with increases in salinity, and this pattern is consistent with other subtropical perennial halophytes like *Cressa cretica* [15]; *Halopyrum mucronatum* [4]; *Haloxylon recurvum* [8] and *Heleochocha setulosa* [16].

The micronutrients such as Fe, Mn, Zn are spatially distributed along the grass leaves [17] and the growing tissues are a strong sink for nutrients. Various studies report the effect of micro-nutrient distribution along the growing leaves of maize [18], maize [19], *Cassia montana*

[20] and *Salvadora persica* [21]. The objective of the present study is to examine the effect of exogenously added NaCl on the growth and mineral constituents of *B. conjugata* and to assess its salt tolerance, a phenomenon commonly occurring in coastal land plants. It is therefore an ideal material for the study of salt tolerance.

## 2. Materials and Methods

*Bruguiera conjugata* seedlings (7 to 10 cm length) were collected from Pichavaram mangrove area of Tamil Nadu. The seedlings were planted in polythene bags containing nutrient free sand soil. The pH of the soil is the 7.2 and E. C 0.59. They were irrigated with tapwater and allowed to establish well for a month. After well established the plants were selected and treated with five salinities (0, 200, 400, 600 and 800 mM NaCl) using the experimental plots. The seedlings above 800mM NaCl could not survive beyond a week after salt treatment. The control plants were maintained without the addition of NaCl. The samples were collected periodically at bimonthly intervals for different mineral analysis. The plants had an approximate 12h photoperiod and mean day temperature of 30°C and night temperature of 26°C.

### Nutrient Analysis

Biomass was determined by destructive sampling techniques. Six plants from the initial plant pools were selected at random for determination of biomass partitioning at the beginning of the experiment (after the acclimation period but prior to treatment initiation). These samples were then partitioned into leaf, stem and root components. The samples were washed in deionized water and dried in an oven at 80°C. Weights were recorded once a constant weight

was reached. Likewise, at the conclusion of the experiment all the study plants were sampled for biomass, partitioned into root, stem and leaf components, dried at 800 °C constant weights and weighed. The leaf, stem and root samples were then finely ground and then take was 0.5 g of dried samples were digested with 10ml of H<sub>2</sub>SO<sub>4</sub> / H<sub>2</sub>O<sub>2</sub> and kept for 24 hours and then heated with sand bath. The inorganic residue was cooled and diluted with 50 ml of deionized water and filtered through whatmann No. 42 filter paper. The filtrate was stored and used for nutrient analysis. Chloride ion was measured with a Beckman specific ion electrode. The Na<sup>+</sup> and K<sup>+</sup> content were determined by flame emission spectrophotometer and Ca<sup>2+</sup>, Mg<sup>2+</sup> levels by atomic absorption spectrophotometer. Nitrogen in the plant tissue was analyzed based on the Dumas dry-combustion method using a Leco Fp-428 nitrogen analyzer (St. Joseph, MI. USA). The phosphorus content was estimated by [22]. The micronutrient analyses were followed by the method of [23].

### Statistical Analysis

The data for fresh and dry weight and ionic content were analysed using two-way ANOVA without replication. The values to determine if significant were present at P<0.05.

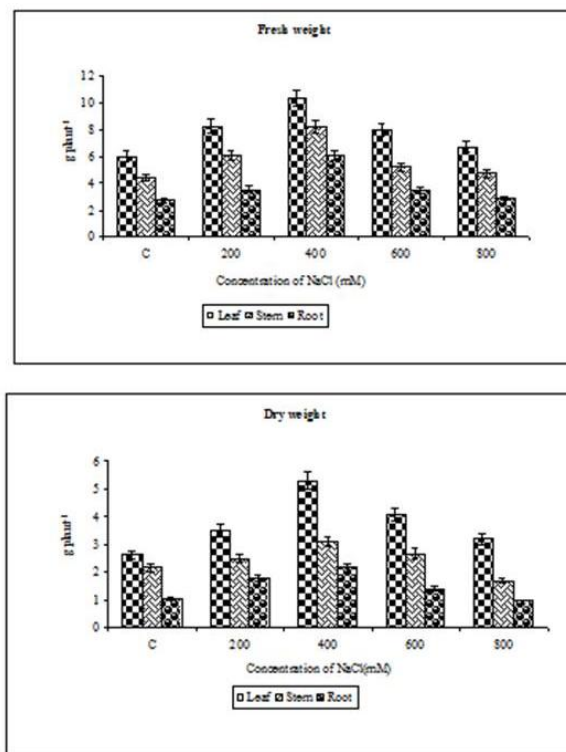
## 3. Results and Discussion

### Growth

The growth of *B. conjugata* was significantly increased by salt treatment when compared to control. The fresh and dry weight of leaf, stem and root showed similar pattern. However, the remarkable increase in the fresh and dry weight it was found at the salt concentration (>200 mM NaCl) when compared to control plants treated with tap

water (Fig. 1). The F values were significant at P<0.05 level.

Fig. 1. Effect of NaCl on fresh and dry weight (g plant<sup>-1</sup>) of leaf, stem and root in *Bruguiera conjugata*. Bar represents mean n=6 ± S.E values are significant at P<0.05.

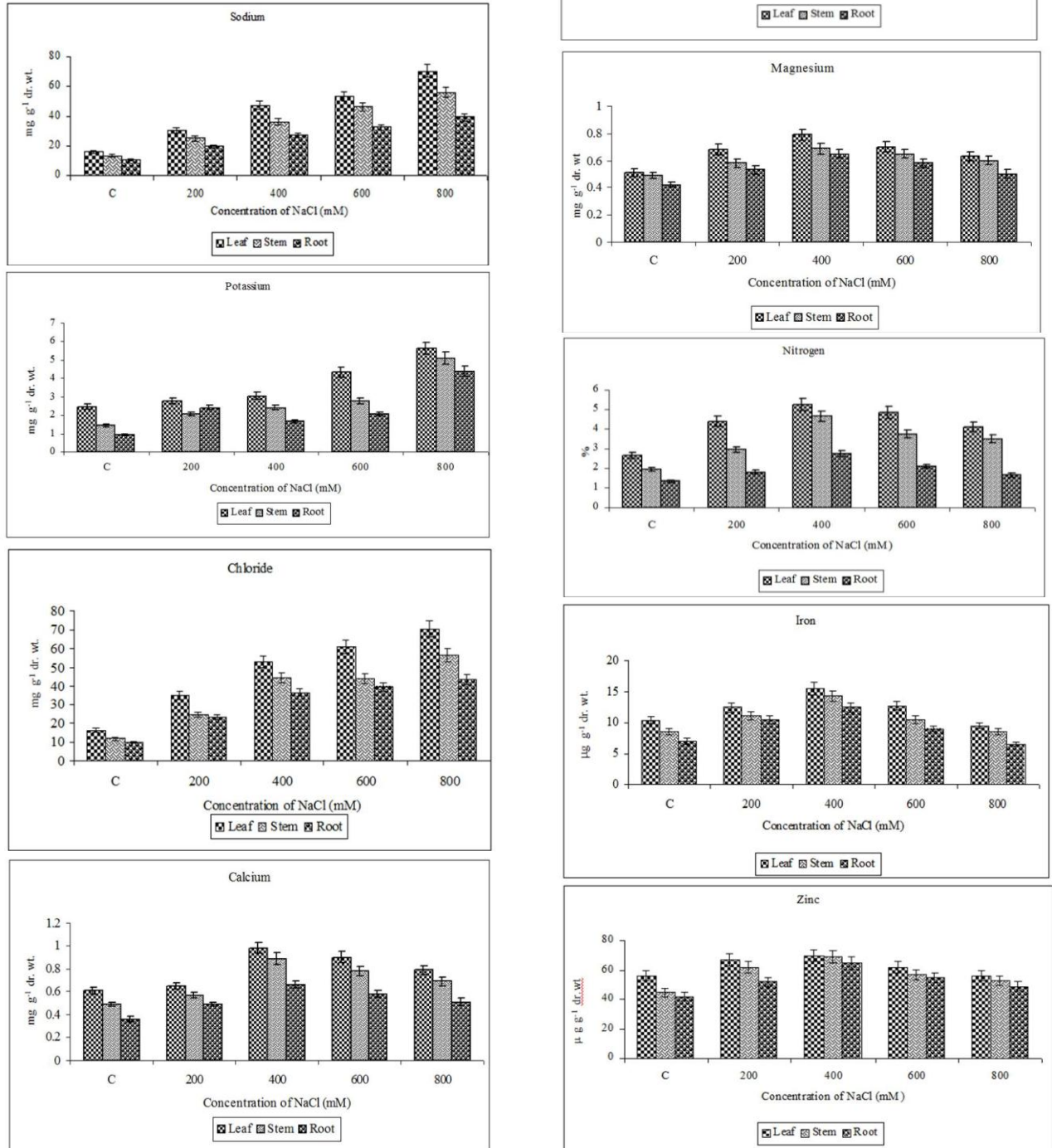


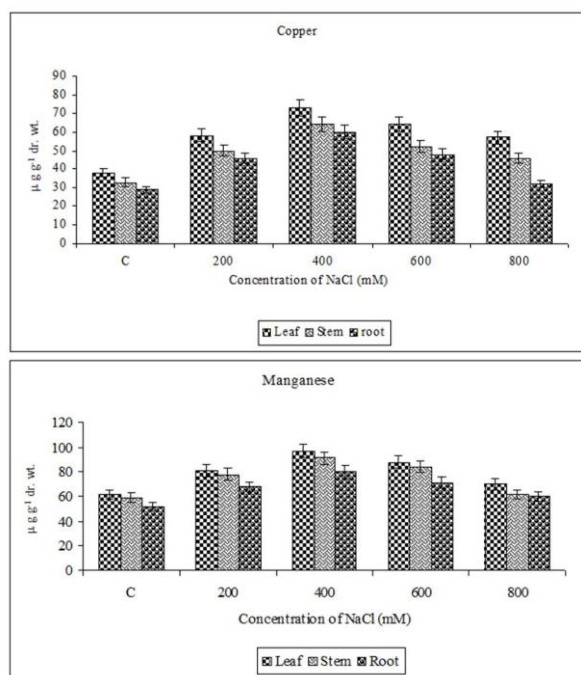
### Ionic Content

Sodium, chloride and potassium content per plant increased significantly with an increasing in salt concentration upto 800 mM NaCl whereas Na<sup>+</sup> content of the leaf and root are significant at P<0.05 level the values. The F values were not significant at stem P>0.05. Salinity induced changes in the concentration of the other element analysed varied with plant organs and element. The K<sup>+</sup> content of stem and root are not significant whereas significant P<0.05 in the leaves. The Cl<sup>-</sup> concentration of leaf and stem are non significant whereas the root Cl<sup>-</sup> concentration significant at P<0.05. The effect of NaCl significantly increased the content of Ca<sup>2+</sup>, P, N and Mg<sup>2+</sup> increased upto optimum level of 400

mM NaCl and above this level all the content drastically reduced (Fig 2).

Fig 2. Effect of NaCl on macronutrient content of leaf, stem and root in *B. conjugata* Bar represents mean  $\pm$  standard error values are significant at  $P < 0.05$





The highest accumulation of  $\text{Ca}^{2+}$ , P,  $\text{Mg}^{2+}$  in 400 mM NaCl was observed at 120th sampling day when compared to that of control. The  $\text{Ca}^{2+}$ , P and  $\text{Mg}^{2+}$  content of stem and root are not significant ( $P > 0.05$ ) whereas the concentration of leaf showed significant  $P < 0.05$ . The nitrogen content of all the organs is significant at  $P < 0.05$ .

The micronutrient such as  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Mn}^{2+}$  contents increased upto optimum level of 400 mM NaCl in all the organs when compared to that of control on 120th day. The F values are significant at  $P < 0.05$  levels.

Salinity might decrease biomass production because it causes a lowering of plant water potentials, specific ion toxicities, or ionic imbalances [24]. Plants protect themselves from NaCl toxicity by minimizing  $\text{Na}^+$  uptake and transport to the shoot [25] osmotic adjustment under saline condition may be achieved by ion uptake, synthesis of osmotica or both [26]. Halophytes differ widely in the extent to which they accumulate ions and overall degree of salt tolerance [11].

Stem and leaf-succulent Chenopods are commonly known as salt-accumulators and have high  $\text{Na}^+$  and  $\text{Cl}^-$  content [24].

### Sodium

Increasing sodium chloride salinity increases the sodium content of several halophytes such as *Aleuropus lagopoides* [27]; *Atriplex griffithii* [6]; *Spartina alterniflora* [28]. The increase is linear with the increasing concentration of exogenous addition of NaCl. The result obtained in this study is in conformity with observations of [29]. The bulk of the sodium ions have been reported to be partitioned in the cell vacuole and thereby depleting the cytoplasmic sodium level and conferring the plant to function as normal [30].

### Potassium

To select  $\text{K}^+$  in preference to  $\text{Na}^+$ , the reasons must lie in the relative effectiveness of the two ions in enzyme activation and this is presumably a property of the co-ordination chemistry of  $\text{Na}^+$  [31]. Since, potassium is more effective than sodium as far as the metabolism is concerned, this has led to the evolution of specific mechanism to maintain the requisite cytoplasmic balance of the two ions and the resulting transport systems presumably discriminated by means of specific carriers in the external membrane. Whatever be the mechanism, the development of the transport system appears to have heightened the dependence of potassium over sodium even though sodium is able to substitute potassium as osmoticum and  $\text{K}^+$  appears to be essential to the overall regulation of the balance of the two ions.

[4] have reported that  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations increase with an increase in salinity, while  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$  decrease. Shoots of *A. lagopoides* maintain relatively constant  $\text{K}^+$  concentration with increasing salinity, resulting in high  $\text{K}^+$  selectively ratios.



Similar responses for shoot  $K^+$  at high salinity have been observed in other halophytic grasses [32, 5]. This contrast with the large drop in shoot  $K^+$  with increasing salinity is frequently observed in dicotyledonous halophytes [33]. The maintenance of fairly constant  $K^+$  concentrations with increasing salinity may be interpreted as the requirement for a minimum cytoplasmic  $K^+$  level, possibly associated with the  $K^+$  requirement of protein synthesis [34].

The  $K^+$  deficiency of salinized plants is inversely correlated to the increased accumulation of  $Na^+$ , indicating the existence of competition effects between  $Na^+$  and  $K^+$  ions which most likely share the same transport system at the root surface [35]. In contrast to  $Na^+$ , we do not find differences between leaf types suggesting that additional transport systems and/or regulatory mechanism may be involved in controlling  $Na^+$  and  $K^+$  homeostasis [35].

#### Chloride

Chloride is required as a macronutrient to support the maximum growth. [36] have found a preferential accumulation of chloride in the inner cells rather than in the chlorenchyma cells of the leaf in *Suaeda monoica*. Chloride uptake is believed to be an active process, since there is a lower electrical potential in the cytoplasm than outside the cell [37]. However, the studies of [38] in *Spergularia marina* show no alteration in the proton efflux when the plants are transferred from non-saline medium to saline environment and this supports the passive uptake of ions. There exists still, controversial opinion on the mechanism of ion uptake [39]. The increase is linear with increasing concentration of NaCl. Chloride, a physiologically natural anion is tolerated over a wide range of concentrations in *Kandelia candel* [40] and *Arthrocnemum indicum* [41]. [42] have suggested that the leaves of *Atriplex*

*littoralis* and *A. calotheca* increase with an increment in external salt concentrations.

#### Calcium

Calcium is also suggested to protect membrane damage and to play a key role in the selective transport of potassium in the presence of excess of sodium, and thereby making a plant more salt tolerant [43]. Absorbance and accumulation of adequate amount of calcium and magnesium from the soil reflect the ability of a species to salt tolerance [44]. Calcium has been shown to reduce the toxic effect of NaCl salinity and enhance calcium level in the tissue. It may protect the plant from NaCl toxicity by reducing displacement of membrane associated calcium ions [45].

#### Phosphorus

Phosphorus along with nitrogen can modify the effect of salinity on the growth of *Rhizophora mangle* and in *Triticum vulgare* [46]. Adequate availability of phosphorus in irrigated water enables the photosynthetic area of salt stressed plants to remain green and these leaves act as a sink for the accumulation of ion, for buffering against potentially toxic ion accumulation in the photosynthetically active leaves [47]. In the present study, phosphorus accumulation in the leaves is associated with enhanced photosynthetic activity and greater accumulation of carbohydrates. Phosphorus plays a key role in the energy metabolism requirements and in the formation of ATP, ADP, NAD and NADP which in turn play key roles in photosynthesis [16]. Phosphorus is required only in the oxidized form as orthophosphate.

#### Magnesium

Sodium chloride salinity upto 400 mM stimulates magnesium accumulation in the plant organs of *Bruguiera conjugata* and the magnesium content gradually decreases at

higher salinity levels. Magnesium, integral photosynthetic pigment, chlorophyll and a factor for several enzyme systems is beneficial to various biochemical processes. The decrease in the magnesium content at higher NaCl salinity is probably due to less translocation of magnesium under salinity stress.

### Nitrogen

Increased nitrogen content with increasing NaCl salinity has been reported in a few halophytes. Higher nitrogen content may be associated with osmotic solute or accumulation of nitrate ions or increased protein degradation of a combination of these, when plants are subjected to high salinity [49]. The control of  $\text{Na}^+$  and  $\text{Cl}^-$  accumulation might be an important physiological process conferring salt tolerance in plants [50] and this control can be related to nitrogen nutrition. Since, the source of nitrogen nutrition can lead to difference in the accumulation patterns of these ions [51]; nitrogen has been found to be a limiting factor for plant growth in coastal halophytes. Increased NaCl salinity had increasing effect in the total nitrogen content of leaf and root of *Plantago maritima* and *Rhizophora mangle* [46]. The response of halophyte, *Halimione portulacoides* to non-saline polyethylene glycon (PEG) – 6000 or saline seawater is a rise in concentration of total soluble nitrogen which has been associated with increased salinity [52].

### Micronutrients

In addition, many nutrient instructions in salt – stressed plants can occur which may have important consequences for growth. Internal concentrations of major nutrients and their uptake have been frequently studied [53], but the relationship between micronutrient concentrations and soil salinity is rather complex and remains poorly understood [54].

The function of  $\text{Mn}^{2+}$  at the cellular level of plant is to bind firmly to lamellae of chloroplasts, possibly to the outer surface of thylakoid membranes affecting the chloroplast structure and photosynthesis, [55]. External application of  $\text{Mn}^{2+}$  increased photosynthesis, net assimilation and relative growth in barley under salinity [56]. The increase in manganese in the plant tissue may be related to the increase in soluble manganese in the soil by the conversion of insoluble manganese oxide to the soluble form [57]. Increase in manganese concentration in the salinised soil and plant tissue has been reported earlier [58]. Manganese is a part of the water-splitting enzyme of photosystem II of photosynthesis [59]. Manganese deficiency may cause a reduction in photosynthesis and which, in turn, may be responsible for the inhibition of growth under salinity. Plants under salinity stress require a higher concentration of manganese [60]. In the present study, manganese content in relation to salinity can be correlated with photosynthesis rates. The increase in the iron content of the tissues under moderate salinity may be due to abrupt changes in the membrane's permeability [61]. Organic acids have shown to be important in the transport of divalent cations which appear to chelate with iron [62].

Zinc content decreases at higher salinity levels. The increase in the zinc content can be correlated with increase in nitrogen and potassium content. The close relationship of zinc with nitrogen has been reported [63]. The increase in the copper content can be explained by the close association of copper with nitrogen ligands. A close parallel relation in the movement of copper and nitrogen has been shown by [63 and 57]. In the present study, the increase in the copper content is parallel with nitrogen and potassium.

## Conclusion

The pattern of mineral nutrition as observed in the present study in *Bruguiera conjugata* and similar studies in other mangrove species may serve as the source of basic information to understand the processes of growth and development of crop species under saline irrigation.

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